APPLICATION OF BACTERIAL BIOFLOCCULANTS FOR WASTEWATER AND RIVER WATER TREATMENT

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Submitted in fulfilment of the academic requirements for the degree of Master of Science in the Discipline of Microbiology, School of Biochemistry, Genetics, Microbiology and Plant Pathology, Faculty of Science and Agriculture at the University of KwaZulu-Natal, Durban.

As the candidate's supervisor, I have approved this dissertation for submission.

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For my parents: Mr and Mrs E. Buthelezi

Declaration by the candidate

This MSc study was carried out in the School of Biochemistry, Genetics, Microbiology and Plant Pathology, University of KwaZulu-Natal, Westville Campus, under the supervision of Professor B Pillay.

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Simphiwe Promise Buthelezi (Candidate)

ACKNOWLEDGEMENTS

The author records her appreciation to the following persons and organizations for their assistance in the completion of this study:

Prof. B. Pillay, Department of Microbiology, University of KwaZulu-Natal for his contribution, supervision, academic stimulation and words of encouragement;

Prof. J. Lin for his support and words of encouragement;

Dr. A. O. Olaniran for his assistance and support;

Dr. R. Shaik for her assistance and support;

Dr. M. E. Setati for her assistance and support;

Mr A. Govender for his assistance and support;

Mr. K. Buthelezi, Mr. L. Buthelezi, Mrs E. Buthelezi, Mr and Mrs Simpson, Mrs Z. Zungu, Mrs D. N. Ngubane, Miss M. Makatini, Mr. G. Makaula, Mr. I. Karodia, Mr. B. Naidoo, Mr. S. Dlamini, Mr. L. Mnguni, Mr and Mrs Mnyeni, and Mr. M. Ngiba for their support;

LIFE*lab*, DAAD and the UKZN for financial assistance;

Staff and postgraduate students of the Discipline of Microbiology (Westville campus), University of KwaZulu-Natal, for their companionship and selfless assistance;

God, for being with me throughout the course of the study;

Family and friends for their support, emotional and spiritual strength throughout the course of the study.

ABSTRACT

Dyes are often recalcitrant organic molecules that produce a colour change and contribute to the organic load and toxicity of textile industrial wastewater. Untreated effluent from such sources is harmful to aquatic life in the rivers and lakes due to reduced light penetration and the presence of highly toxic metal complex dyes. The use of alum as flocculant/coagulant in wastewater treatment is not encouraged as it induces Alzheimer's disease in humans and results in the production of large amounts of sludge. Therefore, the development of safe and biodegradable flocculating agents that will minimize environmental and health risks may be considered as an important issue in wastewater treatment. Bioflocculants are extracellular polymers synthesized by living cells. In this study, bacterial bioflocculants were assessed for their ability to remove dyes from textile wastewater as well as reducing the microbial load in untreated river water. The bacteria were isolated from a wastewater treatment plant and identified using standard biochemical tests as well as the analysis of their 16S rDNA gene sequences. Six bacterial isolates were identified *viz. Staphylococcus aureus, Pseudomonas plecoglossicida, Pseudomonas pseudoalcaligenes, Exiguobacterium acetylicum, Bacillus subtilis,* and *Klebsiella terrigena*. The flocculating activities of the bioflocculants produced by these isolates were characterized. The effect of temperature, pH, cations and bioflocculant concentration on the removal of dyes, kaolin clay and microbial load was also determined. The amount of bioflocculants produced by the bacterial isolates ranged between 5 and 27.66 g/l. According to the findings of the present study, bacterial bioflocculants were composed of carbohydrates, proteins, uronic acid, and hexosamine in varying quantities. The bioflocculants were effective to varying degrees in removing the dyes in aqueous solution, in particular whale dye, medi-blue, fawn dye and mixed dyes, with a decolourization efficiency ranging between 20-99.9%. Decolourization efficiency was influenced by the bioflocculant concentration, pH, temperature, and cations. The bacterial bioflocculants were also capable of reducing both the kaolin clay and the microbial load from river water. The flocculating activity ranged between 2.395–3.709 OD^{-1} while up to 70.84% of kaolin clay and 99% of the microbial load from the river water was removed. The efficiency of kaolin clay flocculation increased with higher

concentration of bacterial bioflocculants. The optimum pH for the flocculating activity was observed between 6 and 9. The best flocculating activity was observed at 28^{o}C. Divalent cations such as Mg^{2+} and Mn^{2+} improved the flocculation while salts such as K_2HPO_4, CH_2COONa, and Na_2CO_3 did not. The findings of this study strongly suggest that microbial bioflocculants could provide a promising alternative to replace or supplement the physical and chemical treatment processes of river water and textile industry effluent.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

A great number of industries such as textile, paper and pulp, printing, iron-steel, coke, petroleum, pesticide, paint, solvent, pharmaceutics, wood preserving chemicals, consume large volumes of water and organic based chemicals. These chemicals show a vast difference in chemical composition, molecular weight, and toxicity. Effluents from these industries may also contain undesired quantities of these pollutants and need to be treated (Aksu, 2005).

1.1 MICROBIAL PROCESSES FOR THE DECOLOURIZATION OF TEXTILE EFFLUENTS

The most common textile-processing set-up (Fig. 1.1) for the decolourization of textile effluents consists of desizing, scouring, bleaching, mercerising, and dyeing processes (Dos Santos *et al*., 2007). Sizing is the first preparation step, which involves the addition of sizing agents such as starch, polyvinyl alcohol (PAVE) and carboxymethyl cellulose. These agents provide strength to the fibres and minimize breakage. Desizing is employed next, to remove sizing materials prior to weaving. Scouring removes impurities from the fibres by using alkali solution (commonly sodium hydroxide) to breakdown natural oils, fats, waxes and surfactants, as well as to emulsify and suspend impurities in the scouring bath. Bleaching is the step used to remove unwanted colour from the fibres by using chemicals such as sodium hypochlorite and hydrogen peroxide. Bleaching is followed by mercerising which is a continuous chemical process used to increase dye-ability, lustre, and fibre appearance. In this step, a concentrated alkaline solution is applied and an acid solution washes the fibres before the dyeing step. Finally, dyeing is the process of adding colour to the fibres, which normally requires large volumes of water not only in the dye bath, but also during the rinsing step. Depending on the dyeing process, many chemicals like metals, salts, surfactants, organic processing assistants, sulphide and formaldehyde, may be added to improve dye

adsorption onto the fibres. Figure 1.1 shows some potential pollutants from cotton processing operations in which the desiring/scouring and dye bath/rinsing wastewaters are mainly composed of organic pollutants and colour-causing pollutants, respectively (Dos Santos *et al*., 2006; 2007).

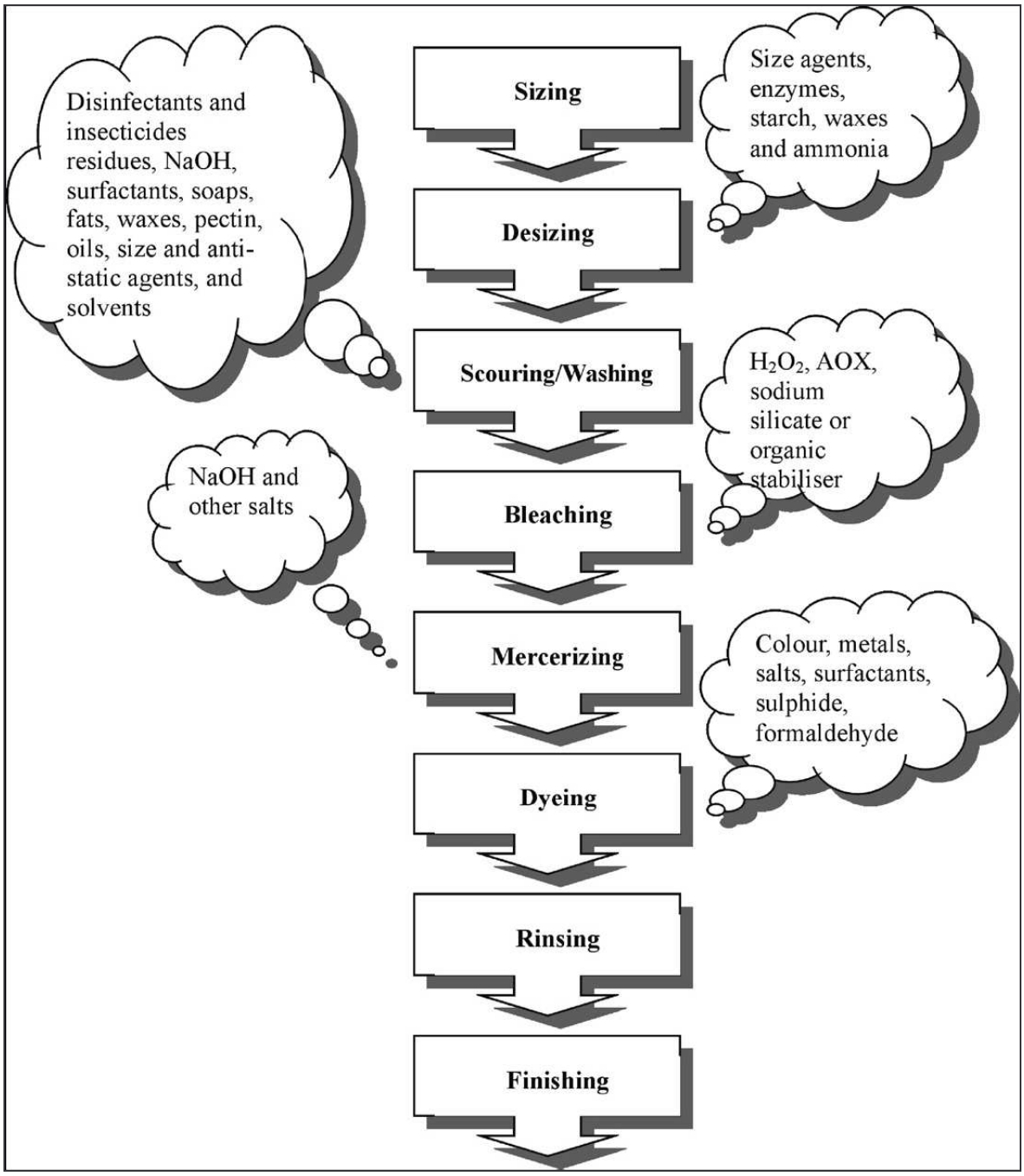

Fig. 1.1: Scheme of operations involved in textile cotton industry and the main pollutants from each step (Dos Santos *et al.,* 2007).

Synthetic dyestuffs are a group of organic pollutants, which are used extensively in textile, paper, printing industries, and dye houses. It has been reported that there are over 100 000 commercially available dyes with a production of over 7×10^5 metric tonnes per year (Clarke and Anliker, 1980). Effluents from dyeing industries constitute one of the most problematic wastewaters because of their high chemical and biological oxygen demands. It has been recognized that the public perception of water quality is greatly influenced by colour. Colour is the first contaminant recognized in wastewater. The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and undesirable (Banat *et al*., 1996; Robinson *et al*., 2001). Dyes may significantly affect photosynthetic activity in aquatic life due to reduced light penetration and may be toxic to some aquatic life due to the presence of aromatics, metals, and chlorides, in them. Dyes usually have a synthetic origin and complex aromatic molecular structures, which make them more stable and more difficult to biodegrade (Clarke and Anliker, 1980; Mishra and Tripathy, 1993; Banat *et al*., 1996; Fu and Viraraghavan, 2001; Robinson *et al*., 2001).

1.1.1 Classification of dyes

Dyes are classified as follows: anionic-direct, acid, and reactive dyes; cationic-basic dyes; and non-ionic disperse dyes. The chromophores in anionic and non-ionic dyes are mostly azo groups or anthraquinone types. The reductive cleavage of azo linkages is responsible for the formation of toxic amines in the effluent (Mishra and Tripathy, 1993; Fu and Viraraghavan, 2001). Anthraquinone-based dyes are more resistant to degradation due to their fused aromatic structures and thus remain coloured for a longer time in the wastewater. Reactive dyes are typically azo-based chromophores combined with different types of reactive groups e.g., vinyl sulfone, chlorotriazine, trichloropyrimidine, difluorochloropyrimidine. They differ from all other classes of dyes in that they bind to the textile fibres such as cotton through the covalent bonds. They are widely used in textile industries because of their favourable characteristics such as bright colour, water-fast, simple application techniques with low energy consumption. Water-soluble reactive and acid dyes are the most problematic, as they tend to pass through conventional

treatment systems unaffected. Hence, their removal is also of great importance (Hu, 1992; Juang *et al*., 1997; Moran *et al*., 1997; Karcher *et al*., 1999; Aksu and Tezer, 2000; Sumathi and Manju, 2000; Robinson *et al*., 2001; O'Mahony *et al*., 2002).

Basic dyes have high brilliance and intensity of colours and are highly visible even in a very low concentration (Clarke and Anliker, 1980; Banat *et al*., 1996; Mittal and Gupta, 1996; Fu and Viraraghavan, 2001; Chu and Chen, 2002; Fu and Viraraghavan, 2002). Metal complex dyes are mostly based on chromium, which is carcinogenic (Gupta *et al*., 1990; Mishra and Tripathy, 1993). Disperse dyes do not ionize in an aqueous medium and some disperse dyes have also been shown to have a tendency to bioaccumulate (Baughman and Perenich, 1988; Srivastava and Prakash, 1991). Due to the chemical stability and low biodegradability of these dyes, conventional biological wastewater treatment systems are inefficient in treating dye wastewater. Physical or chemical treatment processes are usually employed to treat dye wastewater. These include chemical coagulation/flocculation, ozonation, oxidation, ion exchange, irradiation, precipitation, and adsorption. Some of these techniques are effective, but have their limitations. These include: excess amount of chemical usage, or accumulation of concentrated sludge with obvious disposal problems; expensive plant requirements or operational costs; lack of effective colour reduction; and sensitivity to a variable wastewater input (McKay and Poots, 1980; El-Geundi, 1991; Juang *et al*., 1997; Lambert *et al*., 1997; Low and Lee, 1997; Ramakrishna and Viraraghavan, 1997; Slokar and Le-Marechal, 1997; Ho and McKay, 1999; Lee *et al*., 1999; Morais *et al.*, 1999; Otero *et al.*, 2003).

In recent years, a number of studies have focused on some microorganisms, which are able to biodegrade or bioaccumulate azo dyes in wastewaters (Dhodapkar *et al*., 2006). A wide variety of microorganisms including bacteria, fungi, and algae are capable of decolourizing a wide range of dyes via anaerobic, aerobic, and sequential anaerobic–aerobic treatment processes. Cytoplasmic azo reductases play an important role in the anaerobic biodegradation of azo dyes to produce colourless aromatic amines although complete mineralization is difficult and the resulting aromatic amines may be toxic and

carcinogenic. These amines are resistant to further anaerobic mineralization. Fortunately, once the xenobiotic azo component of the dye molecule has been removed, the resultant amino compounds are good substrates for aerobic biodegradation suggesting a choice of a sequential anaerobic–aerobic system for wastewater treatment (Razo-Flores *et al*., 1997; Fu and Viraraghavan, 2001; Manu and Chaudhari, 2001). A number of aerobic biological processes for the removal of dyes from textile effluents exist. This includes decolourization through liquid fermentations by white-rot fungi (such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Coriolus versicolor*); bacterial cultures (such as *Pseudomonas* strains, mixed bacterial cultures, *Bacillus subtilis*) and yeasts (such as *Klyveromyces marxianus*, *Candida zeylanoides*). Biochemical oxidation suffers from significant limitations since more dyestuffs found in the commercial market have been intentionally designed to be resistant to aerobic microbial degradation (Pearce *et al*., 2003).

Reactive azo dyes are electron deficient in nature and this property makes them less susceptible to oxidative catabolism (Banat *et al*., 1996). Research has shown that the efficiency of biological treatment systems are greatly influenced by the operational parameters, the composition of textile wastewater and the structure and substituents of dye molecule. The level of aeration, temperature, pH, and redox potential of the system are the variables that can be optimized to produce the maximum rate of dye reduction. The ability of microorganisms to reduce dyes from wastewater depends on the classes of dyes used (acidic, basic, direct, disperse, metal-complex, and reactive). The composition of textile wastewater varies and can include organics, nutrients, salts, sulphur compounds and toxicants as well as colour. Therefore, the inhibitory effect of any of these compounds on the dye reduction process should be investigated (Glenn and Gold, 1983; Knapp and Newby, 1999; Kapdan *et al*., 2000; Meehan *et al*., 2000; Fu and Viraraghavan, 2001; Manu and Chaudhari, 2001; Ramalho *et al*., 2002). Another biological treatment method is bioaccumulation. Bioaccumulation is defined as the accumulation of pollutants by actively growing cells by metabolism; temperature-independent; and metabolism-dependent mechanism steps. Although bioaccumulations of dyes by yeasts were accomplished, significant practical limitations exist. These include

the inhibition of cell growth at high dye concentrations and requirement of metabolic energy externally provided (Dönmez, 2002; Aksu, 2003). Therefore, there is a need to find alternative treatment methods that are effective in removing dyes from large volumes of effluents and are low in cost, such as biosorption or bioflocculation.

1.2 THE NEED FOR WASTEWATER TREATMENT

Matter in water may be broadly classified according to its origin as inorganic mineral matter or organic carbonaceous material. Substances producing turbidity are often inorganic, while those causing taste, odour, and colour are generally organic compounds. The particles producing turbidity may be further classified according to their size, which may range from molecular dimensions of 50 microns or larger. The fraction greater than 1 micron in diameter is generally referred to as silt and will settle out on standing. The smaller particles, which are classified as colloidal, will remain suspended for very long time. Most attention has therefore been directed towards the use of coagulation for removal of colloidal material, although this process also removes the larger particles (Jones, 1998). It is important to keep our water clean because of our environment and health. Some of the reasons include:

- Fisheries; clean water is critical to plants and animals that live in water. This is important to the fishing industry, sport fishing enthusiasts, and future generations;
- Wildlife habitat, rivers and ocean waters teem with life that depends on shoreline, beaches, and marshes. They are critical habitats for hundreds of species of fish and other aquatic life. Migratory water birds use the areas for resting and feeding;
- Recreation and quality of life; water is a great playground for us all. The scenic and recreational values of our waters are reasons many people choose to live where they do. Visitors are drawn to water activities such as swimming, fishing, boating, and picnicking; and
- Health concern; water may carry disease if not properly cleaned. Since we live, work and play so close to water, harmful bacteria have to be removed to make water safe (Cowan and Talaro, 2006).

1.3 WATER SOURCES AND QUALITY

Water is usually withdrawn for drinking and household purposes from the following sources (Navin *et al.,* 2006):

- Ground water (springs, infiltration galleries, and wells)
- Surface water (rivers, lakes, ponds, streams, impounded reservoirs, and stored rainwater).

Ground water is one of the nation's most important natural resources. Ground water provides drinking water for more than one-half of the nation's population and is the sole source of drinking water for many rural communities and some large cities. In 1990, ground water accounted for 39% of the public water supply for cities and towns and 96% for self-supplied systems for domestic use (Twarakavi and Kaluarachchi, 2006). It is also the source of much of the water used for irrigation. Ground water is a major contributor to flow in many streams and rivers and has a strong influence on river and wetland habitats for plants and animals. Ground water withdrawal in the USA in 1995 was estimated to be approximately 77 billion gallons per day, which is about 8% of the estimated 1 trillion gallons per day of natural recharge to the ground water system of the USA (Navin *et al.,* 2006; Twarakavi and Kaluarachchi, 2006).

If groundwater is conveniently located and in sufficient quantities it should be used, as it is less polluted compared with surface water. Groundwater may be aerobic or anaerobic depending on the environmental conditions where it is located. The anaerobic groundwater often contains CO_2, which makes it corrosive. The removal of CO_2 as well as provision of oxygen can be facilitated by aeration. Chlorination also removes CO_2. Some ground water contains excessive amounts of Fe, Mn, hardness, and/or fluoride (Decker and Long, 1992).

The surface waters are generally more polluted than groundwater due to their exposure to the environment. They may require more treatment steps than ground water.

The typical impurities may include turbidity, colour, algae, floating debris, bacteria, and other microorganisms, in addition to the constituents of ground water. Surface water in general contains physical, chemical, and biological impurities (such as clay, sand, colloids, minerals, colour, odour, taste, and microorganisms). Rivers have been used by man since the dawn of civilization as a source of water, for food, for transport, as a source of power to drive machinery, and a means of disposing of waste (Vigneswaran and Visvanathan, 1995).Thus river water requires adequate treatment steps so that it can be suitable for domestic purposes. Figure 1.2 is an illustration of the water cycle.

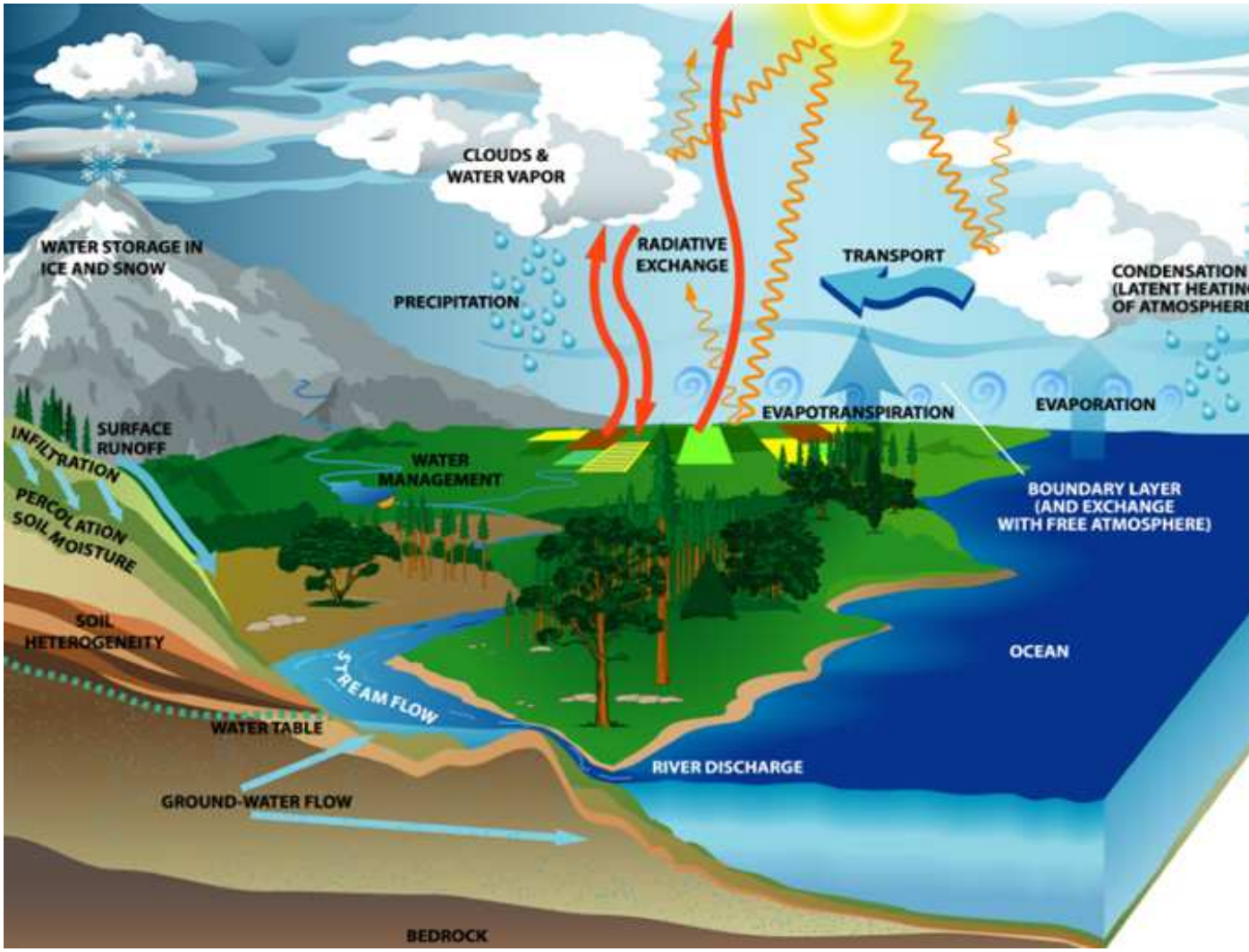

Fig. 1.2: The water cycle (http://www.usgcrp.gov/usgcrp/images/ocp2003/WaterCycle optimized.jpg).

The majority of water supplies require treatment to make them suitable for use in domestic and industrial applications. Although appearance, taste, and odour are useful indicators of the quality of drinking water, suitability in terms of public is determined by microbiological, physical, chemical, and radiological characteristics. Of these, the most important is microbiological quality. In addition, a number of chemical contaminants (both organic and inorganic) are found in water. These may lead to health problems. Therefore, detailed analyses of water are warranted (Vigneswaran and Visvanathan, 1995). The drinking water thus should be; free from pathogenic (disease causing) organisms; clear (i.e., low turbidity, little colour); not saline (salty); free from offensive taste or smell; and free from compounds that may have adverse effects on human health (harmful in the long term) (Vigneswaran and Visvanathan, 1995).

1.4 WASTEWATER TREATMENT PROCESSES

The major aim of wastewater treatment is to remove as much of the suspended solids as possible before the remaining water, called effluent, is discharged back to the environment. As solid material decays, it uses up oxygen, which is needed by the plants and animals living in the water. Conventional wastewater treatment process involves primary, secondary, and tertiary treatment (Prescott *et al.,* 1996; Nester *et al.,* 2001).

1.4.1 Primary treatment

Primary treatment involves aerating (stirring up) the wastewater, in order to replenish oxygen back in. Primary treatment can remove 20 to 30% of the BOD, which is present in particulate form and about 60% of suspended solids from wastewater. In this treatment, particulate material is removed by screening, precipitation of small particles, and settling in basins or tanks. The resulting solid material is called sludge. This treatment of physical removal of settleable solids (primary treatment) and secondary treatment (biological transformation of dissolved organic matter to microbial biomass and carbon dioxide) is shown together with final clarifiers. The final clarifiers separate the

newly formed microbial biomass (sewage sludge) from the processed water stream, which can be returned to the receiving body of water (Prescott *et al*., 1996).

1.4.2 Secondary treatment

Secondary treatment is used after primary treatment for the biological removal of dissolved organic matter. This process removes about 90 to 95% of the BOD and many bacterial pathogens and more than 90% of suspended solids. Several approaches involving similar microbial activities can be used for secondary treatment to biologically remove dissolved organic matter. Under aerobic conditions, dissolved organic matter is transformed into additional microbial biomass plus carbon dioxide (Ford, 1993). When these processes occur with lower oxygen levels or with a microbial community too young or too old, unsatisfactory floc formation and settling can occur. The result is a bulking sludge, caused by massive development of filamentous bacteria such as *Sphaerotilus* and *thiothrix,* together with many poorly characterized filamentous organisms. These important filamentous bacteria form flocs that do not settle well and have effluent quality problems (Prescott *et al*., 1996).

An aerobic activated sludge process is a biological method of wastewater treatment that is performed by a variable and mixed community of microorganisms in an aerobic aquatic environment. These microorganisms derive energy from carbonaceous organic matter in aerated wastewater for the production of new cells in a process known as synthesis. Simultaneously energy is released through the conversion of this organic matter into compounds that contain lower energy, such as carbon dioxide and water, in a process called respiration. A variable number of microorganisms in the system obtain energy by converting ammonia nitrogen to nitrate nitrogen in a process termed nitrification. In this consortium of microorganisms, the biological component of the process is known collectively as activated sludge. The overall goal of the activated-sludge process is to remove substances that have a demand for oxygen from the system (Huang and Shui, 1996).

After the bacteria and fungi in sewage treatment have used certain nutrients, they serve as food for ciliates, protozoa, nematodes, and other forms of life. The end result is a small increase in the mass of organisms present in a given amount of treated sewage and a large decrease in the amount of biodegradable organic material (Bitton, 1994). The increased microbial biomass in the sewage is removed to the digester; a portion is left behind as inoculum to act on a new load of waste material. Within the sewage digester, anaerobic organisms act on the solids remaining in sewage after its aerobic treatment. The digester provides anaerobic conversion of organic to inorganic matter and removes water from the sewage so that a minimum of solid matter remains in it. Various populations act sequentially. In the sewage digester, the anaerobic methane-forming organisms can perform their role of converting the simple organic acids in sewage into the useful end product methane (CH_4). Many sewage treatment plants are equipped to use their own methane, thereby avoiding the cost of other sources of energy to run their equipment (Nester *et al*., 2001). Figure 1.3 illustrates the processes involved for the production of methane on small scale.

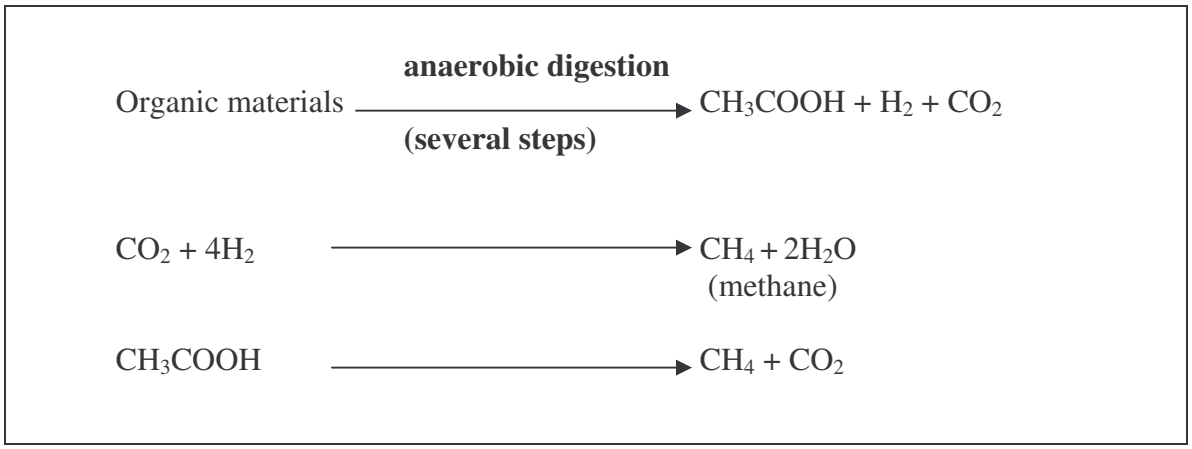

Fig. 1.3: Production of methane on a small scale (Nester *et al.,* 2001).

Pathogenic bacteria are generally removed from sewage during secondary treatment, but disease-producing viruses may survive. Pathogens account for only a small proportion of the total number of bacteria in faeces, and they are diluted by the water in sewage. During the secondary treatment of sewage, these pathogens must compete for nutrients with a huge mass of bacteria that have been adapted to grow best at the

temperature and conditions provided. As a result, most pathogenic bacteria are overgrown and are eliminated by their competitors (Huang and Shui, 1996). Animal viruses lack appropriate host cells in sewage and replicate there, although they may survive for long periods. If large quantities of virus particles are present in raw sewage, some may be recovered after secondary treatment. Although sewage effluents are often chlorinated before being discharged into receiving waters, chlorine treatment at this stage does not inactivate viruses. The virus particles are commonly enclosed within small clumps of effluent materials where they are protected from the chlorine. Because viruses do adhere to large particles, they can be removed along with other solid materials (Huang and Shui, 1996; Nester *et al*., 2001).

1.4.3 Tertiary treatment

Tertiary treatment purifies wastewater more than is possible with primary and secondary treatments. The goal is to remove such pollutants as nonbiodegradable organic matter e.g. polychlorinated biphenyls, heavy metals, and minerals (Prescott *et al*., 1996). Tertiary treatment provides a final stage to raise the effluent quality to the standard required before it is discharged to the receiving environment (sea, river, lake, and ground). The large quantities of phosphates or nitrites remaining in sewage after secondary treatment may increase the growth of microorganism, which gradually deplete the oxygen and thus threaten other forms of aquatic life. The tertiary treatment of sewage, to remove nitrates and phosphates, can greatly alleviate this problem (Cowan *et al*., 1995).

In some designs for the tertiary treatment of sewage, chemical precipitation of phosphates has been combined with biological removal of nitrates. Certain bacteria (particularly species of *Pseudomonas* and *Bacillus*) can completely reduce nitrates (NO_3^-) to N_2 (denitrification). The N_2 gas is inert, non-toxic, and easily removed. More than one tertiary treatment process may be used at any treatment plant. If disinfection is practiced, it is always the final process. It is also called "effluent polishing" (Nester *et al*., 2001). Figure 1.4 is an illustration of the major steps in modern sewage treatment plant.

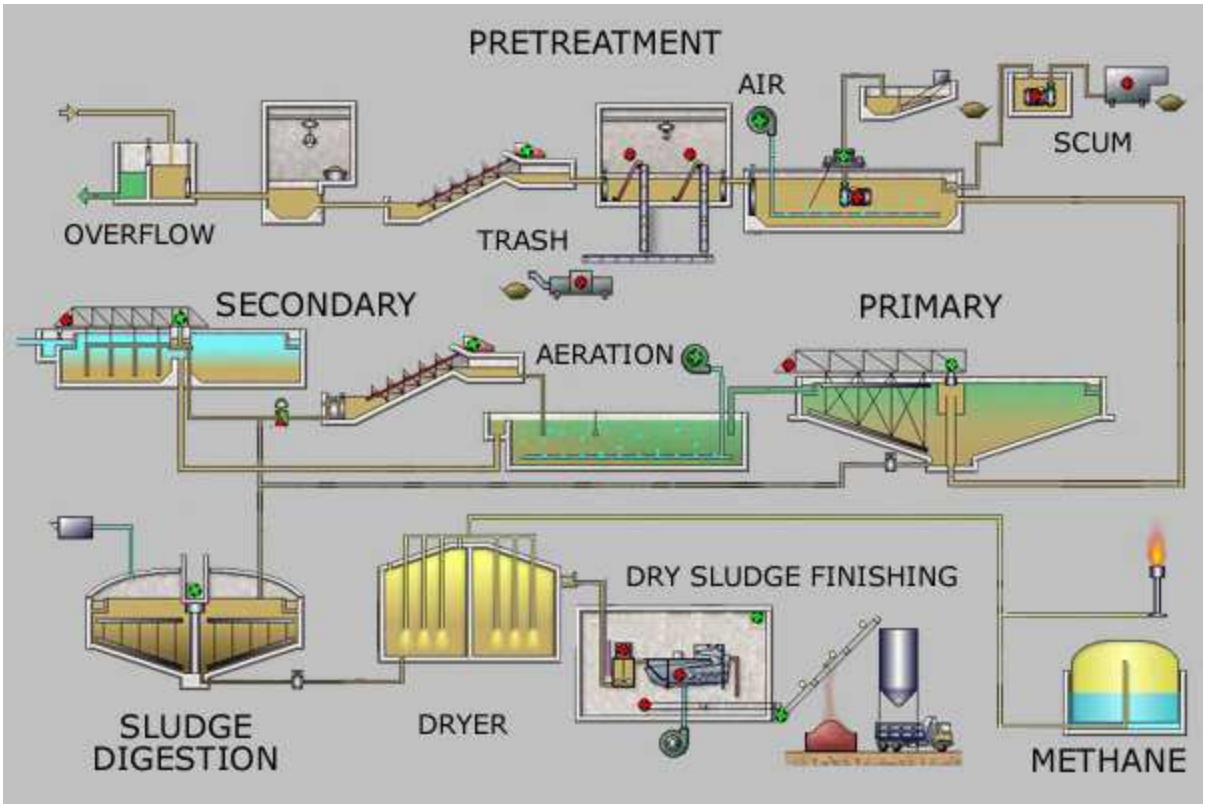

Fig. 1.4: Major steps involved in modern sewage treatment plants (http://web.deu.edu.tr/atiksu/toprak/summary4.html).

1.5 TECHNOLOGIES AVAILABLE FOR COLOUR REMOVAL FROM EFFLUENTS

There are several reported technologies for the removal of dyes from effluents (Table 1.1). The technologies can be divided into three categories: biological, chemical and physical (Robinson *et al*., 2001). Many of these conventional methods for treating dye wastewater have not been widely applied on a large scale in the textile and paper industries because of the high cost and disposal problems (Ghoreishi and Haghighi, 2003). At the present time, there is no single process capable of adequate treatment, mainly due to the complex nature of the effluents (Marco *et al.*, 1997; Pereira *et al*., 2003). In practice, a combination of different processes is often used to achieve the desired water quality in the most economical way. Research is ongoing and in the areas

of combined adsorption-biological treatments in order to improve the biodegradation of dyestuffs and minimize the sludge production (Pearce *et al*., 2003).

Table 1.1: Principal existing and emerging processes for dye removal (Robinson *et al*., 2001)

	Technology	**Advantages**	**Disadvantages**
Conventional treatment processes	Coagulation Flocculation	Simple, economically feasible	High sludge production, handling and disposal problems
	Biodegradation	Economically attractive, publicly acceptable treatment	Slow process, necessary to create an optimal favourable environment, maintenance and nutrition requirements
	Adsorption on activated carbons	The most effective adsorbent, great, capacity, produce a high-quality treated effluent	Ineffective against disperse and vat dyes, the regeneration is expensive and results in loss of the adsorbent, non-destructive process
Established recovery processes	Membrane separations	Removes all dye types, produce a high-quality treated effluent	High pressures, expensive, incapable of treating large volumes
	Ion-exchange	No loss of sorbet on regeneration, effective	Economic constraints, not effective for disperse dyes
	Oxidation	Rapid and efficient process	High energy cost, chemicals required
Emerging removal processes	Advanced oxidation process	No sludge production, little or no consumption of chemicals, efficiency for recalcitrant dyes	Economically unfeasible, formation of by-products, technical constraints
	Selective bioadsorbents	Economically attractive, regeneration is not necessary, high selectivity	Requires chemical modification, non-destructive process
	Biomass	Low operating cost, good efficiency and selectivity, no toxic effect on microorganisms	Slow process, performance depends on some external factors (pH, salts)

1.5.1 Biological treatments

Biological treatment is often the most economical alternative compared to physical and chemical processes. Biodegradation methods such as fungal decolourization, microbial degradation, adsorption by (living or dead) microbial biomass and bioremediation systems are commonly applied to the treatment of industrial effluents because many microorganisms such as bacteria, yeasts, algae and fungi are able to accumulate and degrade different pollutants (Fu and Viraraghavan, 2001; McMullan *et al.*, 2001). However, their application is often restricted because of technical constraints. Biological treatment requires a large land area and is constrained by sensitivity toward diurnal variation as well as toxicity of some chemicals, and less flexibility in design and operation (Bhattacharyya and Sarma, 2003). Biological treatment is incapable of obtaining satisfactory colour elimination with current conventional biodegradation processes (Robinson *et al*., 2001). Moreover, although many organic molecules are degraded, many others are recalcitrant due to their complex chemical structure and synthetic organic origin. In particular, due to their xenobiotic nature, azo dyes are not totally degraded (Ravi-Kumar *et al.*, 1998).

1.5.2 Chemical treatments

Chemical methods include coagulation or flocculation combined with flotation and filtration, precipitation–flocculation with Fe(II)/$Ca(OH)_2$, electroflotation, electrokinetic coagulation, conventional oxidation methods by oxidizing agents (ozone), irradiation or electrochemical processes. These chemical techniques are often expensive, and although the dyes are removed, accumulation of concentrated sludge creates a disposal problem. There is also the possibility that a secondary pollution problem may arise because of excessive chemical use. Recently, other emerging techniques, known as advanced oxidation processes, which are based on the generation of very powerful oxidizing agents such as hydroxyl radicals, have been applied with success for pollutant degradation. Although these methods are efficient for the treatment of waters contaminated with pollutants, they are costly and commercially unattractive. The high

electrical energy demand and the consumption of chemical reagents are common problems (Crini, 2006).

1.5.3 Physical treatments

Different physical methods are also widely used, such as membrane-filtration processes (nanofiltration, reverse osmosis, electrodialysis) and adsorption techniques. The major disadvantage of the membrane processes is that they have a limited lifetime before membrane fouling occurs. The cost of periodic membrane replacement must thus be included in any analysis for their economic viability. In accordance with the literature, liquid-phase adsorption is one of the most popular methods for the removal of pollutants from wastewater. Proper design of the adsorption process may produce a high-quality treated effluent. This process provides an attractive alternative for the treatment of contaminated waters, especially if the sorbent is inexpensive and does not require an additional pre-treatment step before its application (Dabrowski, 2001).

Adsorption is a well known equilibrium separation process and an effective method for water decontamination applications. Adsorption has been found to be superior to other techniques for water re-use in terms of initial cost, flexibility and simplicity of design, ease of operation and insensitivity to toxic pollutants. Adsorption also does not result in the formation of harmful substances (Dabrowski, 2001).

1.6 MECHANISM OF COLOUR REMOVAL BY BACTERIA

The simplest mechanism of colour removal by whole bacterial cells is that of the adsorption of the dye onto the biomass (Bras *et al*., 2001). However, this mechanism is similar to many other physical adsorption mechanisms for the removal of colour. It is not suitable for long-term treatment and during adsorption; the dye is concentrated onto the biomass, which will become saturated with time. The dye-adsorbent composition must also be disposed off. Bio-association between the dye and the bacterial cells tends to be

the first step in the biological reduction of azo dyes, which is a destructive treatment technology (Southern, 1995).

Biodegradation processes may be anaerobic, aerobic or involve a combination of both. In the reaction between bacterial cells and azo dyes, there are significant differences between the physiology of microorganisms grown under aerobic and anaerobic conditions. For aerobic bacteria to be significant in the reductive process, the bacteria must be specifically adapted. This adaptation involves long-term aerobic growth in continuous culture in the presence of a very simple azo compound. The bacteria synthesise an azoreductase specific for this compound, which, under controlled conditions, can reductively cleave the azo group in the presence of oxygen. In contrast, bacterial reduction under anaerobic conditions is relatively unspecific with regard to the azo compounds involved, and is, therefore, of more use for the removal of colour in azo dye wastewater (Stolz, 2001).

It is thought that, as most azo dyes have sulphonate substituent groups and a high molecular weight, they are unlikely to pass through cell membranes. Therefore, the reducing activity referred to above is not dependant on the intracellular uptake of the dye (Robinson *et al*., 2002). This was shown by Russ *et al*. (2000), who also suggested that bacterial membranes are almost impermeable to flavin-containing cofactors and, therefore, restrict the transfer of reduction equivalents by flavins from the cytoplasm to the sulphonated azo dyes. Thus, a mechanism other than reduction by reduced flavins formed by cytoplasmic flavin-dependent azoreductases might be responsible for sulphonated azo dye reduction in bacterial cells with intact cell membranes (Russ *et al*., 2000).

One such mechanism involves the electron transport-linked reduction of azo dyes in the extra-cellular environment. To achieve this, bacteria must establish a link between their intracellular electron transport systems and the high molecular weight, azo dye molecules. For such a link to be established, the electron transport components must be localised in the outer membrane of the bacterial cells (in the case of gram-negative

bacteria), where they can make direct contact with either the azo dye substrate or a redox mediator at the cell surface (Myers and Myers, 1992). In addition, low molecular weight redox mediator compounds can act as electron shuttles between the azo dye and an NADH (nicotinamide adenine dinucleotide)-dependent azo reductase that is situated in the outer membrane. These mediator compounds are either formed during the metabolism of certain substrates by the bacteria or they may be added externally (Gingell and Walker, 1971).

The addition of synthetic redox mediators such as anthraquinone sulphonates, even at very low concentrations, will facilitate the non-enzymatic reduction of the azo dyes in the extra-cellular environment (Plumb *et al*., 2001; Yoo *et al*., 2001). However, if the extra-cellular environment is aerobic, this reduction mechanism would be inhibited by oxygen, due to the preferential oxidation of the reduced redox mediator by oxygen rather than by the azo dye. Membrane-bound azo reductase activity, mediated by redox compounds, is different from the soluble cytoplasmic azo reductase that is responsible for the reduction of non-sulphonated dyes that permeate through the cell membrane. Their results show that a thiol-specific inhibitor almost completely inactivates the membrane-bound azo reductase in *Sphingomonas* sp. but had no effect on the cytoplasmic azo reductase. Therefore, the membrane-bound and the cytoplasmic azo reductases are two different enzyme systems (Kudlich *et al*., 1997).

Figure 1.5 illustrates a proposed mechanism for the redox-mediator-dependent reduction of azo dyes using whole bacterial cells, under anaerobic conditions. Although the final reduction of the azo dyes in the cell supernatants is a dominantly chemical redox reaction, the redox mediators depend on cytoplasmic reducing enzymes to supply electrons (Yoo *et al*., 2001). It is also possible that this chemical redox reaction works in conjunction with a direct enzymatic reaction involving an azo reductase, which may be a dehydrogenase enzyme that is synthesized throughout the cytoplasm and secreted without accumulation inside the cell (Bragger *et al*., 1997).

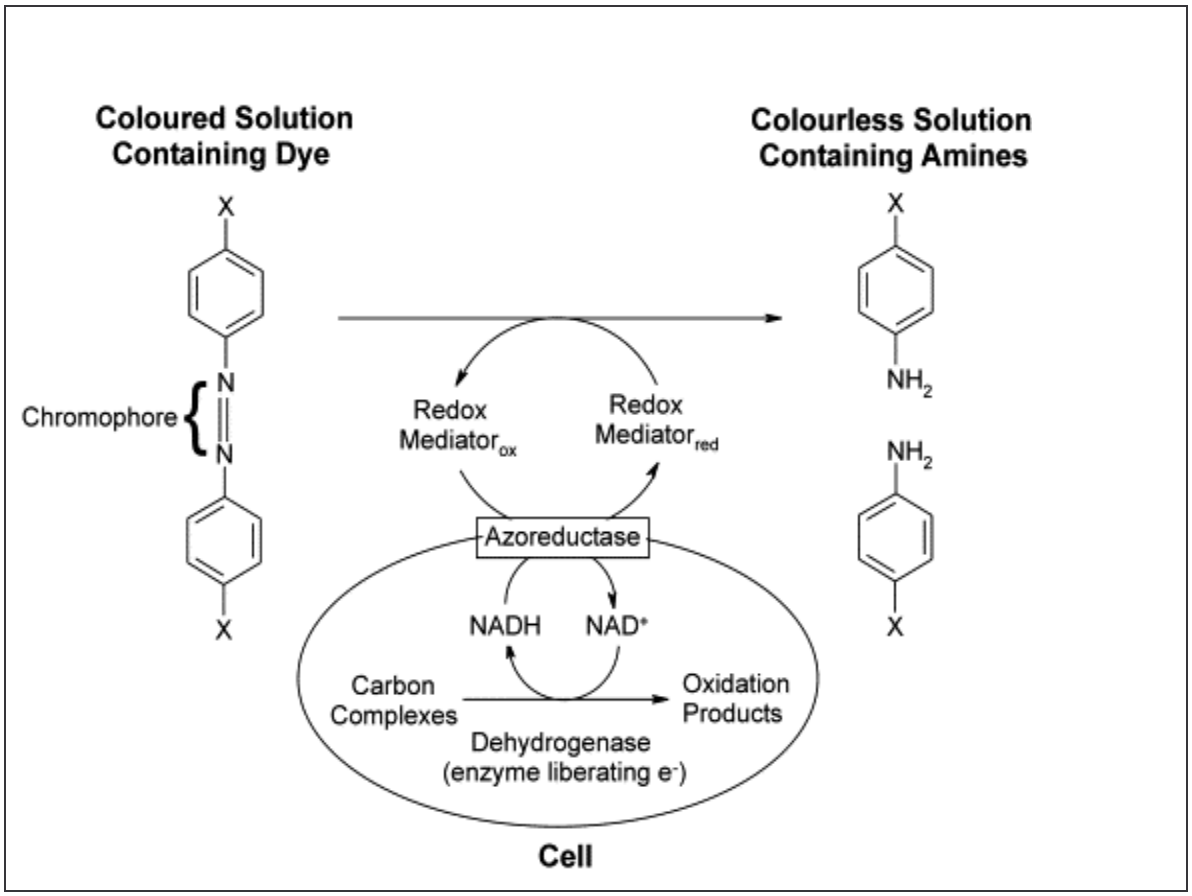

Fig. 1.5: Proposed mechanism for reduction of azo dyes by whole bacterial cells (Keck *et al*., 1997).

A study into the kinetics of anaerobic colour removal indicated that the transfer, rather than the production of reduced redox mediators was the rate-limiting step in the reduction of azo dyes. The reduction rate was also governed by the redox potential of the dyes and the redox mediators. In the same study, it was found that amino quinone compounds that are formed during the reduction of certain azo dyes were involved in autocatalysis and contributed substantially to the reduction process (Van der Zee *et al*., 2000). Another possible mechanism for colour removal involves the reduction of the azo bond by reduced inorganic compounds, such as Fe^{2+} or H_2S that are formed as the end product of certain anaerobic bacterial metabolic reactions (Kudlich *et al*., 1997).

In summary, it is probable that there are at least two mechanisms for the decolourization of azo dyes in bacterial systems:

- Direct electron transfer to azo dyes as terminal electron acceptors via enzymes during bacterial catabolism, connected to ATP-generation (energy conservation); and
- Reduction of azo dyes by the end products of bacterial catabolism not linked to ATP-generation.

Organics or inorganics may be involved in both mechanisms by acting as electron shuttles between the reducing equivalents and the azo dyes (Yoo *et al*., 2000).

1.6.1 Colour removal using whole bacterial cells

Alternative approaches to colour removal, utilizing microbial biocatalysts to reduce the dyes that are present in the effluent, offer potential advantages over physio-chemical processes. In particular, the ability of whole bacterial cells to metabolise azo dyes has been extensively investigated (Pearce *et al*., 2003). Under aerobic conditions, azo dyes are not readily metabolized (Robinson *et al.,* 2001). However, under anaerobic conditions, many bacteria reduce the highly electrophilic azo bond in the dye molecule, reportedly by the activity of low specificity cytoplasmic azo reductases, to produce colourless aromatic amines. These amines are resistant to further anaerobic mineralization and can be toxic or mutagenic to animals. Fortunately, once the xenobiotic azo component of the dye molecule has been removed, the resultant amino compounds are good substrates for aerobic biodegradation. According to Lourenco *et al*. (2000), if a sequential anaerobic–aerobic system is employed for wastewater treatment, the amines can be mineralised under aerobic conditions by a hydroxylation pathway involving a ring opening mechanism. Degradation of dyes in coloured wastewater involves the use of whole cells rather than isolated enzymes. This approach is advantageous because of the high costs associated with enzyme purification. In addition, degradation is often associated with a number of enzymes working sequentially (Pearce *et al*., 2003).

1.6.2 Colour removal using mixed bacterial cultures

Degradation of xenobiotics such as azo dyes is often carried out by mixed cultures (Knackmuss, 1996; Pearce *et al*., 2003). Pearce *et al*. (2003) have reported that a higher degree of biodegradation and mineralization can be expected when co-metabolic activities within a microbial community complement each other. Knackmuss (1996) gives an example of this using the degradation of naphthalene sulphonates by a two-species culture. *Sphingomonas* strain BN6 was able to degrade naphthalene-2-sulphonate, a building block of azo dyes, into salicylate ion equivalents. The salicylate ion cannot be further degraded and is toxic to strain BN6. Therefore, naphthalene-2-sulphonate can only be degraded completely in the presence of a complementary organism that is capable of degrading the salicylate ion (Knackmuss, 1996). In addition, it can be difficult to isolate a single bacterial strain from dye-containing wastewater samples and, in some instances, long term adaptation procedures are necessary before the isolate is capable of using the azo dye as a respiratory substrate (Pearce *et al*., 2003).

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1.6.3 Colour removal using single bacterial cultures

The advantages of mixed cultures are apparent as some microbial consortia can collectively carry out biodegradation tasks that no individual pure strain can undertake successfully (Nigam *et al*., 1996). In addition, mixed culture studies may be more comparable to practical situations. However, mixed cultures only provide an average macroscopic view of what is happening in the system and results are not easily reproduced, making thorough, effective interpretation difficult. For these reasons, a substantial amount of research on the subject of colour removal has been employed using single bacterial cultures. The use of a pure culture system ensures that the data are reproducible and that the interpretation of experimental observations is easier (Chang and Lin, 2000).

1.7 NOVEL AND ESTABLISHED APPLICATIONS OF MICROBIAL POLYSACCHARIDES

Flocculation in microbial systems was first reported by Louis Pasteur 1876 (cited by Salehizadeh and Shojaosadati, 2001) for the yeast *Levure casseeuse*. Two years later, this phenomenon was observed in bacterial cultures (Salehizadeh and Shojaosadati, 2001). Butterfield (1935) isolated *Zoogloea*-forming bacteria from activated sludge in 1935. Later, bioflocculation was investigated extensively and a correlation was established between the accumulation of extracellular biopolymeric flocculants (EBFs) and cell aggregation (McKinney, 1956; Tenny and Stumm 1965). Many EBF-producing microorganisms including bacteria, fungi, yeast, and algae have since been isolated from soil and wastewater (Bar-or and Shilo, 1987; Bender *et al*., 1989; Huang, 1990; Kakii *et al*., 1990; Morgan *et al*., 1990; Fumio, 1991; Hantula and Bamford, 1991a, b; Dube, 1992; Guirand, 1992; Sousa *et al*., 1992; Kim, 1993; Seo, 1993; Kurane *et al*., 1995; Yokoi *et al*., 1995; Suh *et al*., 1997; Salehizadeh *et al* ., 1998; Tong *et al*., 1999; Misra, 2002).

Many microorganisms synthesize exopolysaccharides (EPS) or EBFs, which either remain attached to the cell surface or are found in the extracellular medium in the form of amorphous slime. In the natural environments in which the microorganisms are found, such polymers may either be associated with virulence, as in the case of plant or animal pathogens, with plant microbial interactions or even protect the microbial cell against desiccation or attack by bacteriophages and protozoa. In both natural and man-made environments, the EPS play a major structural role in 'biofilms', the normal habitat of many microbial communities, in which varying numbers of prokaryotic and eukaryotic microorganisms grow while attached to solid-liquid interfaces (Yokoi *et al*., 1995; Salehizadeh *et al*., 1998). Several such microbial polysaccharides are now widely accepted products of biotechnology, while others are in various stages of development. The uses of such polymers vary widely; some are employed because of their unique or superior physical properties relative to traditional plant polysaccharides. In this category are xanthans, from *Xanthomonas campestris* pv. *campestris* and gellan (Gelrite) from

Sphingomonas paucimobilis strains (Fig. 1.6). These two polysaccharides have found various food and non-food applications. Xanthan is, in many ways, the 'benchmark' product, having received food approval many years ago and being a relatively inexpensive product because of the high conversion of 60-70% substrate to polymer (Sutherland, 1995).

Other microbial polysaccharides are more expensive to produce, some markedly so. The microbial products always have to compete against other natural or synthetic polymers, which may be inferior in their physical or ecological properties but are much cheaper to produce and market. Alteration of the chemical properties of the original exopolysaccharide may also greatly enhance their value and extend their range of applications, as exemplified by the dextran-derived Sephadex products (Sutherland, 1998).

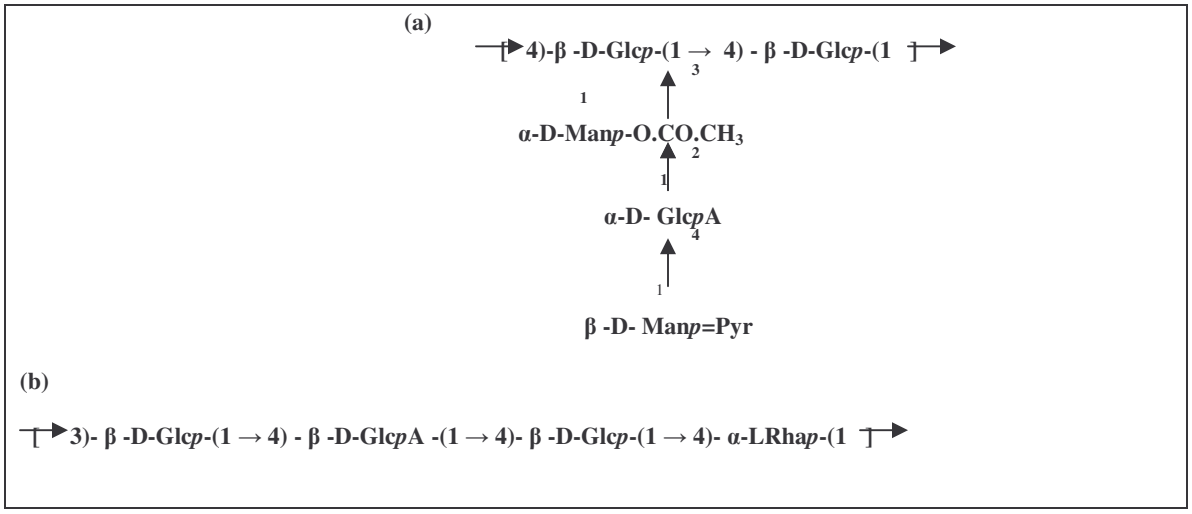

Fig. 1.6: (a) The structure of the exopolysaccharide from *Xanthomonas campestris* pv *campestris* (xanthan). **(b)** The structure of gellan from*Sphingomonas paucimobilis* (Sutherland, 1998).

Although a better understanding of the relationships that exist between structure and function have been ascertained, it is still difficult to predict which microbial polymers will be worth developing. Many initial reports in the literature have proved overoptimistic. On the other hand, two products have proved to be valuable, i.e. bacterial cellulose and hyaluronic acid, despite the fact that both were already available from

nonbacterial sources. Another example of biological properties that have led to a polysaccharide application can be found in the range of fungal 1.4-β-D-glucans, which have proved to be potent immune-system modulators, a property that is still poorly understood (Sutherland, 1990).

1.7.1 β-D Glucan

1.7.1.1 Bacterial cellulose

Two groups of β-D-glucans are of biotechnological interest. Bacterial cellulose is perhaps the more surprising, given the universal availability and cheapness, of plant cellulose. In contrast to its role in the wall of plants, cellulose is produced as an exopolysaccharide by *Acetobacter xylinum* and others, mainly Gram-negative bacterial species. It is excreted into the medium where it rapidly aggregates as microfibrils, yielding a surface pellicle. Fermenter design and the degree of aeration are important factors in optimizing yield. Bacterial cellulose is essentially a high-value speciality chemical with specific applications and usage. Some are produced commercially as a source of highly pure polymer in the so-called cellulose-I form (60% Iα: 40% Iβ), free from lignin and other noncellulosic material. The fibrils form a unique ribbon 3-8 nm thick and approximately 100 nm wide, which differ in morphology from other native celluloses (Fig. 1.6). Bacterial cellulose also forms the basis for high-quality acoustic-diaphragm membranes, in which the distribution of the fibrils containing a parallel orientation of the glucan chains yield fibres possessing high tensile strengths. Bacterial cellulose can also be used as a binder for ceramic powders and minerals and as a thickener for adhesives (Yoshinaga *et al*., 1997).

1.7.1.2 (1 → 3) β-D- glucans from bacteria and fungi

Several bacteria, including *Agrobacterium* and *Rhizobium* species, can each produce several EPS under appropriate physiological conditions. One of these is curdlan, a neutral gel forming 1.3-β -D-glucan of relatively low molecular weight

(approximately 74 000 g/mol). Curdlan is formed in the stationary phase following depletion of nitrogen and is insoluble in cold water but can be dissolved in hot water or in dimethylsulphoxide. Curdlan forms a weak gel on heating above 55°C followed by cooling. Further heating to 80-100°C increases the gel strength and produces a firm, resilient gel, while autoclaving at 120°C converts the molecular structure to a triple helix. The gel formed by this high-temperature treatment no longer melts when heated and, unlike the similar alginate gels, is independent of the presence of divalent cations. The gels are intermediate in properties between the high elasticity of gelatine and the brittleness of agar. Those formed at higher temperature do not melt below 140°C. They are very susceptible to shrinkage and syneresis but resistant to degradation by most β-D-glucanases (Vossoughl and Buller, 1991).

1.7.2 Pullulan

Aureobasidium pullulans synthesizes a α-D-glucan in which maltotriose and a small number of maltotetraose units (1.4- α -linked) are coupled through 1.6 α –bonds to form an essentially linear polymer. The molecular mass is between 10^3 and 3 x 10^6 and is dependent on the physiological conditions and the culture strain used (Wiley *et al*., 1993). Pullulan is not degraded by most amylases, but specific pullulanase enzymes (isolated from sources including *Enterobacter aerogenes*) can be used to hydrolyze the polysaccharide to its component maltotriose (and maltotetraose) units and thus provide a useful means of preparing these oligosaccharides. Similar products are formed by several other fungal species including *Tremella mesenterica* and *Cyttaria harioti*. Pullulan is highly water soluble, forming viscous solutions that are stable in the presence of most cations, but does not form gels. Esterification can be used both to increase its range of physical properties and to reduce its susceptibility to enzyme attack. A proposed use of pullulan is to form oil-resistant, water-soluble films with low oxygen permeability. This novel packaging material assists in flavour retention and maintains the fresh appearance of foods, which can then be cooked directly. Solutions of the polymer can also be used to form odourless and tasteless coating directly onto food. These applications have

apparently been made in Japan, but usage of the polymer in other countries appears to be limited (Nguyen *et al*., 1988).

1.7.3 Gellan and related polysaccharides

In its native form gellan carries O-acetyl and glyceryl substituents on a linear polymer of 500 kDa that is composed of tetrasaccharide repeat units (Fig. 1.6). The acyl groups inhibit crystallization of localized regions of the gellan chains and weak elastic thermoreversible gels are formed. Deacylation causes extensive intermolecular association, and strong, brittle gels form with various cations. Control of the degree of acylation of the polymer yields a range of gel textures with properties similar in some respects to agar, alginate or carrageenan gels. Gellan forms thermoreversible gels and concentrations as low as 0.75% provides high gel strength. Marketed as Kelcogelm or Gelrite, gellan has approval in the USA and the EU for food use as a gelling, stabilizing, and suspending agent for a wide range of foods, either on its own or in combination with other hydrocolloids. The gels give good flavour release and are stable over the wide pH range found in food products. As a replacement for agar, Gelrite can be incorporated into microbiological and cell-culture media. It may even lead to some growth enhancement when compared with agar-based bacterial culture media. The high clarity of the gels may have distinct advantages, as may the lower concentration required to provide gels of equivalent strength (Pollock, 1993).

1.7.4 Xanthan

Xanthan, from *Xanthomonas campestris*, is a major commercial biopolymer. Production from several commercial sources probably exceeds 20 000 tonnes per annum. Alternate glucose residues of a cellulose backbone carry the side-chains composed of D-mannose and D-glucuronic acid (Fig. 1.6). Mutants, different *X. campestris* pathovars and different nutrient conditions yield a range of polysaccharides that conform to the same general structure but differ in the completeness of carbohydrate side chains and extent of acylation. Many of the rheological properties of xanthan derive from the double-helical

ordered conformation adopted in solution. The trisaccharide side chains align with the cellulosic backbone, stabilizing the conformation by noncovalent interactions. Several strains of *A. xylinum* also yield xanthan-like polysaccharides (acetans). One product has a cellulosic main chain together with a pentasaccharide side chain on alternate main-chain sugars (Jansson *et al*., 1993).

Solutions of xanthan are highly pseudoplastic, rapidly regain viscosity on removal of shear stress and show very good suspending properties; they show high viscosity at low shear rates. The polysaccharide is incorporated into foods to alter the rheological properties of the water present, and has found applications that take advantage of many of its physical properties (Table 1.2). In many foodstuffs, xanthan possesses further useful attributes, including rapid flavour release, good 'mouthfeel' and compatibility with other food ingredients such as proteins, lipids and polysaccharides [most foodstuff already contain polysaccharides such as starch or pectin in addition to proteins and lipids, and any added polymer such as xanthan should be compatible with them] (Jansson *et al*., 1993).

1.8 SCOPE OF THIS STUDY

Various flocculants such as inorganic flocculants, organic high-polymer flocculants and naturally occurring flocculants have been used in wastewater treatment, dredging, and industrial downstream processes. Although organic high-polymer flocculants such as polyacrylamide are frequently used because they are inexpensive and highly effective, some of them are not easily degraded in nature and some of the monomers derived from synthetic polymers are harmful to the human body. In recent years, to solve these environmental problems, use of microbial flocculants has been anticipated due to their biodegradability and the harmlessness of their degradation intermediates to the environment (Yokoi *et al*., 1995).

Table 1.2: Established applications of microbial exopolysaccharides (Sutherland, 1998)

	Use	**Polymer**
Biological properties:	Antitumour agents	β-D-Glucans
	Eye and joint surgery	Hyaluronic acid (*Streptococcus* EPS)
	Heparin analogues	*Escherichia coli* K5 EPS
	Wound dressings	Bacterial cellulose
Chemical properties:	Enzyme substrates	*Escherichia coli* K4 and K5 EPS
	Oligosaccharide preparation	Curdlan, pullulan, scleroglucan
Physical properties:	Foods, thixotropic paints	Xanthan
Emulsions stabilization	Acoustic membranes	Bacterial cellulose
Fibre strength	Food coatings	Pullulan
Film formation	Water clarification, ore extraction	Various
Flocculant	Beer, fire-fighting fluids	Xanthan
Foam stabilization	Cell and enzyme technology	Gellan
Gelling agents	Foods	Curdlan, gellan
	Oil recovery (blockage of permeable zones)	Curdlan, xanthan
Hydrating agent	Cosmetics pharmaceuticals	Hyaluronic acid
Inhibitor of crystal formation	Frozen foods, pastilles and sugar syrups	Xanthan
Shear thinning and viscosity control	Oil-drilling 'muds'	Xanthan
Suspending agent	Food	Xanthan
	Paper coatings	Various
	Agrochemical pesticides and sprays	Xanthan
Viscosity control	Jet printing	Xanthan

Colour pollution in aquatic environments is an escalating problem, despite the fact that there has been substantial research into the modification of the dyeing process to improve the level of affinity/fixation of the dyestuffs onto the substrate. The recalcitrant nature of modern synthetic dyes has led to the imposition of strict environmental regulations. The need for a cost-effective process to remove colour from wastewater produced by the textile industries has been recognised and several strategies have been investigated (Willmott *et al*., 1998).

Chlorine is widely used in the treatment of water for both industrial and domestic purposes. Chlorination of water results in formation of an array of disinfection by-products (DBPs). Trihalomethanes (THMs) are the most common volatile DBPs and haloacetic acids (HAAs) are the major non-volatile DBPs. Other DBPs, such as, haloacetonitriles (HANs), chloropicrine and chlorinated furanones, are usually present at lower concentrations. Health effects of exposures to DBPs include various cancers and reproductive health effects, including spontaneous abortions, stillbirths, congenital malformations and retarded fetal development (Egorov *et al*., 2003). Therefore, the development of safe and biodegradable flocculant agents that will minimize the environmental and health risk is of paramount importance in various industries. Hence, the current study will focus on investigating the role of bacterial bioflocculants in the reduction and removal of microbial load and textile industrial effluents.

1.8.1 Hypotheses to be tested

It is hypothesized that bacterial bioflocculants can significantly reduce the microbial load in river water as well as remove a variety of dyes and chemicals from textile industrial effluents.

1.8.2 Objectives

a. To isolate and characterize the properties of the bioflocculants from bacteria.
b. To evaluate the efficacy of the bacterial bioflocculant on decreasing the microbial load of river water and compare the findings to alum.
c. To evaluate the ability of the bacterial bioflocculants to remove dyes and chemicals from the industrial effluents.

1.8.3 Key questions

a. Do all bacterial strains found in wastewater produce extracellular polysaccharides?
b. What is the chemical composition and properties of these bacterial bioflocculants?

c. What are the factors that influence bioflocculation?

d. Can these bioflocculants be used as an alternative to alum?

e. Can these bacterial bioflocculants remove dyes from textile industrial effluents?

CHAPTER TWO

PRODUCTION AND CHARACTERIZATION OF BACTERIAL BIOFLOCCULANTS

2.1 INTRODUCTION

Microbial extracellular polysaccharides (EPS) or extracellular biopolymeric flocculants (EBFs) are either associated with, and often covalently bound to, the cell surface in the form of capsules, or secreted into the environment in the form of slime. They are referred to as capsular (CPS) or slime (EPS) exopolysaccharides, respectively. Cell wall polysaccharides (WPS) are another type that, in contrast to EPS, are not released into the medium and are associated with the cell envelope, and may again be either covalently bound to the peptidoglycan layer or loosely associated with it. The lack of economical production limits their use and consequently they represent a small fraction of today's biopolymer market. Efficient production and reduction in recovery costs requires knowledge of biosynthesis and adoption of appropriate bioprocess technologies (De Vuyst *et al*., 2001).

Understanding and controlling the important environmental variables affecting polymer synthesis can be advantageous in the design of an economic process. The basic carbohydrate structure of most exopolysaccharides does not change with growth conditions, but the content of groups attached to the basic carbohydrate structure, can vary widely and may have a dramatic effect on the properties of the polymer and hence their effectiveness in various applications (Lopez *et al*., 2003). For the production of these biopolymer flocculants, sugars such as glucose, fructose or sucrose (Takagi and Kodowaki, 1985; Kurane *et al.*, 1986b; Kurane and Nohata, 1991; Toeda and Kurane, 1991), casein, L-glutamate or citrate are usually required as the main substrate(s).

Polysaccharides, which constitute the outermost layer of cells, are thought to mediate interaction between cells or the adherence of cells to surfaces. Some

polysaccharides are liberated outside of the cell, and some are bound to the cell envelope, but under certain physiological conditions, extracellular polysaccharide molecules may remain bound and associated with the cell surface without detectable membrane anchoring. The function of the extracellular polysaccharide in flocculation and adhesion is an important theme as is the function of envelope bound polysaccharides (Nakata and Kurane, 1999).

Bioflocculants are essentially polymers produced by microorganisms during growth, with their flocculating activities being dependent on the characteristics of the flocculants. Most bioflocculants are reported to comprise of polysaccharides and proteins. For example, *Bacillus subtillis* (Yokoi *et al*., 1996a), *Bacillus licheniformis* (Shih *et al*., 2001), *Paecilomyces* sp. (Shubo *et al*., 2005) and *Nocardia amarae* YK1 (Takeda *et al*., 1992) produce proteinaceous bioflocculants. *Alcaligenes latus* KT201 (Toeda and Kurane, 1991) and *Enterobacter* sp. (Yokoi *et al*., 1997) produce polysaccharide bioflocculants, while glycoprotein bioflocculants are produced by *Arcuadendron* sp. TS-4 (Lee *et al*., 1995).

In proteinaceous bioflocculants, the amino and carboxyl groups are the effective groups for flocculation, and their molecular weights are usually low (Kurane *et al*., 1994a). In contrast, polysaccharide bioflocculants have high molecular weights and many functional groups (Kurane *et al*., 1991). The molecular weight of most bioflocculants reported in the literature is in the range of 10^5 to 2.5×10^6 Da (Salehizadeh and Shojaosadati, 2001). The components and structures of bioflocculants are complex, with different organisms producing various bioflocculants with diverse properties. The bridging mechanism was found to play an important role in flocculating organic particles in wastewater and yeast cells using the bioflocculants produced by *Bacillus mucilaginosus* and *Aspergillus sojae* (Deng *et al*., 2003). Charge neutralization occurs when the flocculant is oppositely charged compared to the particles. As most bioflocculants and particles are negatively charged, charge neutralization seldom occurs in the bioflocculation process. Compared with conventional chemical and synthetic flocculants, which are relatively well developed, and their flocculating mechanisms

(including bridging and charge neutralization) well understood, the flocculating mechanisms of bioflocculants still needs to be investigated (Zouboulis *et al.,* 2004).

Many microorganisms secrete EBFs in the culture broth. Bioflocculation resulting from synthesis and secretion of EBFs by microorganisms has been well known in activated sludge (Salehizadeh and Shojaosadati, 2001). Generally, soil and activated sludge samples are the best sources for isolating EBF-producing microorganisms. *Rhodococcus erythropolis* was isolated from activated sludge using phthalic acid assimilation as an indicator (Kurane and Matsuyama, 1994).

Many factors influence the production of EBFs and the bioflocculation process. These include genotypic, physiological and environmental aspects. The environmental aspects involve physical, chemical and biological factors. The carbon and nitrogen concentration (C/N ratio), culture pH, temperature, and agitation speed used in the fermentor need to be optimized for efficient production (Salehizadeh and Shojaosadati, 2001). This optimization is essential because productivity and distribution of EBFs depend on the culture conditions. The pH of the culture medium can influence the production of EBFs. For example, in one case, the localization of the flocculant on the cell's surface of *Aspergillus sojae* at pH 6 was greater than at pH 8. In the case of *C. xerosis*, the flocculant was produced at relatively low pH whereas the optimum pH for production of EBF by *A. sojae* was in the alkaline range. Temperature is another physical factor that affects the production of EBFs. The best production of EBFs by *A. sojae* was obtained within the temperature range of 30–34^{o}C. The interaction between different microorganisms in a mixed culture is another biological parameter that can have a positive effect on aggregation of cells and the production of EBFs (Nakamura *et al.,* 1976c). By adjusting the growth conditions, the adsorption of the flocculant F-1 on the cell's surface could be raised by 5% of the concentration in the filtrate (Nakamura *et al*., 1976c).

The importance of carbon and nitrogen sources and the C/N ratio has been emphasized for EBFs production (Nakamura *et al*., 1976c; Kurane *et al*., 1994a). The

addition of sugars to the medium reduced the pH of the culture broth and inhibited the accumulation of the flocculant. Ethanol is also a good carbon source for flocculant production on an industrial scale (Nakata and Kurane, 1999). The canning factories wastes and spillage from distilleries are alternative inexpensive carbon sources. Urea, ammonium sulphate, yeast extract, and casamino acids are good nitrogen sources for EBF production and growth of *R. erythropolis* (Tong *et al*., 1999).

The application potential of EBF is determined by their physical and rheological properties. Factors influencing these properties are molecular mass, stiffness of the polymer, presence of side chains, and presence of nonsaccharide components, such as organic (e.g., acetyl, pyruvyl, or succinyl groups) or inorganic (e.g., sulphate or phosphate groups) substituents. Genetic engineering may be applied as a tool to direct the EPS synthesis and introduce desired properties by altering the composition or chain length. This requires a proper understanding of the genetics and biochemistry of EPS biosynthesis (Van-Kranenburg *et al*., 1999).

Inorganic and organic synthetic polymer flocculants are frequently used in water and wastewater treatment because they are economical and highly effective. However, their use often gives rise to environmental and health problems in that some of them are not readily biodegradable and some of their degraded monomers, such as acrylamide, are neurotoxic and even strong human carcinogens. Residual alum concentration in treated water can also impose health problems apart from the production of high amount of sludge (Letterman and Driscoll, 1988). There is also a problem of reaction of alum with natural alkalinity present in water leading to a reduction of pH and a low efficiency in coagulation of cold waters (Degremont, 1989 – cited by Ndabigengesere and Narasiah, 1998). Thus, the development of safe biodegradable flocculants that will minimize environmental and health risks is urgently required (Shih *et al*., 2001). Hence, the objective of this chapter were to isolate and identify bacteria capable of bioflocculant production; to quantify the amount of flocculant produced by the bacteria; and to characterize the properties of the bioflocculants produced by these bacteria isolated from the wastewater treatment plant.

2.2 MATERIALS AND METHODS

2.2.1 Isolation and identification of bioflocculant producing bacteria

2.2.1.1 Isolation and biochemical characterization of the bacterial isolates

Microorganisms were isolated from the effluent samples collected from various points at the Northern Wastewater Treatment Plant, Durban, including; activated sludge (aerobic), activated sludge (anaerobic), digested sludge, and effluent clarifier. Pure cultures were obtained by using four way streaks on nutrient agar and dilution methods (Prescott *et al*., 1996). Bacterial isolates were maintained on YMPG agar. Bacterial strains were identified and characterized using standard biochemical tests with reference to Bergey's Manual of Systematic Bacteriology (Peter *et al*., 1986; Garrity, 2005) and the API Kit (Biomerieux).

2.2.1.2 DNA isolation and 16S rDNA gene amplification and sequencing

Total genomic DNA was isolated from LB-grown cells using QIAamp DNA Miniprep Kit (Qiagen) following manufacture's instructions, and used directly as the template for PCR amplification. The 16S rDNA of the bacterial isolates were amplified with the oligonucleotide primers: 63f (5'–CAGGCCTAACACATGCAAGTC-3') and 1387r (5'–GGGCGG(A/T)GTGTACAAGGC-3') [numbering based on the *E. coli* 16S rRNA gene (Brosius *et al*., 1978)] described by Marchesi *et al*. (1998). The amplification reaction mixture contained standard *Taq* amplification buffer, 100 μM (each) deoxyribonucleotide triphosphate, 0.5 μM (each) primer, 100 ng of genomic DNA and 2.5 U of *Taq* DNA polymerase in a 50 μl reaction volume. The cycling parameters were 94^{o}C for 2 min followed by 30 cycles of denaturation at 92^{o}C for 30 s, annealing at 55^{o}C for 30 s, and elongation at 75^{o}C for 45 s, with a final elongation step of 75^{o}C for 5 min. The PCR products obtained above were visualized in 1% agarose by horizontal

electrophoresis at 80 V for 2 hrs. The agarose gels were stained in 0.5 μg/ml ethidium bromide solution for 15 min and fluorescent bands visualized under a UV transilluminator (UVP Inc.). The PCR products were sent to Inqaba Biotech for sequencing. Similarity searches in DNA data bases were perfomed using BLAST analysis (Pubmed).

2.2.2 Measurement of bioflocculant production

Microorganisms were cultivated in 250 ml Erlenmeyer flasks containing 30 ml YMPG medium for 20 hrs at 28^{o}C on a rotary shaker at 220 rpm. A 0.7 ml aliquot was added into a 500 ml Erlenmeyer flask containing 70 ml of production medium (0.5% yeast extract, 0.5% polypeptone, 2% ethanol, 1% glycerol, 0.05% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.2% NaCl, and 0.2% $CaCO_3$). The flasks were incubated for 3 days at 28^{o}C before determining the amount of bioflocculant produced by the ethanol precipitation method (Kurane *et al*., 1994a).

2.2.3 Bioflocculant purification

In order to purify the biopolymer flocculant, the viscous culture broth was diluted with an equal volume of distilled water and centrifuged at 2800 ×*g* for 30 min to remove cell pellets. Two volumes of cold ethanol (4^{o}C) was added to the supernatant and the crude bioflocculant precipitate was dried in a dessicator overnight. Thereafter, the crude bioflocculant was dissolved in distilled water and 2% (w/v) cetylpyridinium chloride solution (CPC) was added until no more insoluble CPC-bioflocculants complex were formed. After several hours, the precipitate collected by centrifugal separation of the CPC-bioflocculants complex was dissolved in saline solution [0.85% (w/v) NaCl], washed with cold ethanol three times and lyophilized (Salehizadeh and Shojaosadati, 2002).

2.2.4 Analysis of the bioflocculants

2.2.4.1 Determination of carbohydrate, protein, uronic acid, and amino sugar content of the purified bioflocculants

The total sugar content of the purified bioflocculants was determined by the phenol-sulphuric acid method (Chaplin, 1994). The Folin-Lowry method was used to measure the total protein content of the bioflocculants (Plummer, 1978). Aromatic amino acids and α-amino acids were determined by xanthoprotein and ninhydrin reactions (Plummer, 1978). Amino sugars were determined by the Elson-Morgan method (Chaplin, 1994). The carbazol method was used to measure uronic acid (Chaplin, 1994). Full description of the methodology and data sets obtained are given in Appendix B.

2.3 RESULTS

2.3.1 Bacterial isolation and identification

Of the thirty bioflocculant producing bacteria initially isolated from the wastewater treatment plant, only six were selected on the basis of the amount of bioflocculant produced (the highest, intermediate and the lowest producers) were used in subsequent experiments. The biochemical tests and the API Kit (Appendix A Table 6.1) were used to tentatively identify the organisms up to the genus level, and then confirmed by the analysis of their 16S rRNA gene sequences. Figure 2.1 shows the amplicons of 16S rDNA genes of the bacterial isolates. The analysis of the gene sequences (shown in Appendix A) identified the bacterial isolates as indicated in Table 2.1

Table 2.1: Identity of bacterial isolates based on 16S rDNA sequencing data (Appendix A)

Isolate code	Identification
A22	*Staphylococcus aureus*
A14	*Pseudomonas plecoglossicida*
A17	*Pseudomonas pseudoalcaligenes*
D1	*Exiguobacterium acetylicum*
E1	*Bacillus subtilis*
R2	*Klebsiella terrigena*

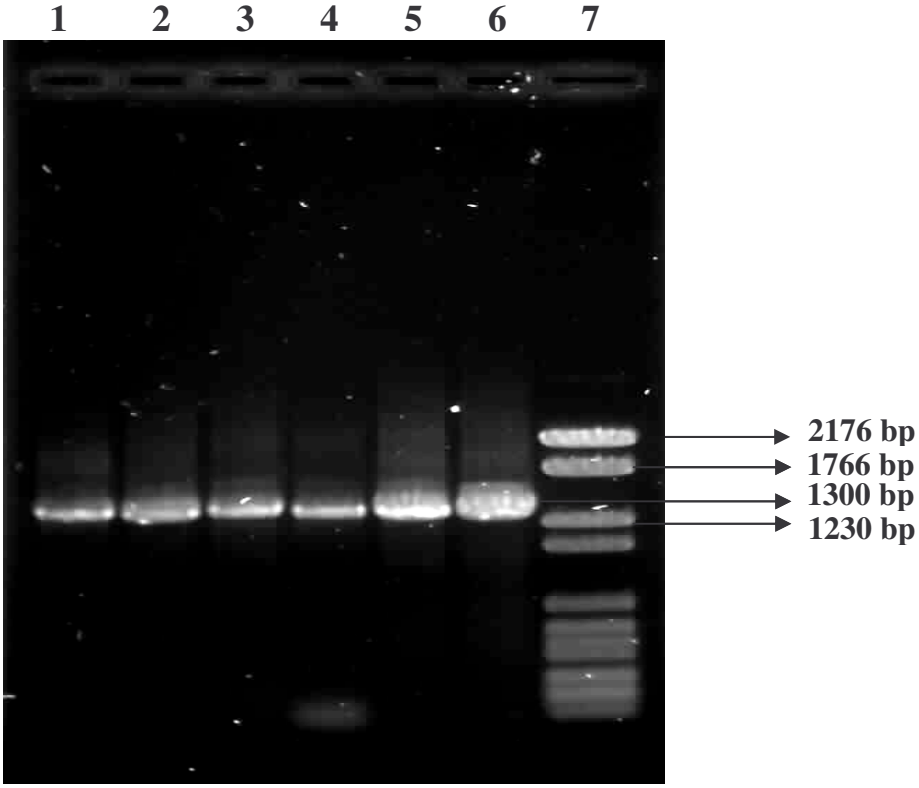

Fig. 2.1: PCR products after amplification of the 16S rDNA sequences of the bacterial isolates. Lanes 1–6: E1; A17; D1; A22; R2; A14; and lane 7: Molecular weight marker VI (Roche Biochemicals).

2.3.2 Measurement of bioflocculant production

Temperature was maintained at 28°C for the production of bacterial polysaccharides (Fig. 2.2). The bacterial isolates produced bioflocculants in varying amounts, ranging from 6.33 to 27.66 g/l, with isolate R2 being the highest producer and isolate E1 the lowest producer.

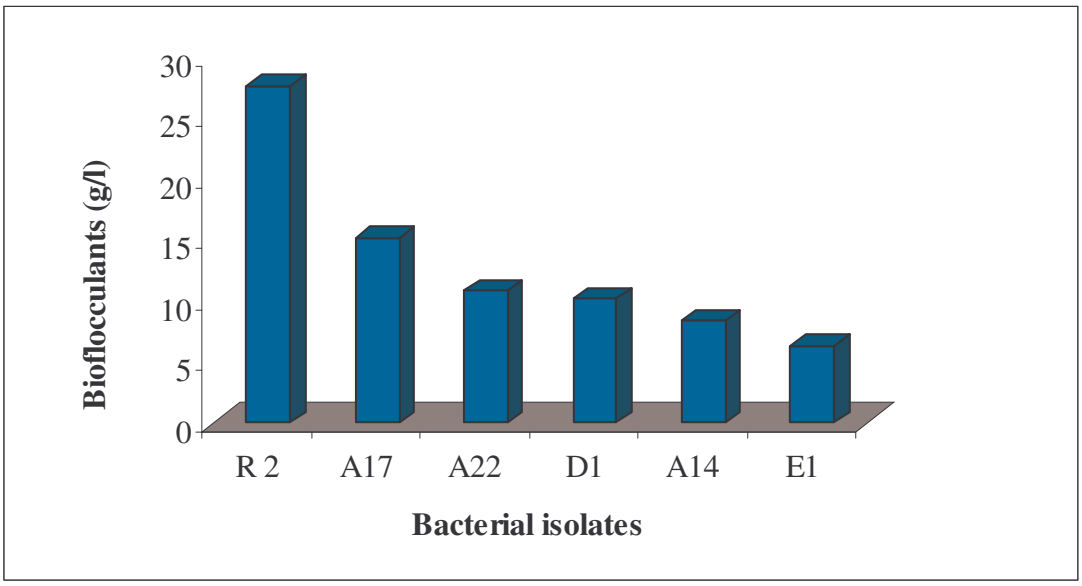

Fig. 2.2: Bioflocculant produced by the bacterial isolates

2.3.3 Purification and analysis of the composition of the bacterial bioflocculants

The standard curves for carbohydrate (CHO), protein, uronic acid, and amino sugars (hexosamine), from which the values below were derived, are shown in Appendix B.

2.3.3.1 Carbohydrate and protein

Bioflocculants from all the bacterial isolates were composed of both CHO and proteins in varying quantities (Fig. 2.3). The flocculants of isolates R2, A22 and A14 had more carbohydrate than protein. Isolates A17 and D1 had approximately similar concentration of CHO and protein in their bioflocculants. Isolate E1 had 45% carbohydrates and 55% proteins.

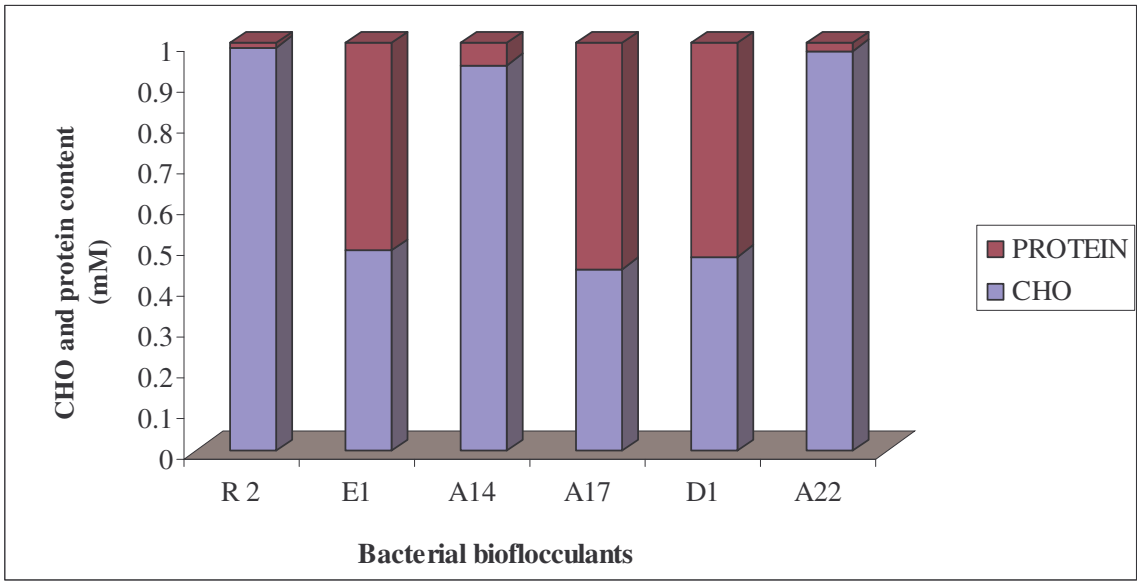

Fig. 2.3: Carbohydrate and protein content of the bacterial bioflocculants.

2.3.3.2 Uronic acid and Amino sugars

The uronic acid content of the bacterial bioflocculants is shown in Fig. 2.4. All the bacterial bioflocculants were composed of uronic acid in varying quantities. Isolate R2 (45.5 mM) had more uronic acid compared to the other isolates. Isolates E1, A17, A14, A22, and D1 had similar concentration of uronic acid (25.5 mM). The amino sugar content of the bioflocculants is depicted in Fig. 2.5. Isolate A17 contained the highest concentrations of hexosamine (amino sugars) followed by isolate E1. Isolate R2 and A14

had the same concentration of amino sugars and only isolate D1 lacked the presence of hexosamine.

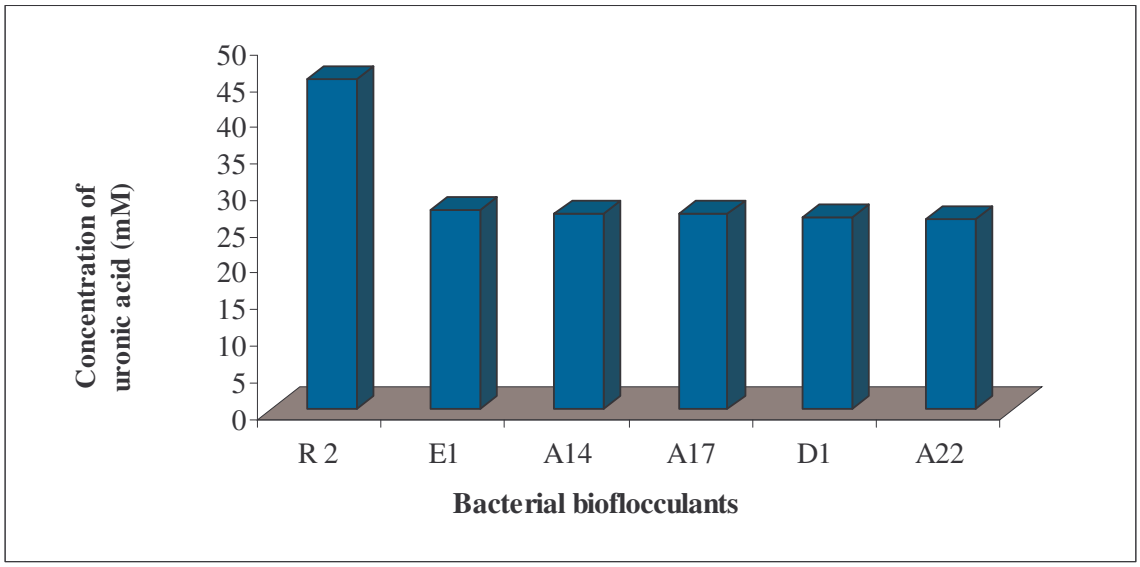

Fig. 2.4: Uronic acid content of the bacterial bioflocculants.

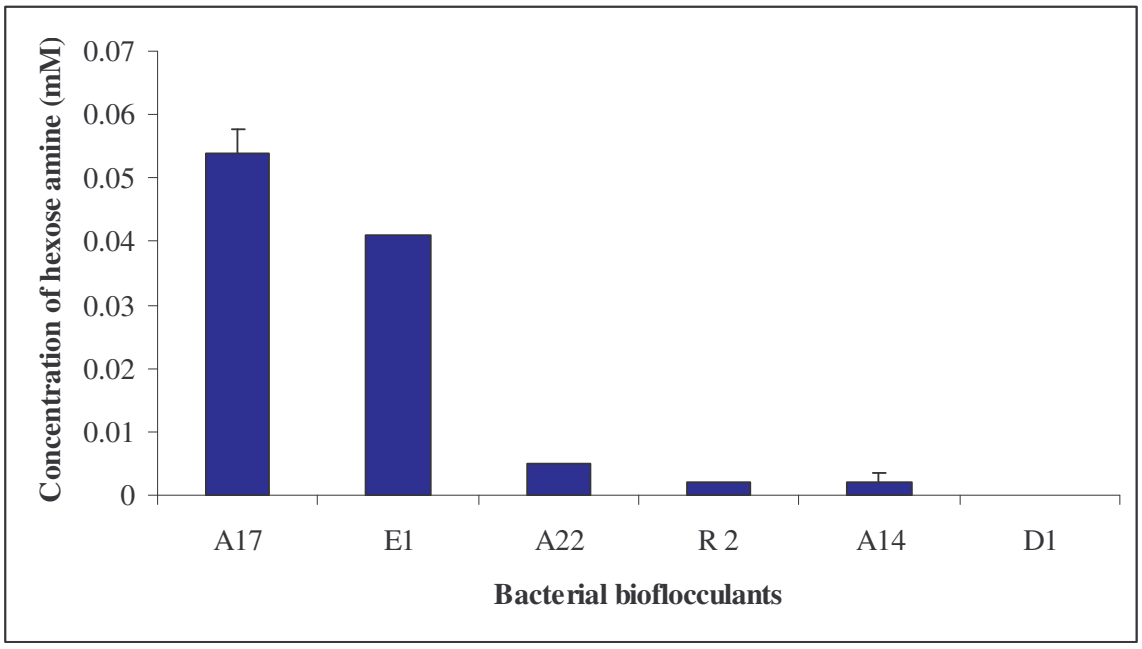

Fig. 2.5: Amino sugar content of the bacterial bioflocculants.

2.4 DISCUSSION

In this study the YMPG medium which contained yeast extract, polypeptone, glycerol, and ethanol were used as substrates for the production of bacterial bioflocculants. Yeast extract was a very good organic nitrogen source for the production of bioflocculants (Fig. 2.2). Nataka and Kurane (1999) indicated that polypeptone and yeast extract were good nitrogen sources for the production of EPS by *Klebsiella pneumoniae* H12. Kurane *et al.* (1994a) determined ethanol to be the cheapest substrate for bioflocculant production. According to Nakamura *et al.* (1976c) and Kurane *et al.* (1994a) the production of EPS by *A. sojae* was enhanced when casein, yeast extract, polypeptone, and some amino acids (e.g. glutamic acid and alanine) were added to the medium.

The six bacterial isolates investigated in this study produced extracellular polysaccharide/bioflocculants in varying quantities (Fig. 2.2). Isolate R2 (*K. terrigena*) produced 27.66 g/l while the production by D1 (*E. acetylicum*) was 10.167 g/l. The lowest producer was isolate E1 (*B. subtilis*) which produced only 6.33 g/l. Salehizadeh and Shojaosadati (2002) reported that 1.36 g/l of crude bioflocculant was produced by *Bacillus firmus*. This amount is much lower than that produced by *B. subtilis* in this study. In a similar study done by Shih *et al.* (2001), 14 g/l of crude bioflocculant was produced by *Bacillus licheniformis* CCRC 12826 which was higher than that produced by isolate *B. subtilis*. Only 3 g of bacterial bioflocculant was produced by *K. pneumoniae* H12 in a study done by Nakata and Kurane (1999). The amount produced by this bacterium is lower than that produced by isolate R2 (*K. terrigena*). This may be due to the fact that different species of *Klebsiella* were used. Zouboulis *et al*. (2004) reported on the production of bioflocculant by the bacterium *Rhizomonas* sp.; and have shown that only 2 g of bioflocculant were produced. Amongst the five genera used in the present study (Table 2.1) only four have been extensively studied previously, namely, *Bacillus* sp. (Kim, 1993; Yokoi *et al.,* 1995, 1996a; Suh *et al.,* 1997; Salehizadeh *et al.,* 2000); *Pseudomonas* sp. (Tago and Aida 1977); *Klebsiella* sp. (Dermlim *et al*., 1999) and

Staphylococcus sp. (Nakamura *et al*., 1976b). Currently there are no reports regarding the production of bioflocculants by *E. acetylicum*.

According to the findings of the present study, bacterial bioflocculants were composed of carbohydrates, proteins, uronic acid, and hexosamine. Isolate R2 and A22 had 99% carbohydrate and 1% protein while isolate D1 and A17 had 40% carbohydrate and 60% protein in their bioflocculants (Fig. 2.3). In a study done by Fujita *et al*. (2000) on the characterization of a bioflocculant produced by *Citrobacter* TKF04 from acetic and propionic acids, the total sugar content of the bioflocculant was 10% and no proteins were detected. A similar study was carried out by Salehizadeh and Shojaosadati (2002) on the isolation and characterization of bioflocculant produced by *B. firmus*. The bioflocculant was composed of 87% total sugar, 38% uronic acid and no proteins were detected (Fig. 2.4). Shubo *et al.* (2005) indicated that bioflocculant produced by *Aspergillus parasiticus* had 76.3% sugar content and a 21.6% protein. In general, bacterial bioflocculants contain proteins in minute quantities. Higgins (1995) reported that extracellular proteins in the floc are associated with improvements in settling and dewatering properties.

To date, many studies on the microbial production of flocculating substances have been reported. Microbiologically-produced bioflocculants are generally high molecular-weight polymers, and have been identified or presumed to be proteins (Takeda *et al*., 1991; Takeda *et al*., 1992), glycoproteins (Kurane *et al.*, 1986b), polysaccharides (Kurane and Nohata, 1991; Toeda and Kurane, 1991), glycolipids (Kurane *et al*., 1994a), cellulose (Napoli *et al*., 1975), DNA (Sakka and Takahashi, 1981) or complex hetero-polymers (Nakamura *et al.,* 1976a). The primary structure of proteins and polysaccharides describes the arrangement of the different building blocks, amino acids and monosaccharides, respectively, along the polymer chain. The possible structural variability due to available units and connecting patterns is estimated to be about three orders of magnitude larger for polysaccharides than for proteins (Sletmoen *et al.,* 2003).

In this study, all the bacterial bioflocculants were composed of uronic acid in varying quantities. Isolate R2 had the highest concentration of uronic acid [45.3 mM] (Fig. 2.4) compared to the other bacterial isolates. Nakata and Kurane (1999) showed that the bioflocculant produced by *K. pneumoniae* was composed of only 10% uronic acid. This amount was very low when compared to that produced by isolate R2 (*K. terrigena*) in the current study. Salehizadeh *et al*. (2000) analyzed the composition of the bioflocculant produced by *Bacillus* sp As 101, and indicated that it was composed of only 11.4% uronic acid, this amount was lower than that produce by *B. subtilis* (25%) in this study. Uronic acids appear to be the most universal and specific indicators of extracellular polymers that are used by the invertebrates in feeding nets, faecal pelletization, or feeding tube structures in the benthic environment and of the polymers that protect and regulate the ionic traffic at the surface of the bacteria. These polymers are important in the stabilization of marine sediments. The uronic acid content of the extracellular polymers can be measured by the formation of the ester and the reduction of the carboxylic acid moiety to an alcohol. This process eliminates the problems of resistance to hydrolysis and of quantitative recovery in separation from the neutral carbohydrates (Fazio *et al*., 1982). They are also found in the polysaccharide polymers of higher plant cell walls, in gram-positive microbes under conditions of phosphate limitation (Elwood and Tempest, 1972), and in some gram-negative microbial lipopolysaccharides. Polymers containing uronic acid are resistant to quantitative hydrolysis, and the uronic acid, once released, form lactones irreproducibly and are difficult to separate from the neutral sugars. Uronic acids are often estimated by their acid catalyzed decarboxylation under controlled conditions (Kiss, 1974). The known microbial exopolysaccharides contain D-glucuronic acid, D-galacturonic acid, D-mannuronic acid, and L-gulonic acid (Dudman, 1977).

Amino sugars are important structural components of bacterial cell walls, and neutral carbohydrates make up a basic unit of plant cells (Cheng and Kaplan, 2003). Five of the six bacterial bioflocculants used in this study were composed of hexosamine or amino sugars in very low concentrations except for isolate D1, which is *E. acetylicum*. The small yield might be due to the unstability of the amino sugar or the presence of

some reducing substance other than amino sugar in the bioflocculant. This finding is consistent with that done by Nakata and Kurane (1999); who showed that no hexosamine was detected in the bioflocculant produced by *K. pneumoniae* H12. Generally, amino sugars are present in very small quantities in bacterial cell walls. Fujita *et al*. (2000) reported that bioflocculant produced by *Citrobacter* sp. TKF04 was composed of 29.4% of hexosamine. In this study, bacterial bioflocculants were produced and characterized. They were found to be composed of carbohydrate, protein, uronic acid, and hexosamine in varying quantities.

CHAPTER THREE

COAGULATION-FLOCCULATION BY MICROBIAL BIOFLOCCULANTS

3.1 INTRODUCTION

Historically, the term "coagulation" and "flocculation" have been used indiscriminately to describe the process of removal of turbidity from water. There is however a clear distinction between the two terms. The term "coagulation" comes from the Latin *coagulare,* meaning to drive together (Faust and Aly, 1998; Nester *et al.*, 2001). This process describes the effect produced by the addition of chemicals to a colloidal dispersion resulting in particle destabilization by a reduction of the forces tending to keep the particles apart. Colloidal particles have a net negative surface charge. The size of colloids (0.001 to 1 μm) is such that the attractive forces between particles are considerably less than the repelling forces of the electrical charge. Under these stable conditions, particle growth does not occur, and Brownian motion keeps the particles in suspension. Operationally, coagulation is achieved by adding appropriate chemicals, which causes particles to stick together when contact is made. Rapid mixing is important at this stage to obtain uniform dispersion of the chemical and to increase the opportunity for particle-to-particle contact. The entire process occurs in a very short time, probably less than a second, and initially results in particles submicroscopic in size (Cohen *et al*., 1972; Prescott *et al*., 1996). The settling velocities of finely divided and colloidal particles under gravity alone are so small that ordinary sedimentation is not practical. It is therefore necessary to use procedures, which agglomerate the small particles into larger aggregates, which then have the settling velocities required for various applications. Formation of larger particles from smaller ones is also required for their removal by filtration (Nester *et al*., 2001).

The second stage of the formation of settleable particles from destabilized colloidal-sized particles is termed flocculation. This term also has its derivation from Latin, *flocculare*, meaning to form a floc, which visually resembles a tuft of wool or

highly fibrous porous structure. In contrast to coagulation, where the primary force is electrostatic, flocculation occurs by a chemical bridging of physical enmeshment mechanism. Flocculation is operationally obtained by gentle and prolonged mixing which converts the submicroscopic coagulated particles into discrete, visible, suspended particles. At this stage, the particles are large enough to settle rapidly under gravity and may be removed from suspension by filtration. The more usual practice has been to physically separate the unit processes into coagulation-flocculation, sedimentation, and filtration (Cohen *et al*., 1972; Faust and Aly, 1998).

The chemical coagulation of turbid water or naturally coloured surface water involves the interaction of particulates and/or colloids with a destabilizing agent. The essential purpose of coagulation is to aggregate these particles into larger sizes that will settle quickly within an hour or two and/or will be filtered by sand or other media. This aggregation process is also called destabilization of colloidal systems. Colloids are characterized by their size and by the mechanism by which they are stabilized in water. Another characteristic of colloids is their affinity for the solvent in which stabilization occurs. This is a process of salvation. Lyophilic is the general term given to colloids "loving" the solvent. In water, this becomes hydrophilic, and such colloids are stabilized by the formation of adherent thick layers of oriented water molecules around the particle. Lyophobic is the general term given to colloids "hating" the solvent, which in water becomes hydrophobic; such colloids are stabilized by an electrostatic repulsion between the particles arising from ions that are attracted to the surface from bulk solution or dissolved out of the solid's surface (Bitton, 1994).

Recently there has been a large increase in the utilization of synthetic organic polymers in the treatment of water and wastewaters as coagulants or aids to coagulation. Optimum treatment is frequently obtained with anionic and polymeric destabilization of negatively charged particles. It is obvious that an electrostatic mechanism is not the only means of destabilization. A bridging theory was proposed by La-Mer and Healy (1963) to account for the destabilization of colloidal systems by high molecular weight organic polymers. Adsorption of the polymer on specific sites of the colloid plays an important

role in the bridging theory. This theory resembles the binder theory proposed for alum precipitation and coagulation. In order for a polymer molecule to be an effective destabilizer, it must contain constituents that can interact with sites on the colloidal particle (Singh *et al*., 2000).

A number of methods and/or chemicals are used either as aids to coagulation or as the primary coagulant. Polyelectrolytes are mostly synthetic organic compounds of high-molecular weight. They are polymers composed of a chain of monomers. In turn, these monomers are varied frequently within a given polymer, which results in compounds with different molecular weights. These polymers are linear or branched. If the monomer contains an ionisable group, such as carboxyl, amino, or sulfonic, then the polymer is called a polyelectrolyte. There are cationic, anionic, or ampholytic (has both positive and negative) groups which, of course, depends on the nature of the functional groups within the monomer. Non-ionic polymers are those compounds without any ionisable groups. These polymers and polyelectrolyte are able to flocculate colloidal particles due to adsorption. In most cases, the bonding mechanism between a functional group on the polymer and a site on a colloid's surface is quite specific. In addition, molecular weight and degree of branching of the polymer play a mechanistic role in their ability to flocculate (Bitton, 1994; Faust and Aly, 1998).

The rate of flocculation is determined by the collision frequency induced by the relative motion. Because Brownian movement causes this, it is called perikinetic flocculation. That which is caused by velocity gradients is called orthokinetic flocculation. If there is no surface, repulsion between the particles, then every collision leads to aggregation and the process is called rapid flocculation. If a significant repulsion exists, then only a fraction of the collisions results in aggregation. This is called slow flocculation. The floc blanket clarifier provides a special case of orthokinetic flocculation. In addition to the fluidized bed giving rise to velocity gradients, the fluidized particles are participating in the process of agglomeration. If particles are settling at different velocities, then the faster settling particles may collide with slower settling particles, leading to aggregation. The aggregates will then settle faster due to

their increased mass, and possibly experience further collisions and aggregations (Yan *et al.*, 2004).

Compared with present understanding of floc formation through perikinetic and orthokinetic mechanisms, understanding of floc breakup in agitated systems is qualitative and speculative, despite research efforts (Faust and Aly, 1998; Nester *et al*., 2001). Proper characterization of floc disruption is an important problem since it is well documented that break-up can appreciably affect the performance of solid-liquid separation processes downstream. Floc break-up in dilute agitated suspensions is governed by the interaction of individual flocs with fluid forces. Depending on its constituent materials, a floc can be viewed roughly as an aggregate of primary micro particles that are bound together to form a matrix possessing a substantial fraction of fluid within its framework. The size and compactness of the matrix, size, and shape of the microparticles contribute to floc structure and the ability to withstand disruption by fluid forces. This also includes the number and strength of bonds that the microparticle contacts (DiTerlizzi and Fall, 1994; Singh *et al*., 2000).

The biopolymers in activated sludge flocs appear to affect the physico-chemical properties associated with the flocs such as floc density, floc particle size, specific surface area, charge density, bound water content, and hydrophobicity. These physicochemical floc characteristics express themselves among other things as activated sludge settling and dewatering properties. Research indicates that an increase in floc density and floc particle size increases settling velocity. The theoretical basis for improved settling through an increase in floc density and floc particle size is presented in Stokes Law. An increase in floc density results in improved dewatering properties through a decrease in bound water associated with the flocs (Eriksson and Alm, 1991). Calcium may create denser sludge flocs through a decrease in bound water associated with the floc. The percentage bound water associated with the floc is also an indicator of the maximum dryness that can be achieved in the sludge cake by mechanical means (Foster, 1983).

A variety of flocculants, including inorganic flocculants (polyaluminium chloride and aluminium sulphate), organic flocculants (polyacrylamide, polyethylene imine), and natural flocculants or bioflocculants (gelatin, chitosan guar gum and microbial flocculants) have been widely used for tap water production, wastewater treatment, dredging, downstream processing, fermentation, and in the food industry (Zouboulis *et al*., 2004, Crini, 2006).

Kaolin's are white raw materials, their essential constituent being fine grained white clay which are amenable for beneficiation that make them ideal for an assortment of industrial applications. Kaolin deposits can be classified into two types, primary (residual) and secondary (sedimentary) (Prasad *et al*., 1991). Kaolin or china clay is a versatile industrial mineral and one of the highest value additions is achieved when it is beneficiated to pigment grade suitable for paper and paint industries. In kaolin, minor quantities of transition elements such as iron, titanium, and manganese are generally present as ancillary minerals, which adversely affect its optical properties (Chandrasekhar and Ramaswamy, 2006). The valence state of the ion and atomic position in the structure depend on the conditions of formation of the mineral (Muller *et al*., 1995). Extensive research has been carried out on the nature of iron impurities in kaolin, which leads to the conclusion that iron is present as a part of the kaolinite or ancillary mineral structure i.e., "structural iron" or as separate iron minerals such as oxides, hydroxides, oxy-hydroxides, sulphides and carbonates i.e., free iron. Clay particles are strongly anisotropic and exhibit faces and edges, which are very different in surface area and in chemical behaviour. The explanation for dispersion of clay minerals suspensions in water is usually done by considering that the surfaces are electrically charged. Kaolin clay has been widely used as a test sample or material for flocculation because of its characteristics (Konan *et al*., 2006). Therefore, the objective of this chapter was to evaluate the ability of the bacterial bioflocculants to flocculate kaolin clay and determine the effects of temperature, pH and metal salts on the flocculating activities.

3.2 MATERIALS AND METHODS

3.2.1 Determination of the growth patterns of bacterial isolates and measurement of flocculating activity

The flocculating activity of the bacterial isolates was evaluated by measurement of the turbidity of a kaolin suspension. In all the experiments, 9 ml of kaolin suspension (5.5 g/l) was mixed with 1 ml of 0.5 M $CaCl_2$ in 9 mM glycine-NaOH buffer (pH 7.0) in a test tube. The six bacterial cultures used to produce bioflocculants in this study were grown in 250 ml of YMPG medium for 84 hrs, at 28^{o}C with shaking (150 rpm). Culture broth (50 µl) was then added to the kaolin suspension every 12 hrs for 84 hrs. The test tube was mixed vigorously for 20 s and left to stand, without shaking, for 5 min. The turbidity of the sample supernatant (A) and a control experiment without the culture broth (B) were measured at 550 nm with spectrophotometer (LKB ultrospec II) (Salehizadeh and Shojaosadati, 2002). The flocculating activity was expressed as the concentration of the flocculant in parts per million (ppm) when the OD_{550} was (1/10) x OD_{550} of the control (Nakata and Kurane, 1999). The percentage removal of kaolin suspension was calculated by the equations of Kurane and Matsuyama (1994) and Kurane *et al*. (1994b) as follows:

$$\text{Removal } (\%) = \frac{B - A}{B} \times 100$$

In addition, flocculating activity was also determined using various concentrations of purified bioflocculants instead of the culture broth.

3.2.2 Flocculation of microbial cultures

The following microbial cultures, obtained from the stock culture collection of the Discipline of Microbiology (Westville campus) UKZN, South Africa were used: *Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens, Alcaligenes faecalis,*

Citrobacter freundii, Bacillus coagulans, Bacillus cereus, Clostridium hystolyticum, Streptococcus pyogenes, and *Saccharomyces cerevisiae*. The bacterial isolates used for the production of flocculants were also used to measure the flocculation of these cultures. Microbial cultures were cultivated in 30 ml of YMPG medium in 250 ml Erlenmeyer flasks for 20 hrs at 28^{o}C with shaking (220 rpm). A 10 ml aliquot of the culture was washed with and suspended in an equal volume of deionised water in a test tube. One-hundred microlitres of flocculant solution (1 g/l) and then 100 µl of $CaCl_2$ solution (3 g/l) were added to the test tube. The mixture was vortexed for 5 s and left to stand for 5 min and the OD measured with a spectrophotometer at 550 nm. Flocculating activity was compared to the control without the flocculant or $CaCl_2$ (Nakata and Kurane, 1999). The flocculating activity was determined as described in Section 3.2.1.

3.2.3 Flocculation inhibition assay

One-hundred microlitres of different concentrations of potential inhibitors (K_2HPO_4, $NaNO_3$, CH_2COONa, Na_2CO_3, and D-GLU), 100 µl of bioflocculant solution (1 g/l), and 100 µl of $CaCl_2.2H_2O$ (30 g/l) were added to 10 ml of kaolin suspension (5 g/l) in a test tube. The mixture was vortexed for 5 s and left to stand for 5 min and the OD measured with a spectrophotometer at 550 nm. Flocculation activity was compared to the control without the salts. The flocculating activity was expressed as described in Section 3.2.1.

3.2.4 Flocculating activities of bacterial bioflocculants and alum

In order to compare the flocculating activity of the bioflocculants with alum the former was substituted with 10 ppm (0.01 g) alum. Different concentrations (10–50 ppm) of bacterial bioflocculants were added to 500 ml of raw water in 1-litre Erlenmeyer flasks and left to stand at room temperature for 1 hr. The control was performed without the addition of bacterial bioflocculants and the OD was determined spectrophotometerically at 550 nm for all the samples. The flocculating activity was calculated as described in Section 3.2.1.

3.2.5 Determination of the effect of pH, temperature, and cationic/metal salts on the flocculating activity of bioflocculants

Flocculating activity was determined as described in Section 3.2.1. The pH of the kaolin suspension was adjusted using 2 N HCl or NaOH to between 6 and 10 in order to examine the effect of pH on flocculating activity. To determine the effect of temperature on flocculating activity, the flocculation experiment was conducted at 28^{o}C and 37^{o}C. Cationic compounds ($MgSO_4.7H_2O$, $MnCl_2.7H_2O$ and CTAB) were added to the mixture instead of $CaCl_2.2H_2O$ in order to determine their effect on flocculating activity (Nakata and Kurane, 1999).

3.3 RESULTS

3.3.1 The growth patterns of the bacterial isolates in YMPG media and flocculating activities

Figure 3.1 shows the growth patterns of the bacterial isolates in the YMPG medium. Maximum cell growth was observed for isolate A22 after 24 hrs, after which the growth decreased and then remained constant. The other five isolates displayed similar growth patterns. The flocculating activity of the culture broth increased during the logarithmic phase of growth in YMPG medium and decreased during the late log phase. The best flocculating activity was observed in isolate A17 after 24 hrs (Fig. 3.2).

Generally, the flocculating activity of the bioflocculants increased to a maximum before decreasing to a constant level. However, there was no decrease in activity after 84 hrs for isolate E1. The effect of time on the removal of kaolin suspension by crude bioflocculants is depicted in Fig. 3.3. Isolate A17 removed more kaolin (65%) after 24 hrs compared to the other isolates. The pattern of removal first increased and reached a maximum after which it decreased and remained constant except for isolate E1 where the removal of kaolin did not decrease after 84 hrs.

The flocculating activity of the bacterial bioflocculants is shown in Fig. 3.4. Lower concentrations of bioflocculants were required for flocculation using isolates A22, A17, and A14. The flocculating activity of isolates D1, R2, and E1 required more bioflocculants for flocculation. Therefore, low concentrations of bioflocculants led to better flocculation as compared to higher concentrations.

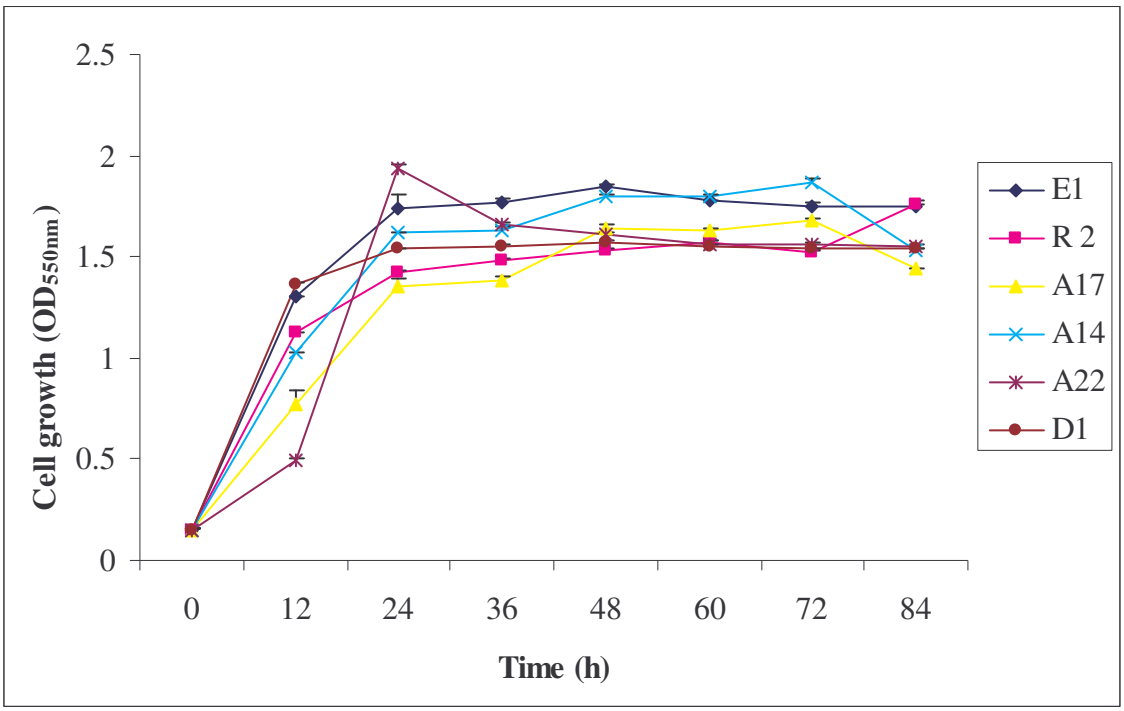

Fig. 3.1: Growth patterns of the bacterial isolates in YMPG medium.

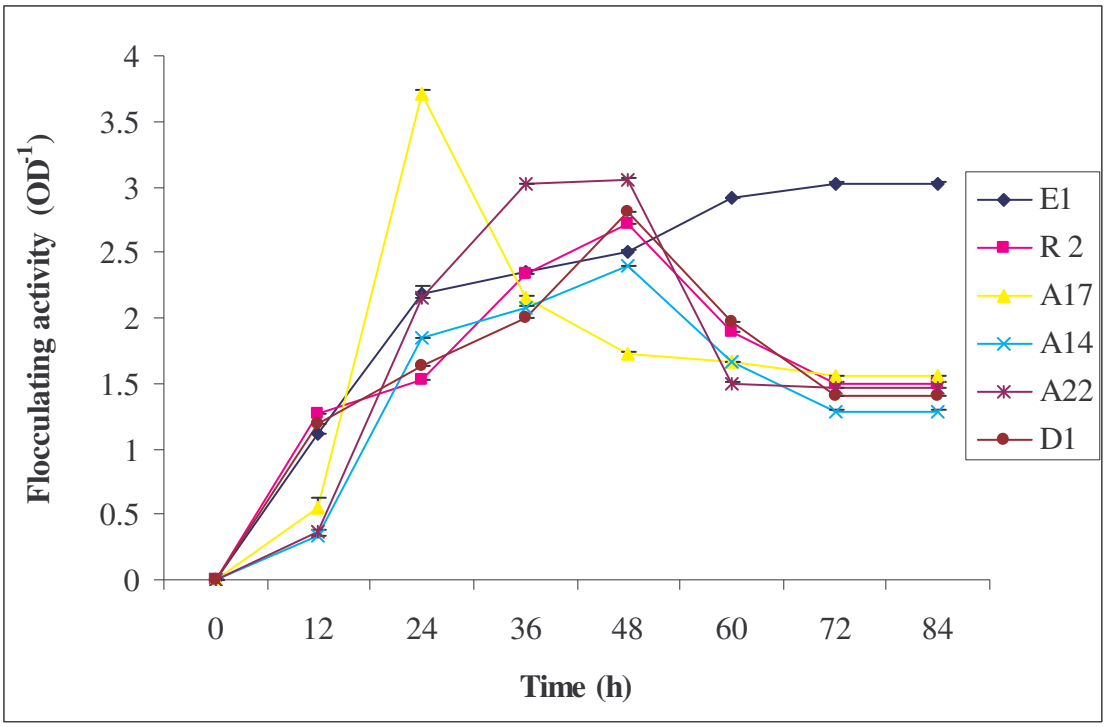

Fig. 3.2: Effect of time on the flocculating activity of crude bioflocculants using a kaolin suspension.

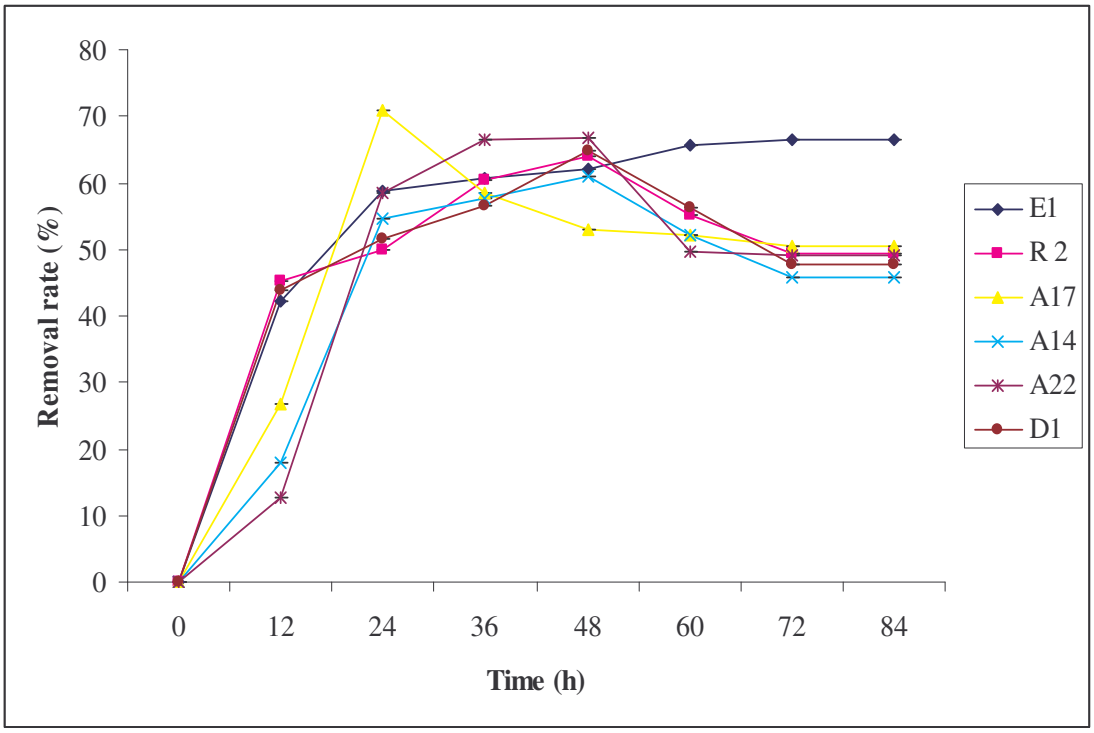

Fig. 3.3: Effect of time on the removal of a kaolin suspension by crude bioflocculants.

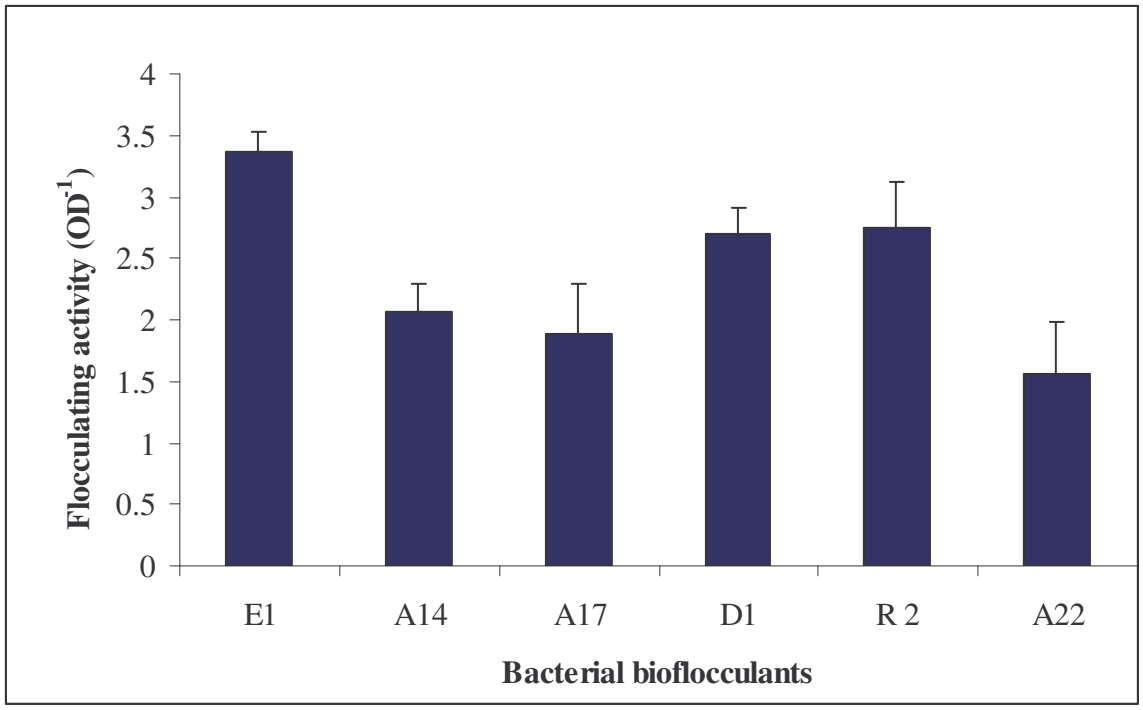

Fig. 3.4: Flocculating activity of bioflocculants using a kaolin suspension.

3.3.2 Flocculation of microbial cultures

The flocculating activity of the bacteria by their bioflocculants is depicted in Fig. 3.5. The best flocculating activity was shown by isolate R2 while that for isolate E1 was the lowest. Figure 3.6 shows the flocculation of Gram-positive and Gram-negative bacteria by the bioflocculants. All the bacterial bioflocculants were very effective in flocculating *C. hystolyticum, S. cerevisiae,* and *A. faecalis.* Other microbial cultures flocculated but to a lesser extent.

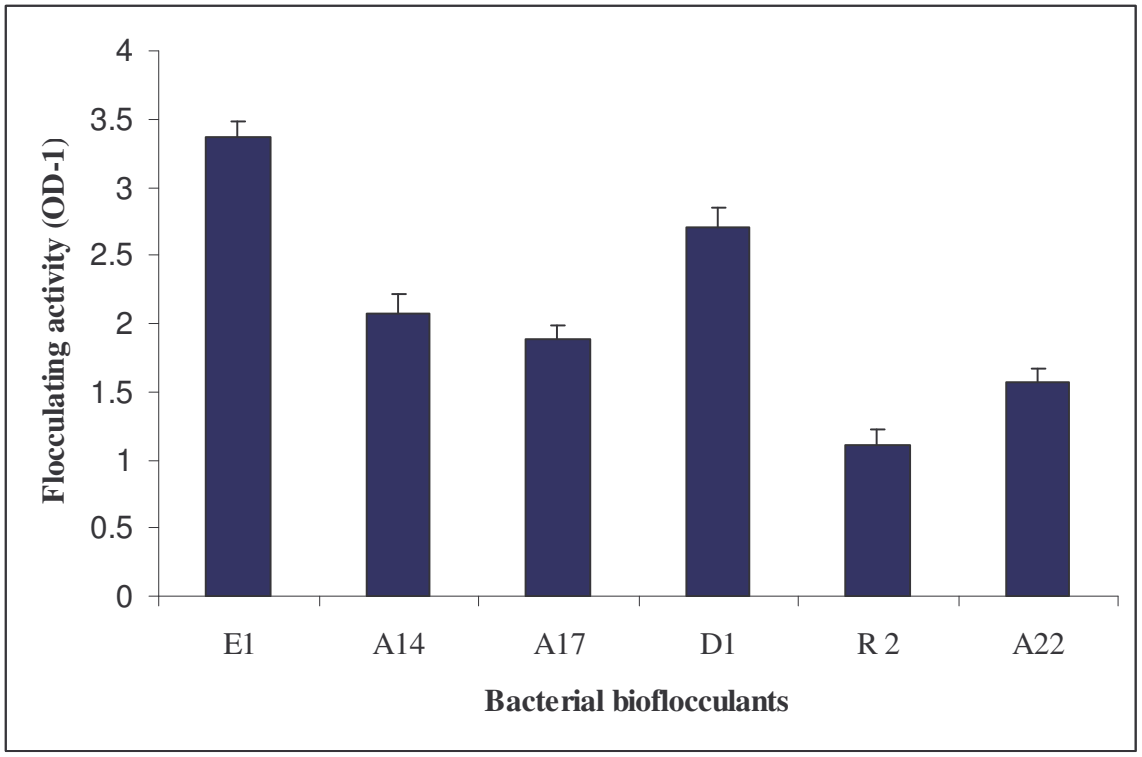

Fig. 3.5: Flocculating activity of bacteria by their bioflocculants.

3.3.3 Inhibition of flocculating activity

The inhibition of flocculating activity was tested by adding different concentrations of salts to the kaolin suspension, which was used as the test material (Table 3.1). High concentrations (10 000 ppm) of K_2HPO_4, CH_2COONa, and Na_2CO_3 inhibited flocculation. D-GLU and $NaNO_3$ improved the flocculating activity at both high (10 000 ppm) and low (10 ppm) concentrations. These observations were similar for all the bacterial isolates used in this study.

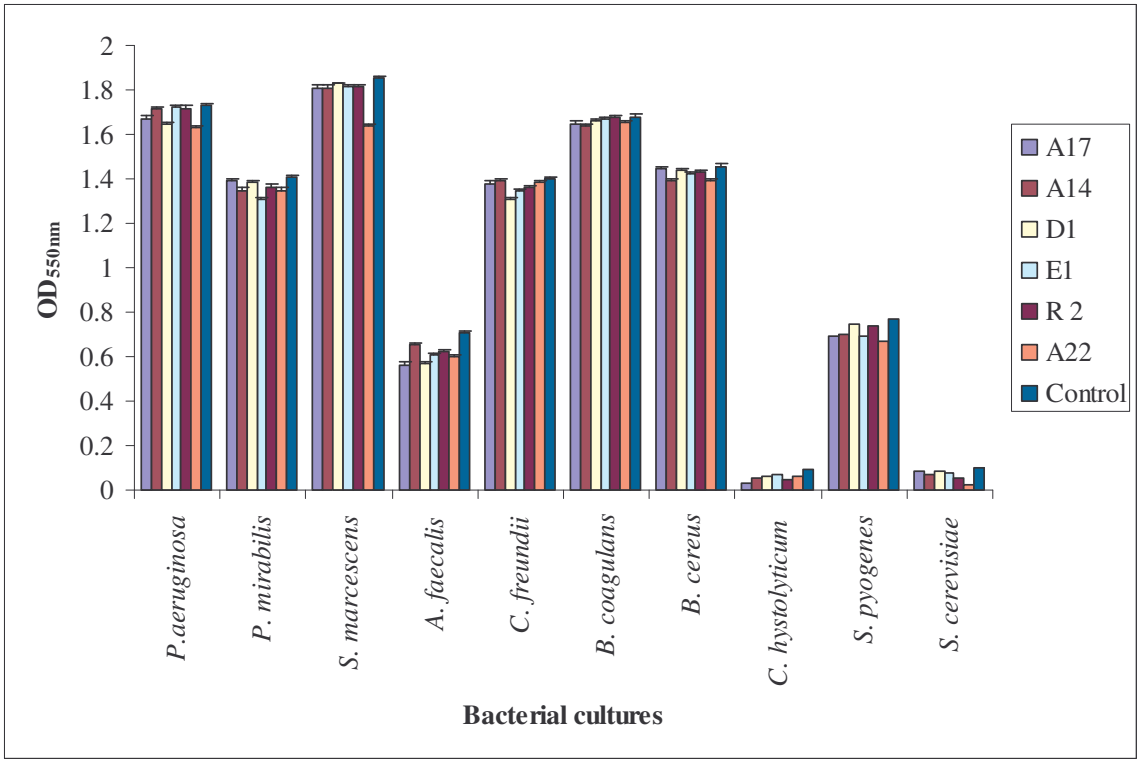

Fig. 3.6: Flocculation of microbial cultures using bacterial bioflocculants.

3.3.4 Comparison of flocculating activities between bacterial bioflocculants and alum

The effect of bioflocculant concentration on the flocculating activity with respect to 10 ppm alum using pond water is depicted in Fig. 3.7. In this case, the flocculating activity of alum was taken as 100% at 10 ppm. The activity of the bacterial bioflocculants was 70-75% at 20-30 ppm when compared to that of alum at 10 ppm. With an increase in bioflocculant concentration up to 50 ppm, the flocculating activity increased significantly. This was achieved by isolates D1, A17, A22, and R2 (Fig. 3.7).

Table 3.1: Inhibition of flocculation activity by salts and constituents of media

Isolates	Additive	Flocculation of kaolin clay with additive (ppm)							
		10000	**7500**	**5000**	**2500**	**1000**	**500**	**100**	**10**
E1	K_2HPO_4	-	-	-	-	-	+	+	+
	$NaNO_3$	+	+	+	+	+	+	+	+
	CH_2COONa	-	-	-	-	+	+	+	+
	Na_2CO_3	-	-	-	-	-	+	+	+
	D-GLU	+	+	+	+	+	+	+	+
A14	K_2HPO_4	-	-	-	-	+	+	+	+
	$NaNO_3$	+	+	+	+	+	+	+	+
	CH_2COONa	-	-	-	+	+	+	+	+
	Na_2CO_3	-	-	-	-	-	-	+	+
	D-GLU	+	+	+	+	+	+	+	+
A17	K_2HPO_4	-	-	-	-	+	+	+	+
	$NaNO_3$	+	+	+	+	+	+	+	+
	CH_2COONa	-	-	-	+	+	+	+	+
	Na_2CO_3	-	-	-	-	-	+	+	+
	D-GLU	+	+	+	+	+	+	+	+
R2	K_2HPO_4	-	-	-	-	+	+	+	+
	$NaNO_3$	+	+	+	+	+	+	+	+
	CH_2COONa	-	-	+	+	+	+	+	+
	Na_2CO_3	-	-	-	-	-	+	+	+
	D-GLU	+	+	+	+	+	+	+	+
D1	K_2HPO_4	-	-	-	-	+	+	+	+
	$NaNO_3$	+	+	+	+	+	+	+	+
	CH_2COONa	-	-	-	+	+	+	+	+
	Na_2CO_3	-	-	-	-	-	-	+	+
	D-GLU	+	+	+	+	+	+	+	+
A22	K_2HPO_4	-	-	-	-	+	+	+	+
	$NaNO_3$	+	+	+	+	+	+	+	+
	CH_2COONa	-	-	-	+	+	+	+	+
	Na_2CO_3	-	-	-	-	-	-	+	+
	D-GLU	+	+	+	+	+	+	+	+

+ = flocculated;

- = did not flocculate

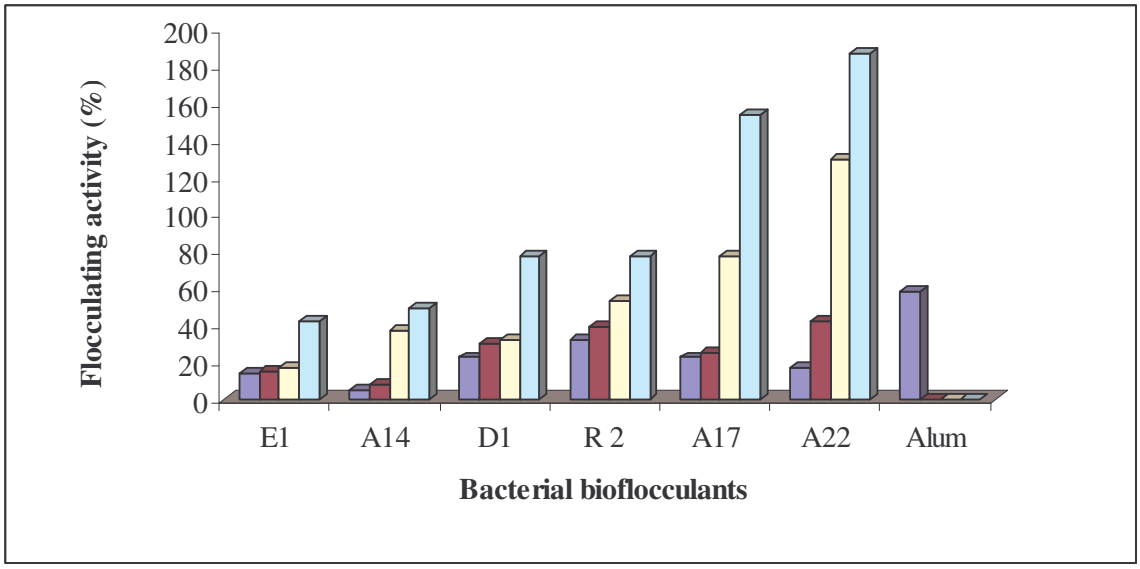

Fig. 3.7: Effect of flocculant concentration on the flocculating activity with respect to 10 ppm alum using pond water. Symbols: ■, 10 ppm; ■, 20 ppm_4; ■, 30ppm; ■, 50 ppm.

3.3.5 Effect of pH, temperature and cationic compounds on flocculating activity

The flocculating activities of the bacterial bioflocculants were found to be dependent on the pH and temperature of the kaolin clay (Fig. 3.8 and 3.9). Optimum pH for the flocculating activity was at pH 6 for isolates D1, A17, and A22 (Fig. 3.8). Isolate E1 and R2 showed the best flocculation at pH 8 while for isolate A14 best flocculation was achieved at pH 9. The best flocculating activity for each isolate was observed at 28^{o}C (Fig. 3.9). These observations were based on the amount of bioflocculant required for flocculation. Low concentrations (2 ppm) of bioflocculants were required for flocculation at pH between 6 and 9 and at 28^{o}C. Of the cations tested for their ability to enhance the flocculating activity, $MgSO_4$ and $MnCl_2$ improved the flocculating activities of all the bacterial isolates except for that of A22 (Fig. 3.10). CTAB was more effective in increasing the flocculating activity of A22. These observations were also based on the amount of bioflocculant required for flocculation.

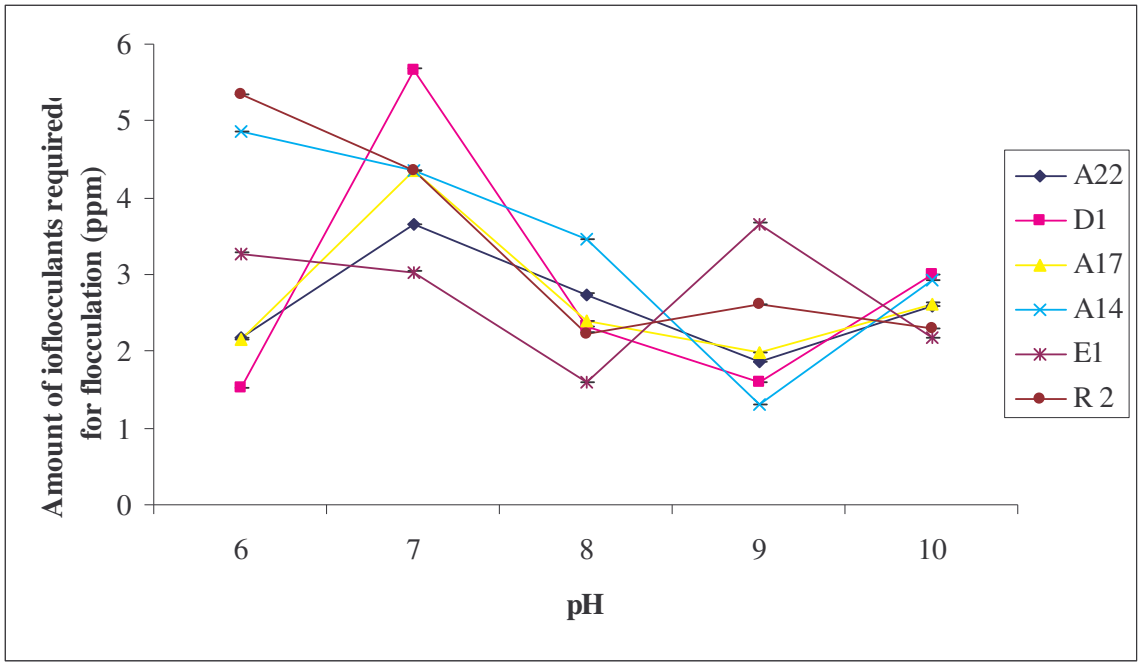

Fig. 3.8: Effect of pH on the flocculating activity of bacterial bioflocculants.

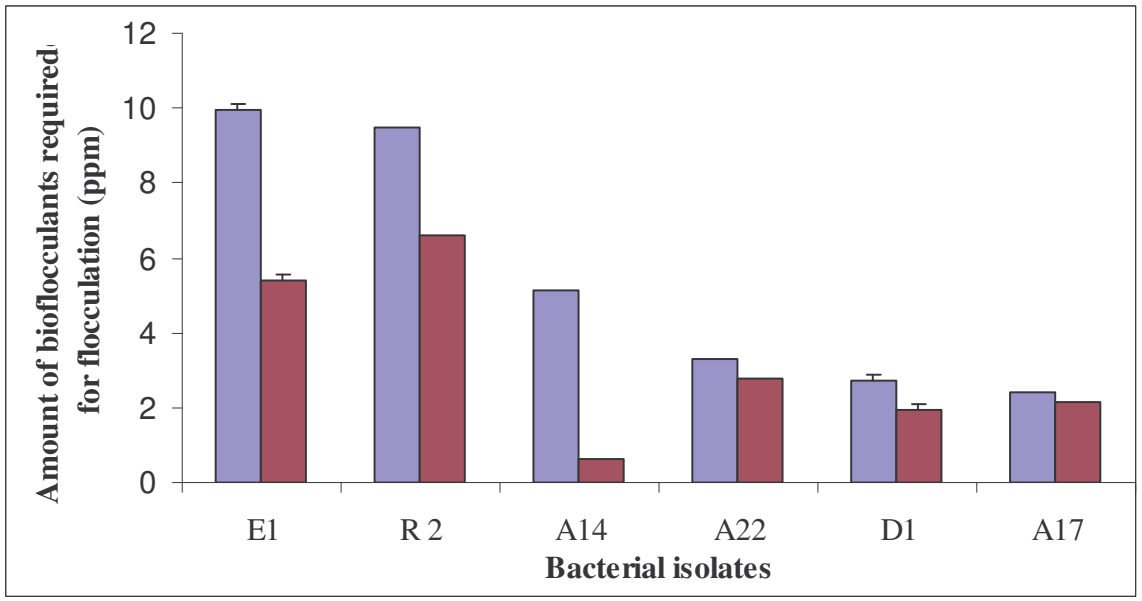

Fig. 3.9: Effect of temperature on the flocculating activity of bacterial bioflocculants. Symbols: ■, 37°C; ■, 28°C.

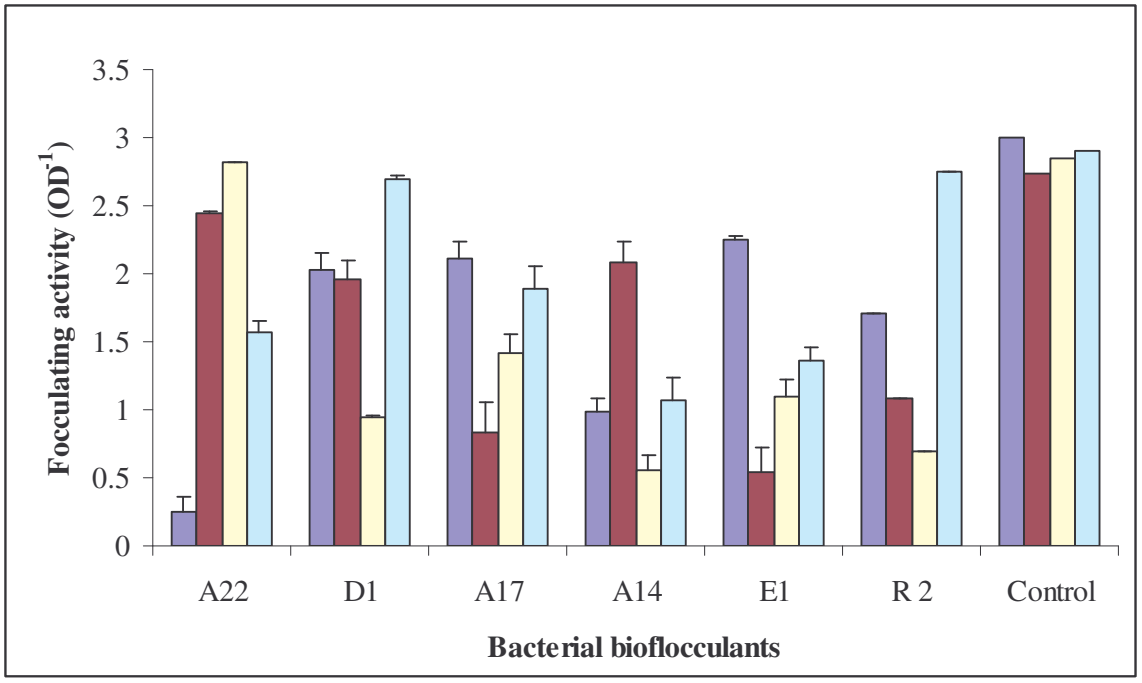

Fig. 3.10: Effect of cationic compounds on the flocculating activity of bacterial bioflocculants. Symbols: ■, CTAB; ■, $MgSO_4$; ■, $MnCl_2$; ■, $CaCl_2$.

3.4 DISCUSSION

The present results indicate that environmental and nutritional parameters play an important role in the production of bioflocculants and in the flocculating activity. The flocculating activities were influenced by temperature, pH, and cationic compounds. The flocculating activity started to decrease rapidly after 36 hrs and this may be due to cell lysis and enzymatic activity. This was also noted by Kurane *et al*. (1994b). Salehizadeh and Shojaosadati (2002) have also reported that the decrease in flocculating activity could be due to over-saturation of the many binding sites of kaolin surface particles, thus the attractive forces of other particles were reduced. During the cultivation of the bacterial strains in YMPG medium, the flocculating activity increased in parallel with its cell growth, indicating that bioflocculants were accumulated extracellularly in the medium during the active phase of growth. This suggests that the bioflocculants were not produced by cell autolysis but by biosynthesis (Nakamura *et al*., 1976b; Kurane *et al*.,

1986b). The correlation between cell growth and secretion of extracellular biopolymeric flocculants (EBFs) was originally reported by McKinney (1956).

Fujita *et al*. (2000) noted that the biological flocculation does not occur until the microorganisms have entered into an endogenous phase. In this study bioflocculant production remained constant after 84 hrs. This could be because carbon and nitrogen sources in the medium were used up. In a study done by Norberg and Enfors (1982) on *Z. ramigera*, the flocculant production ended after 90 hrs in the stationary phase. The production of the flocculant by *S. griseus* was not growth-related. For *S. griseus*, the flocculating activity increased rapidly with increasing time of cultivation after the third day and reached a maximum value after 4 days (Shimofuruya *et al*., 1996). *Flavobacterium* sp. showed flocculating activity at the end of exponential growth phase and the beginning of the stationary phase (Hantula and Bamford, 1991a; b). In contrast to those observations, the flocculant production in *R. erythropolis* (Kurane *et al*., 1991), *A. sojae* (Nakamura *et al*., 1976b), *Zoogloea* MP6 (Unz and Farrah, 1976), and *Alcaligenes latus* (Kurane and Nohata, 1991) have been found to be parallel to cell growth. This observation is consistent with the findings of the present study where flocculant production, flocculating activity and removal of kaolin clay were parallel to the cell growth.

It is clear that with an increase in flocculant concentration the percentage removal of kaolin clay increases and then a decrease in kaolin removal was observed with a further increase in concentration level. This may be because an optimum amount of flocculants in the suspension causes a larger amount of kaolin particles to aggregate and settle. However, according to Chan and Chiang (1995) the amount exceeding the optimum concentration of flocculants is known to cause the aggregated particle to redisperse and would also disturb particle settling. This behaviour could also be caused by an increase in the repulsive energy between the flocculants and kaolin in solution, which causes hindrance in floc formation (Mishra *et al*., 2004). The basal surfaces of kaolinite are believed to carry a constant structural charge due to the isomorphous substitution of Si^{4+} by Al^{3+}, whereas the charge on the edges are due to the

protonation/deprotonation of exposed hydroxyl groups and depend on the pH of the solution. Johnson *et al*. (2000) suggested that the charge on the basal surface is pH dependent. It has been shown that an edge surface which should carry a positive double layer in acid solution, and a negative double layer in alkaline solution, with a point of zero charge (PZC) in the region of pH 7, dependent on the particular kaolinite crystal structure (Hu and Liu, 2003).

In this study, all the bacterial bioflocculants were effective in flocculating *C. hystolyticum, S. cerevisiae,* and *A. faecalis.* Other bacterial cultures flocculated but to a lesser extent. Flocculation in *S. cerevisiae* is due to the presence of dominant flocculation genes *FLO1* and *FLO2* and a recessive gene *flo3* (Lewis *et al*., 1976 – cited by Jin and Speers, 1998). The European Brewery Convention (EBC) suggested that the aggregation of yeast cells into flocs may be due to either non-separation of cells after budding or coalescence of single cells into clumps (EBC Microbiologica, 1981 – cited by Jin and Speers, 1998). Yeast flocculation has been defined as the phenomenon wherein yeast cells adhere in clumps and sediment rapidly from the medium in which they are suspended (Stewart *et al*., 1976 – cited by Jin and Speers, 1998). The responsible bonding has been proved to involve lectin-like protein-carbohydrate recognition and interaction (Jin and Speers, 1998). Bacterial bioflocculants flocculated 76 bacterial species in a floc and caused 15 species to float in a floc. The remaining 9 species were flocculated little if at all. Bacteria that precipitated included *P. aeruginosa* IFO 3924, *P. fluorescens* S272 and *E. coli* K12 (Nakata and Kurane, 1999).

The application of bioflocculants for the removal of suspended fine particles and organic matter from raw water was examined in comparison with a conventional coagulant agent, alum. The results indicated that the bioflocculants were efficient for the removal of these particles and organic matter. Furthermore, the bioflocculants produced similar results, as did the use of alum, but at a slightly higher dosage. Fujita *et al.* (2000) indicated that the flocculation of kaolin by the *Citrobacter* sp. TKF04 was comparable to or slightly lower than that of PAA (polyacrylamide) and much higher than that of PAC (polyaluminum chloride).

Of the various cations tested on the flocculating activity of the bioflocculants the most effective was Mn^{2+} and Mg^{2+}. The flocculating activity was not enhanced by the addition of Ca^{2+}. Shimofuruya *et al.* (1996) reported that a high concentration of Ca^{2+} led to the decrease in flocculating activity. The divalent cations accelerate the initial adsorption of the biopolymer on kaolin particles by decreasing the negative electrical charge of kaolin particles and the biopolymer flocculants (Salehizadeh and Shojaosadati, 2002). Divalent cations have a significant effect on the coagulation of natural colloidal particles. Black *et al.* (1966) suggested that Mg^{2+} affects the stability of anionic polymer-clay. Three mechanisms are possible: firstly, divalent cations compress the double layer of colloid; secondly, the repulsive forces between polymers and clay particles are reduced by the cations that enhance adsorption of the polymer on the clay particle, and finally the range of repulsive barrier between adsorbed anionic polymers is probably reduced by Mg^{2+}. This is reasonably strong evidence that indicates that divalent ions are necessary for polymers to flocculate negative colloids. In this study, anions such as carbonic and phosphate ions prevented proper flocculation by forming weak or loose structures often decreasing the floc size (Faust and Aly, 1998) (Table 3.1).

The optimum pH for the flocculating activity was observed between pH 6 and 9 (Fig. 3.8). In contrast to the finding of the current study Kurane *et al.* (1994a) reported that flocculation did not decrease with an alkaline pH of up to 10. The possible reason for this observation could be that different genera and species of bacteria was used in this study. Also, these bacteria were isolated from different environments. With respect to temperature the optimum flocculating activity was observed at 28^{o}C (Fig. 3.9). Yokoi *et al*. (1996a) showed that the effective flocculation of kaolin suspension by *Bacillus licheniformis* occurred between the temperatures of 4-90^{o}C. Lian *et al*. (2007) found that the flocculation ratio varies from approximately 85% to 89% in the temperature range of 23 to 70^{o}C, and only begins to decrease noticeably with further temperature increase from 70 to 90 ^{o}C. The slight increase in the flocculation ratio from 23 to 40^{o}C may be explained by the higher random motion of the flocculant molecules, and hence, higher collision frequency with kaolin particles. Thus, it appears that temperature has little impact on the physical and chemical properties of the flocculant molecules in the

temperature range of 23–70^{o}C. Fujita *et al*. (2000) indicated that efficient kaolin removal by the bioflocculant produced by *Citrobacter* sp. TKF04, could be performed in a pH range of 2-8 and temperature range of 3-95^{o}C and it could flocculate a variety of organic and inorganic particles.

In conclusion, the bioflocculants produced by these bacterial strains have a satisfactory level of flocculating activity. These results suggest that these bioflocculants can be successfully applied for the clarification of river water or wastewaters under various environmental conditions. Overall, it can be concluded that these bioflocculants exhibit flocculating activity comparable or superior to that of existing inorganic flocculants (alum).

CHAPTER FOUR

MICROBIAL DECOLOURIZATION OF EFFLUENTS CONTAINING TEXTILE-DYES

4.1 INTRODUCTION

The use of synthetic chemical dyes in various industrial processes has increased considerably over the last few years. Some areas where these chemicals are frequently used are paper and pulp manufacturing, plastics, dyeing of cloth, leather treatment and printing (Aksu, 2005). Industrial wastewater containing such dyes is generally discarded as effluents. Since some of these dyes are toxic in nature, their presence in from the industrial effluents is a major environmental problem, because they are recalcitrant to microbial degradation (Pagga and Brown, 1986). The wastewater, which is highly coloured, can block the penetration of sunlight and oxygen, which are essential for the survival of various aquatic forms. Moreover, the dye solution can also undergo anaerobic degradation to form potentially carcinogenic compounds, which can end up in the food chain. Many approaches, including physical and/or chemical processes have been used in the treatment of dye containing industrial wastewater; however, such methods are often very costly (Nigam *et al*., 1996; Rauf *et al*., 2006).

In recent times, industries have been faced with more stringent effluent regulations. For instance, the textile, food, and pharmaceutical industry are required to lower the colour content in their wastewater (Pinheiro *et al*., 2004). Flocculation and precipitation processes have proved to be an effective procedure for the decolourization of such effluents (Mishra and Bajpai, 2005). Physical-chemical flocculation with metal hydroxides assisted by polymer flocculants is the most widely used method of treatment for coloured effluents (Choy *et al*., 2001). However, other alternatives such as powdered activated carbon and activated bentonites can also be used (Pala and Tokat, 2002; Yavuz and Aydin, 2002). The major disadvantages of this technique are the large amount of

sludge, which has to be buried, and the low efficiency with respect to some dyes (Pearce *et al*., 2003).

Colour removal is also usually effective and fairly rapid using ozone. However, not all the methods employed give satisfactory results especially for some dispersed dyes. Therefore, new flocculation mechanisms have been attracting attention (Petzold *et al*., 2003a). Salts alone or in combination with polymers were used for the neutralization of charged particles to obtain coagulation. The flocculation mechanism depends on the properties of polymers, especially the type of charge (negative, positive or uncharged), the charge density, and the molecular weight. Highly charged polyelectrolytes with low molecular weight can cause flocculation by interaction between differently charged regions of the particles (patch flocculation); whereas polymers of very high molecular weight (several millions) can cause bridging. The combined use of cationic flocculant and anionic polyelectrolytes of high molecular weight (step by step) is successful because the two flocculation mechanisms can be combined: at first "patching" is obtained by the highly charged polycation and, in a second step, bridging is caused by the high molecular weight polyanion. This mechanism works very well for small particles, such as clay or cellulose-clay mixtures (Petzold *et al*., 2003b; Petzold *et al*., 2004).

Another, more practicable method is the application of pre-mixed polyelectrolyte complexes made by the interaction of aqueous solutions of polycation and polyanion that was first described for clay (Petzold *et al*., 1998). However, because dye molecules or their aggregates are incomparably smaller than such inorganic particles and, in some cases, they are also uncharged, it is necessary to apply other flocculation principles, for instance the inclusion of dye within complexes. Such complex particles, the so-called particle forming flocculants, are able to bind disperse–dyes effectively over large distances due to their size and structure. Complex particles with hydrophobic parts and positive or negative charge are able to bind the dye via hydrophobic as well as electrostatic interaction forces (Buchhammer *et al*., 2001).

Textile industries consume substantial volumes of water and chemicals for wet processing of textiles. These chemicals are used for desiring, scouring, bleaching, dyeing, printing, and finishing. They range from inorganic compounds and elements to polymers and organic products. There are more than 8 000 chemical products associated with the dyeing process listed in the Colour Index (Society of Dyers and Colourists, 1976 – cited by Banat *et al*., 1996). These dyes include several structural varieties of dyes, such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal-complex dyes. The only aspect in common is their ability to absorb light in the visible region (Song *et al*., 2006).

The removal of colour from wastewaters is often more important than the removal of the soluble colourless organic substances, which usually contribute the major fraction of the biochemical oxygen demand (BOD). Methods for the removal of BOD from most effluents are fairly well established; dyes, however, are more difficult to treat because of their synthetic origin and mainly complex aromatic molecular structures. Such structures are often synthesized to resist fading on exposure to sweat, soap, water, light or oxidizing agents (Aksu, 2005; Khan and Husain 2007) and this renders them more stable and less amenable to biodegradation (Fewson, 1988; Seshadri *et al*., 1994).

Dyes with simple structures and low molecular weights exhibit higher rates of colour removal, whereas colour removal is more difficult with highly substituted, high molecular weight dyes (Sani and Banerjee, 1999). The dye removal rates are influenced by changes in electron density in the region of the azo group. The substitution of electron withdrawing groups ($–SO_3H$,$–SO_2NH_2$) in the para position of the phenyl ring, relative to the azo bond, causes an increase in the reduction rate (Yilmaz *et al*., 2007). Nigam *et al.* (1996) established that azo compounds with a hydroxyl group or with an amino group are more likely to be removed than are those with a methyl, methoxy, sulpho or nitro groups. Colour removal is also related to the number of azo bonds in the dye molecule. The colour of monoazo dyes is removed faster than the colour of diazo or triazo dyes (Nigam *et al*., 1996).

Government legislation is becoming more stringent in most developed countries regarding the removal of dyes from industrial effluents, which is in turn becoming an increasing problem for the textile industries. Environmental-protection agencies in Europe are promoting prevention of transferral of pollution problems from one part of the environment to another. This means that for most textile industries, developing on-site or in-plant facilities to treat their own effluents before discharge is fast approaching actuality. Recently, state and federal agencies in the USA have been requiring lower effluent colour limits (< 200 units of American Dye Manufacturers Institute, ADMI) (McCurdy *et al*., 1992).

Interest in the pollution potential of textile dyes has been primarily prompted by concern over their possible toxicity and carcinogenicity. This is mainly because many dyes are made from known carcinogens, such as benzidine and other aromatic compounds, all of which might be reformed because of microbial metabolism (Khan and Husain 2007). It has been shown that azo and nitro compounds are reduced in sediments (Weber and Wolfe, 1987) and in the intestinal environment (Sirianuntapiboon and Srisornsak, 2007), resulting in the regeneration of the parent toxic amines. Anthraquinone-based dyes are most resistant to degradation due to their fused aromatic structures, which remain coloured for long periods. Basic dyes have high brilliance and therefore higher colour intensity, making them more difficult to decolourize, while metal-based complex dyes, such as chromium-based dyes, can lead to the release of chromium, which is carcinogenic in nature, into water supplies. Some disperse dyes have also been shown to have a tendency to bio-accumulate (Baughman and Perenich, 1988) and heavy-metal ions from textile effluents have also been reported at high concentrations in both algae and higher plants exposed to such effluents (Srivastava and Prakash, 1991).

The lack of data on the properties of many dyes has been the main problem in assessing broad classes of dyes to identify common characteristics. Although dyes constitute only a small portion of the total volume of waste discharge in textile processing, these compounds are not readily removed by typical microbial-based waste-treatment processes (Li *et al*., 2007). Furthermore, dyes can be detrimental to the

microbial population present in such treatment works and may lead to decreased efficiency or treatment failure in such plants (Ogawa *et al*., 1988). Similar adverse effects have also been detected for aquatic microbial populations and the aquatic environment in general (Pearce *et al.*, 2003) or for laboratory cultures exposed to such dyes (Ogawa *et al*., 1989).

Azo dyes, which are difficult to degrade biologically and chemically, constitute the largest group of colorants used in industry. They are commonly found in considerable amounts in wastewater released by dye-house effluents. Their presence in dyeing and production of dye wastewater causes high concentration of dissolved organic matter and deep colour. If these wastewaters are not treated properly, they can flow into lakes, rivers, and seas through public sewage and then contaminate the environments where people live. It is reported that azo dyes themselves are not toxic; however, under anaerobic conditions azo dyes are cleaved by microorganisms to form potentially carcinogenic aromatic amines (Li *et al*., 2007). Since many textile plants have rural locations and municipal treatment costs are increasing, both industries and scientists are becoming compelled to search for innovative novel treatments and technologies directed particularly towards the decolourization of dyes in effluents. Dyes usually have a very low rate of removal ratio for BOD to COD (BOD/COD less than 0.1). Biological methods, being cheap and simple to use, have been the focus of recent studies on dye degradation and decolourization (Sirianuntapiboon and Srisornsak, 2007). Therefore, the objective of this chapter was to evaluate the ability of the bacterial bioflocculants to remove dyes and chemicals from the textile industrial effluents.

4.2 MATERIALS AND METHODS

4.2.1 Textile dyes used in this study

The three types of dispersible dyes selected for use in this study were whale, medi-blue, fawn and a mixture of dyes (see Section 4.2.2). The chemical properties of these dyes are described in Table 4.1.

Table 4.1: Chemical properties of the textile dyes used in this study

Colour	Classification	Dyes	State	Bulk density (kg/m^3)	Concentration (%)
Whale	Azo	Dianix yellow S-6G	Powder	≈600	0.3700000%
		Dianix rubine S-3B	Powder		0.0850000%
		Dianix navy CC	Powder		1.700000%
Medi-blue	Anthraquinone	Avolan 15 LIQ	Powder	400-600	0.3000000%
		Dianix turquois blue S-BG	Powder		0.350000%
		Dianix blue KFBL	Powder		0.0084000%
Fawn	Azo	Dianix yellow S-6G	Powder	450-520	0.0480000%
		Tiacron/rubine – C-BT 200	Powder		0.0380000%
		Dianix blue K-FBL	Powder		0.0180000%

4.2.2 Sample collection

The three dyes (whale, medi-blue, and fawn) were collected from a textile industry in Hammarsdale (KwaZulu-Natal). These dyes were collected directly from the large silver storage tanks immediately after the dyes were cooled. Mixed dyes were collected from the textile treatment plant also in Hammarsdale. It was composed of the variety of dyes from all the textile industries around Hammarsdale.

4.2.3 Effect of flocculant concentration on dye removal

Four dyes (whale, medi-blue, fawn and mixed dyes) were used in the decolourization experiment. In a test tube, 9 ml of undiluted dye effluent was mixed with different concentrations of the bacterial bioflocculants (2-10 ppm). After the addition of bioflocculants, the components of the test tube were mixed using a Labcon shaker at 200 rpm for 1 min, and then at 60 rpm for another 5 min. The dyes were left to settle for 60 min and the OD was measured with a spectrophotometer at 550 nm. The flocculating activity was expressed as the concentration of the flocculant in parts per million (ppm) when the OD_{550} was (1/10) x OD_{550c} of the control (Nakata and Kurane, 1999). The decolourization efficiency or the percentage removal of dyes was calculated using the equation:

$$\text{Percentage removal/decolourization} = \frac{C_0 - C}{C_0} \times 100$$

Where: C_0 is the absorbance of the untreated dye and C is the absorbance after the treatment (Mishra and Bajpal, 2005; Shubo *et al*., 2005).

4.2.4 Effect of pH on dye removal

In the experiments on pH effect, the initial pH of dye wastewater in the test tube was adjusted from 6 - 10 using 2 N HCl or NaOH. The decolourization efficiency or the percentage removal of dyes was calculated as described in Section 4.2.3.

4.2.5 Effect of temperature on dye removal

To determine the effect of temperature on the removal of dyes, the test tubes containing dye wastewater were incubated at different temperatures 28^{o}C, 35^{o}C, 40^{o}C, and 45^{o}C. The decolourization efficiency or the percentage removal of dyes was calculated as described in Section 4.2.3.

4.2.6 Effect of cations on dye removal

Different cationic compounds were used to determine the effect of salts. Solutions of $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $MnCl_2 \cdot 7H_2O$ and CTAB were used as the source of cations. The optimum pH and temperature were used to determine the effect of cations on dye removal. The decolourization efficiency or the percentage removal of dyes was calculated as described in Section 4.2.2.

4.3 RESULTS

The results shown in this chapter are only for the experiment using 10 ppm (high concentration) of bacterial bioflocculants except for the experiment on the effect of flocculant concentration on dye removal. However, data for the experiment using 2, 4, 6, and 8 ppm are included in Appendix B. Ten ppm was chosen based on the high percentage of dye removal.

4.3.1 Effect of flocculant concentration on the flocculating activity and the removal of whale dye

Figure 4.1 shows the effect of flocculant concentration on the removal of whale dye. All the isolates were effective in the removal of whale dye even at low concentrations. Bioflocculants from isolates D1, A22, A17, and A14 removed 60% of whale dye at 2 ppm while isolate D1 removed 99.9% at 10 ppm. Therefore, an increase in flocculant concentration led to an increase in dye removal. The flocculating activity of the bacterial bioflocculants on whale dye is depicted in Fig. 4.2. The best flocculating activity was shown by isolate A14 while E1 showed the least.

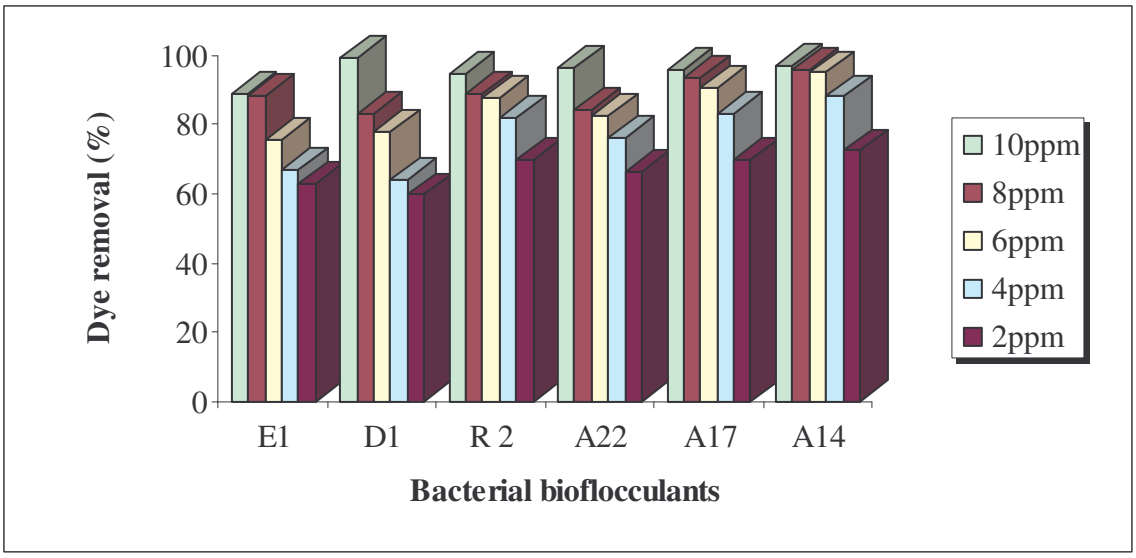

Fig. 4.1: Effect of flocculant concentration on the removal of whale dye.

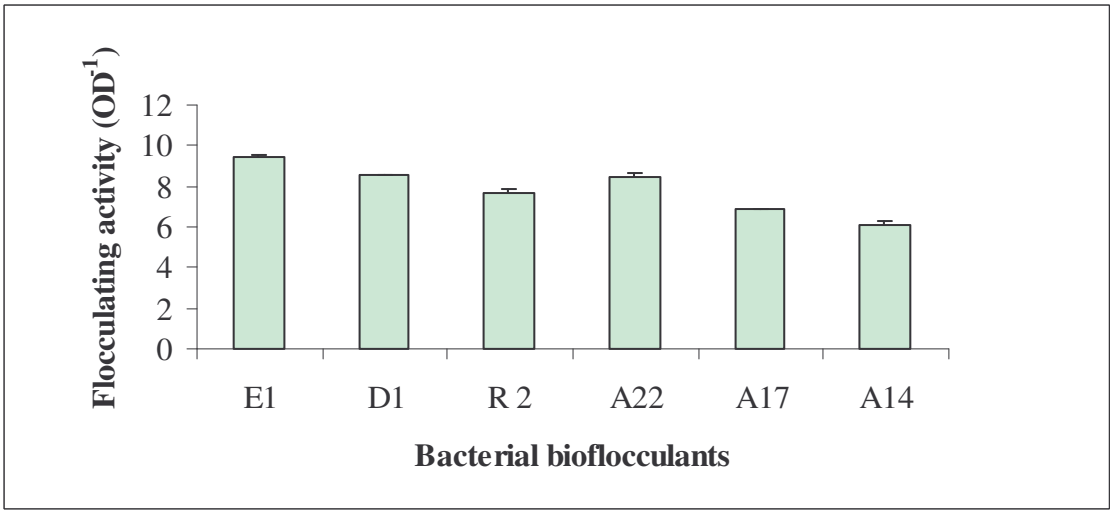

Fig. 4.2: Flocculation of whale dye by the bacterial bioflocculants.

4.3.2 Effect of pH, temperature, and cations on the removal of whale dye

pH is the most important parameter affecting the removal of various dyes from textile industries. The effect of pH on the removal of whale dye at 10 ppm flocculant concentration is shown in Fig. 4.3. The optimum pH for the removal of whale dye was pH 7 for all the isolates. At pH 6, 8, 9, and 10 there was a slight decrease in the removal of whale dye. At pH 10, 70% of whale dye was removed by isolates E1, R2, A17 and A14 while at pH 7, 99% of the dye was removed by all the bacterial isolates.

The optimum temperature for the removal of whale dye was 35^{o}C for all the isolates where 99% of the dye was removed (Fig. 4.4). At 45^{o}C, there was a decrease in the percentage removal of whale dye. This is shown clearly by isolates E1 and A17, where 58% and 62% of the dye was removed, respectively. The effect of cations on the removal of whale dye is depicted in Fig. 4.5. The most effective cation for the removal of whale dye was $MnCl_2$, followed by $MgSO_4$ and $CaCl_2$. Ninety-nine percent of whale dye was removed upon the addition of $MnCl_2$. CTAB was the least effective of all the cations tested.

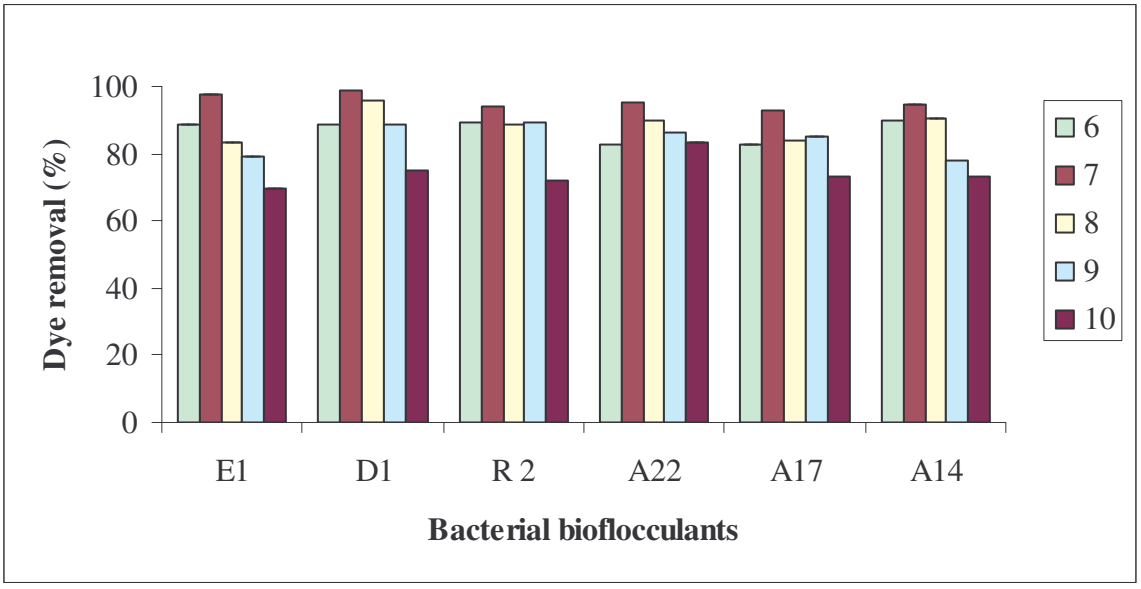

Fig. 4.3: Effect of pH on the removal of whale dye at 10 ppm.

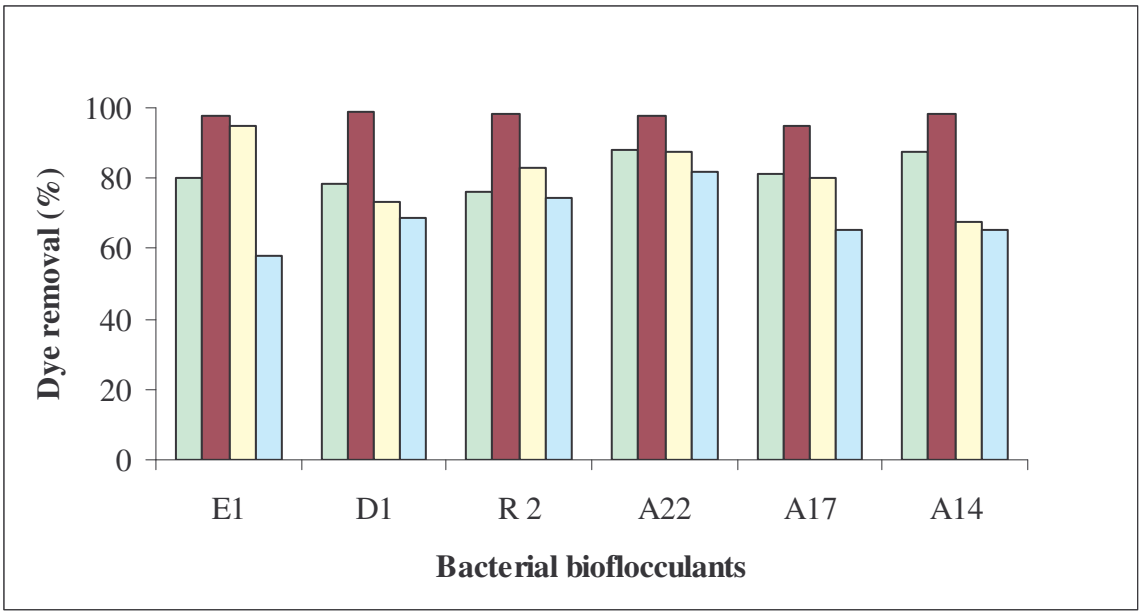

Fig. 4.4: Effect of temperature on the removal of whale dye at 10 ppm. Symbols: ■,28°C; ■, 35°C; ■, 40°C; ■, 45°C.

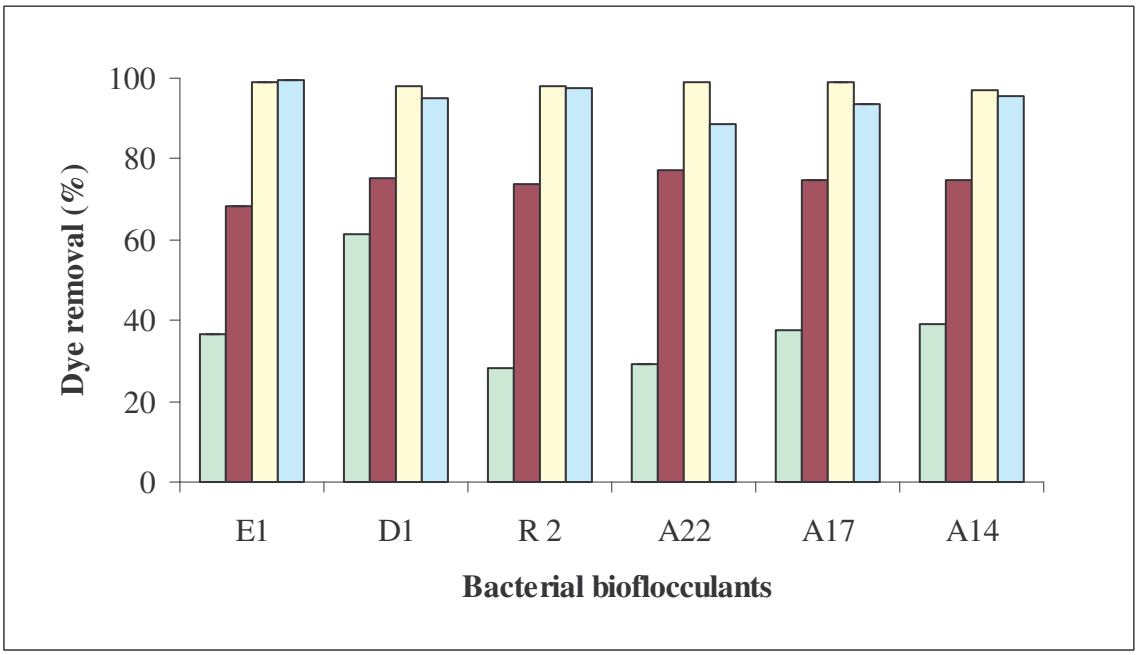

Fig. 4.5: Effect of cations on the removal of whale dye at 10 ppm. Symbols: ■, CTAB; ■, $CaCl_2$; ■, $MnCl_2$; ■, $MgSO_4$.

4.3.3 Effect of flocculant concentration on the flocculating activity and the removal of medi-blue dye

The effect of flocculant concentration on the removal of medi-blue dye is shown in Fig. 4.6. All the isolates were effective in the removal of medi-blue dye, even at low concentrations. At 10 ppm, 80% of medi-blue dye was removed by isolates E1, R2, A17, and A14. Isolate D1 removed 68% of the dye. Therefore, an increase in flocculant concentration led to an increase in the removal of dye. The flocculating activity of the bacterial bioflocculants are shown in Fig. 4.7. The best flocculating activity was achieved by isolate D1 while A22 removed the least dye.

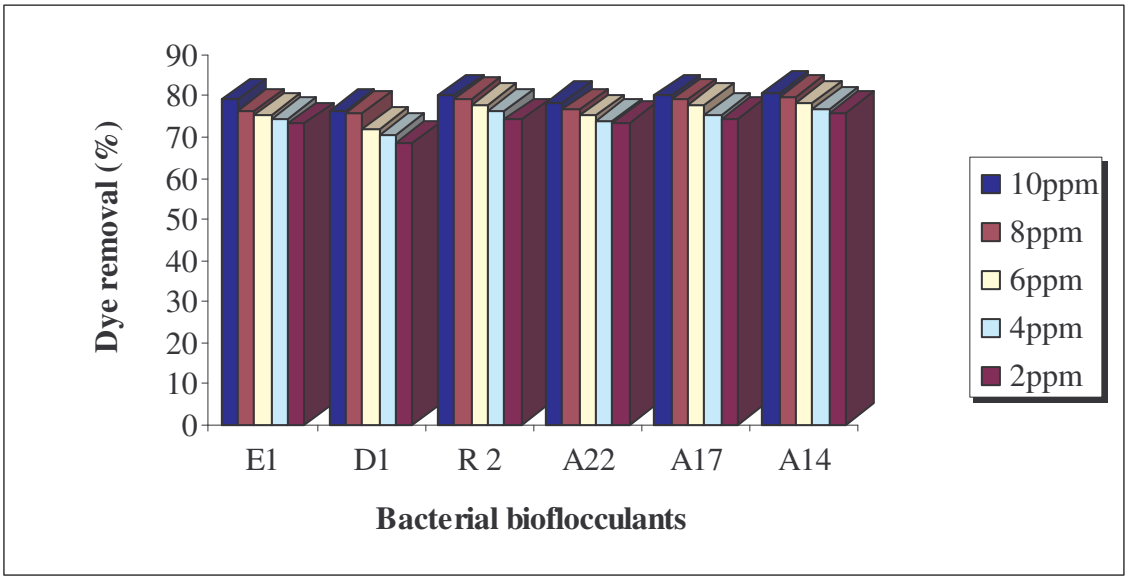

Fig. 4.6: Effect of flocculant concentration on the removal of medi-blue dye.

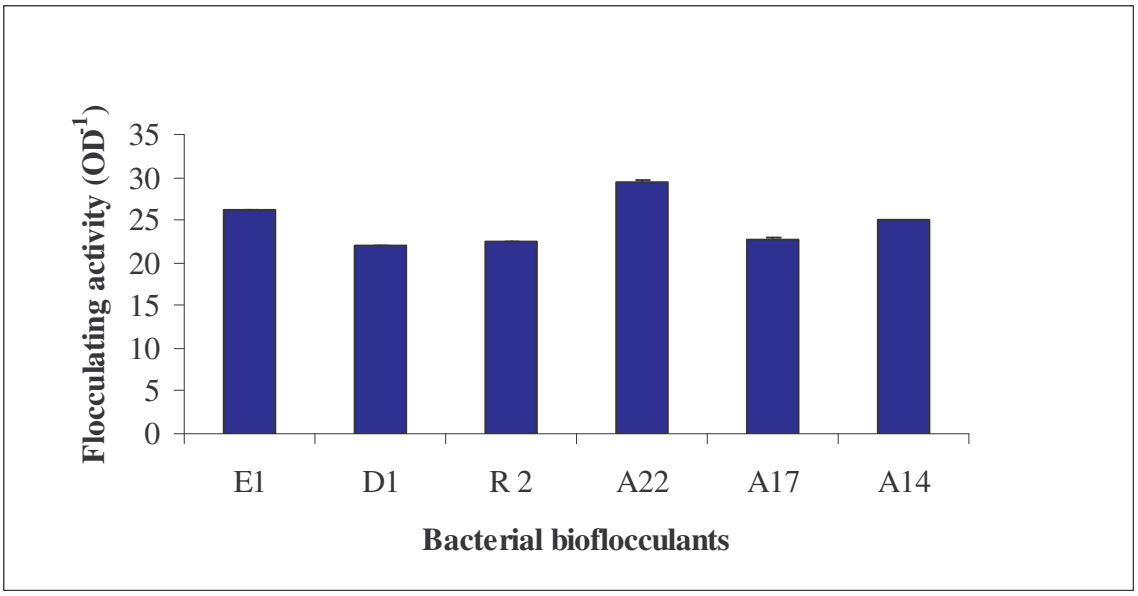

Fig. 4.7: Flocculation of medi-blue dye by the bacterial bioflocculants.

4.3.4 Effect of pH, temperature, and cations on the removal of the medi-blue dye

The effect of pH on the removal of medi-blue dye at 10 ppm is shown in Fig. 4.8. The optimum pH for the removal of medi-blue dye was 7 for all the isolates. At pH 10, 70% of medi-blue dye was removed by isolates E1, R2, A17, and A14. At pH 6, 8 and 9, the removal of medi-blue dye ranged between 70 and 90% while at pH 7, 99.9% of the dye was removed.

Figure 4.9 shows the effect of temperature on the removal of medi-blue dye. The optimum temperature for the removal of medi-blue dye was 35^{o}C for all the isolates, where 70% of the dye was removed by isolates E1, R2, A22, A17, and A14. Isolate D1 removed 75% of the dye. With an increase in temperature from 40^{o}C to 45^{o}C, there was a decrease in the removal of the dye. The effect of cations on the removal of the medi-blue dye is depicted in Fig. 4.10. The most effective cation for the removal of medi-blue dye was $MnCl_2$ for all the isolates followed by $MgSO_4$ and $CaCl_2$. CTAB was the least

effective of all the cations tested. Only 24% of the dye was removed by isolates R2, and A22 in the presence of CTAB and only 32% was removed by isolates A17 and A14.

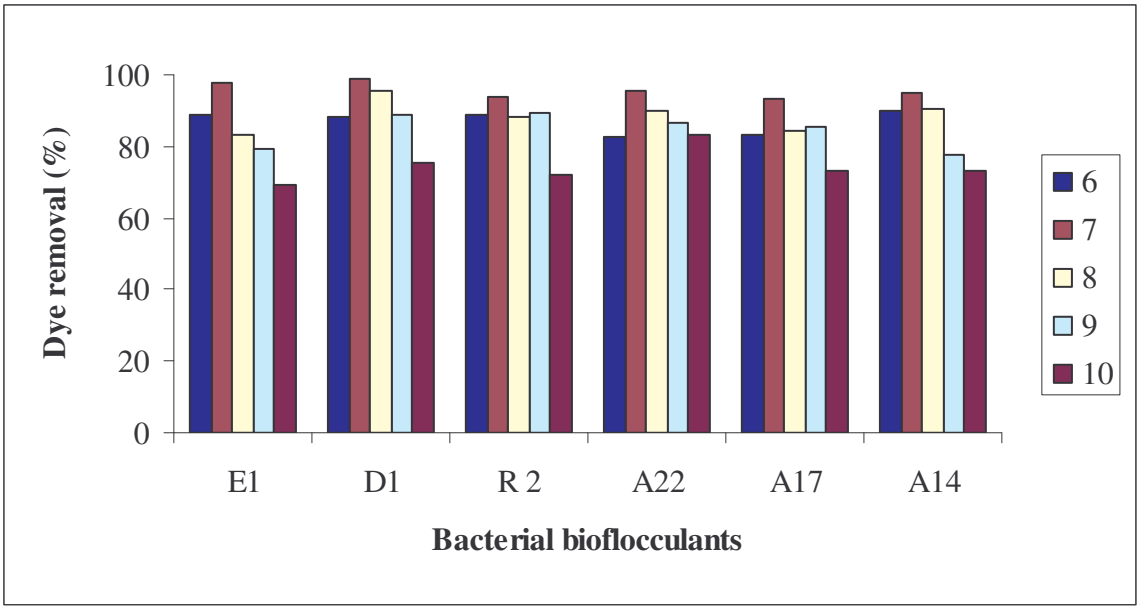

Fig. 4.8: Effect of pH on the removal of medi-blue dye at 10 ppm.

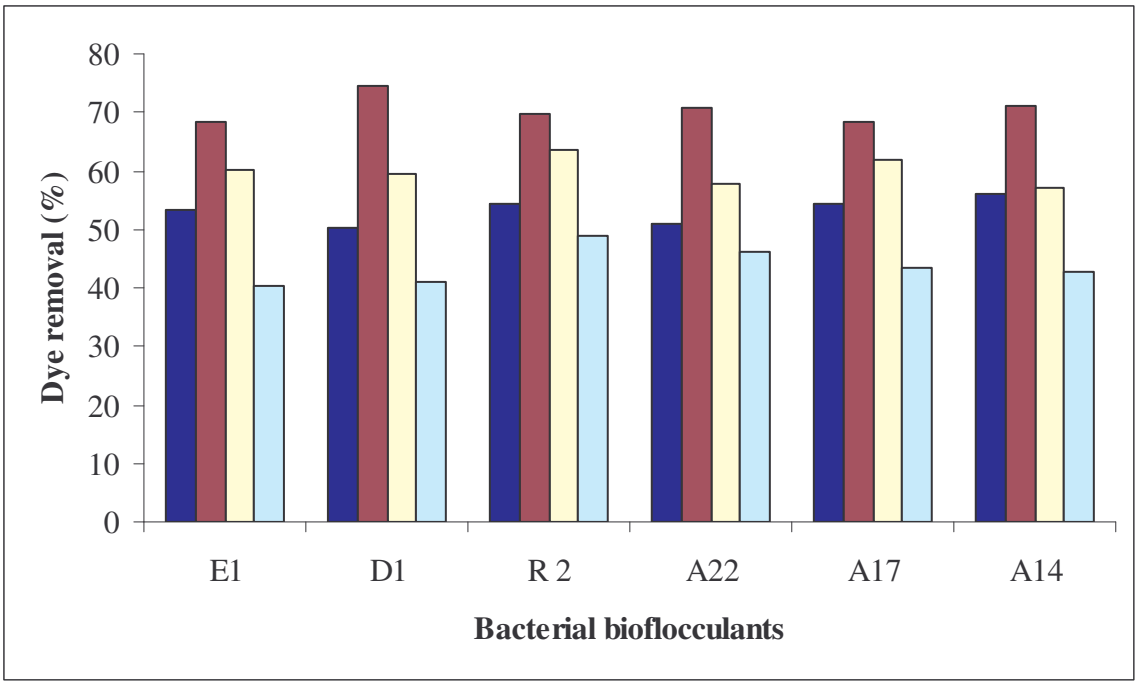

Fig. 4.9: Effect of temperature on the removal of medi-blue dye at 10 ppm. Symbols:■, 28°C; ■, 35°C; ■, 40°C; ■, 45°C.

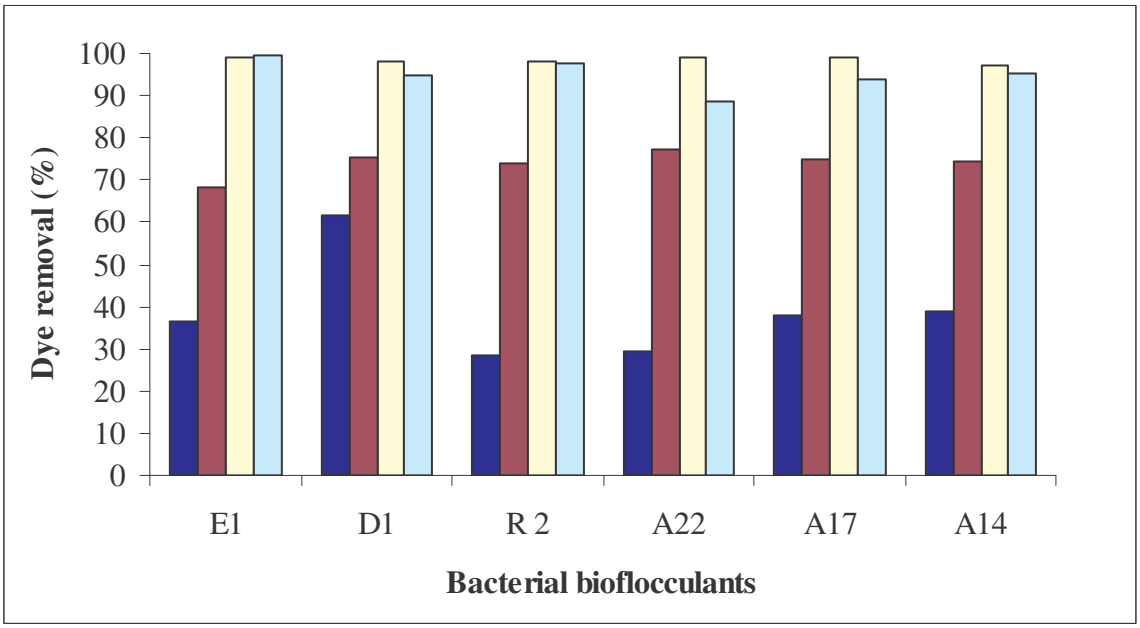

Fig. 4.10: Effect of cations on the removal of medi-blue dye at 10 ppm. Symbols: ■,CTAB; ■, $CaCl_2$; ■, $MnCl_2$; ■, $MgSO_4$.

4.3.5 Effect of flocculant concentration on the flocculating activity and the removal of fawn dye

The effect of flocculant concentration on the removal of fawn dye is shown in Fig. 4.11. Isolate D1 showed the best removal at 10 ppm where 99% of fawn dye was removed followed by isolate E1 where 80% of the dye was removed at 10 ppm. At 2 ppm, the removal of the dye by isolates D1 and E1 were 90% and 70%, respectively. Only 50% of fawn dye was removed by isolate R2 at 10 ppm. The removal of fawn dye by the isolates A22, A17, and A14 was less effective even with an increase in the flocculant concentration. The flocculating activity of the bacterial bioflocculants are depicted in Fig. 4.12. Isolate D1 showed the best flocculating activity while isolate A17 showed the least amount of activity. The flocculating activities of isolates E1, R2, A14, and A22 ranged between 10 – 50 OD^{-1}.

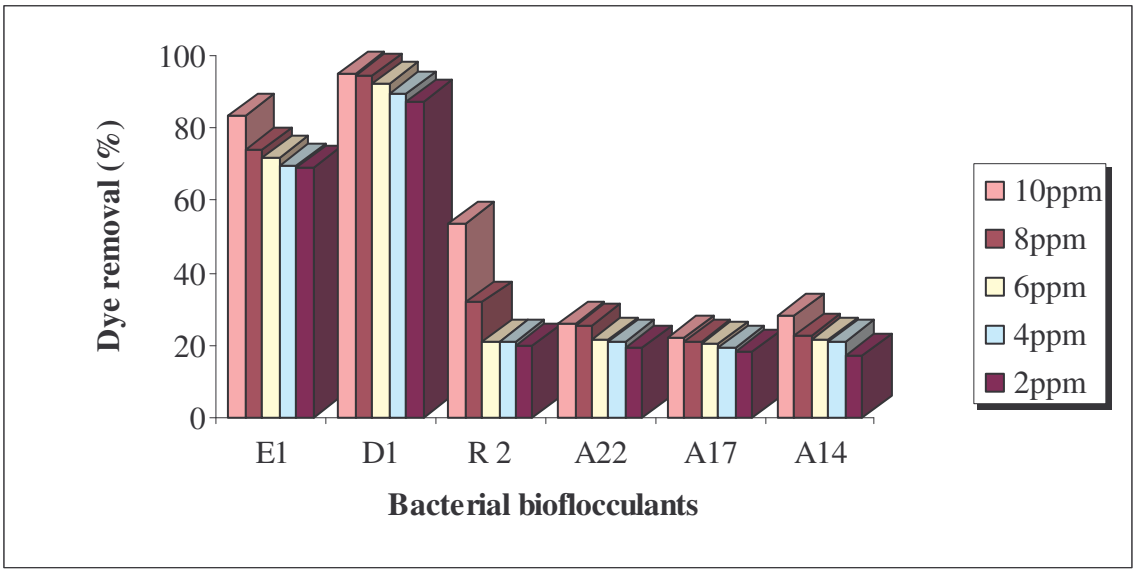

Fig. 4.11: Effect of flocculant concentration on the removal of fawn dye.

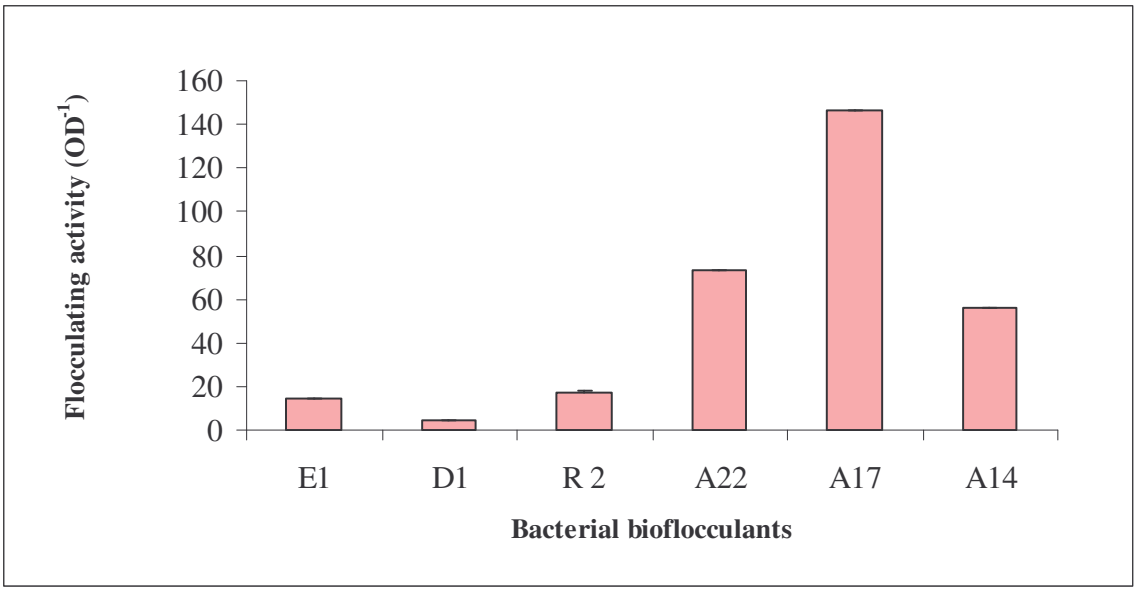

Fig. 4.12: Flocculation of fawn dye by the bacterial bioflocculants.

4.3.6 Effect of pH, temperature, and cations on the removal of fawn dye

The effect of pH on the removal of fawn dye at 10 ppm bioflocculant concentration is depicted in Fig. 4.13. The optimum pH for the removal of fawn dye was 10 for all the isolates. At pH 10, isolates E1 and R2 removed 62% of fawn dye; isolates D1 and A22 removed 58% while A17 and A14 removed only 55%. There was an increase in the removal of fawn dye with an increase in pH. Figure 4.14 shows the effect of temperature on the removal of fawn dye. The optimum temperature for the removal of fawn dye was 40^{o}C for isolates E1 and A22. The optimum temperature for the removal of the fawn dye was 45^{o}C for isolates D1, R2, A17, and A14. The effect of cations on the removal of fawn dye is shown in Fig. 4.15. The most effective cation was CTAB followed by $MnCl_2$, and $MgSO_4$ for isolates E1, D1, R2, and A22 while $CaCl_2$ was the least effective. For isolates A17 and A14, $CaCl_2$ was the most effective cation in the removal of fawn dye followed by $MnCl_2$, and $MgSO_4$ while CTAB was the least effective.

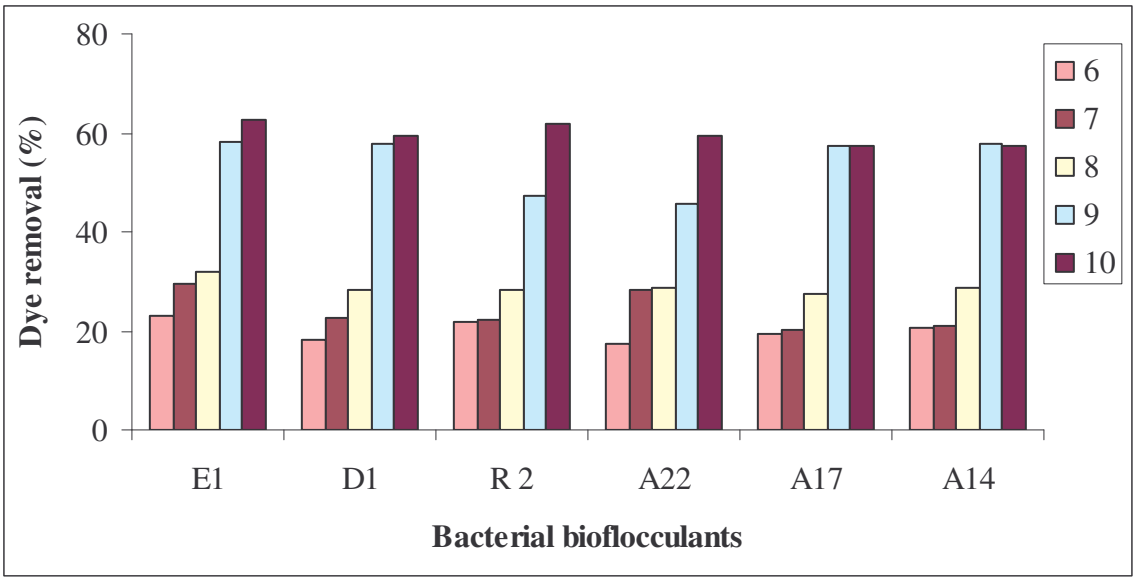

Fig. 4.13: Effect of pH on the removal of fawn dye at 10 ppm.

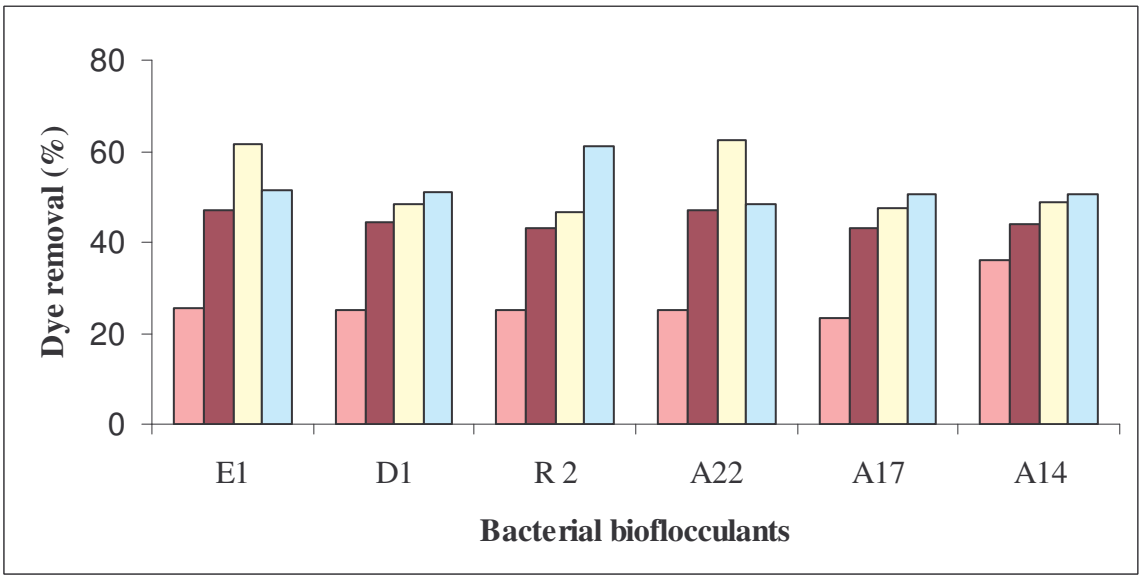

Fig. 4.14: Effect of temperature on the removal of fawn dye at 10 ppm. Symbols: ■,28°C; ■, 35°C; ■, 40°C; ■, 45°C.

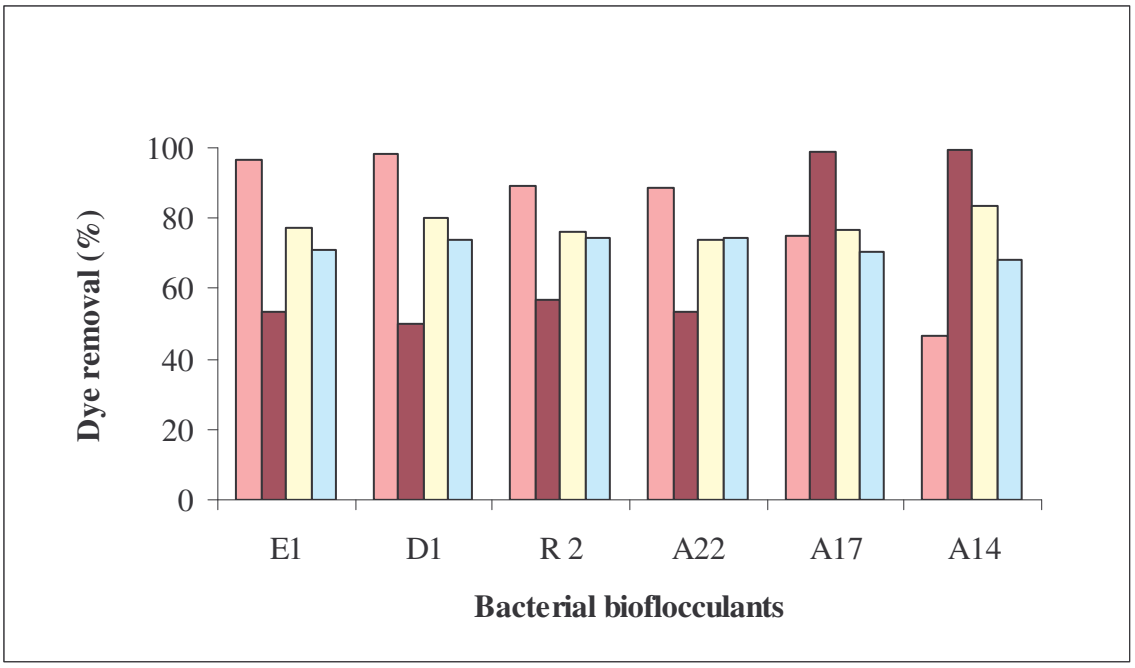

Fig. 4.15: Effect of cations on the removal of fawn dye at 10 ppm. Symbols: ,CTAB;, $CaCl_2$; , $MnCl_2$; , $MgSO_4$.

4.3.7 Effect of flocculant concentration on the flocculating activity and the removal of mixed dyes

Figure 4.16 illustrates the effect of flocculant concentration on the removal of mixed dyes. All isolates were effective in the removal of mixed dyes even at low concentrations. Therefore, an increase in flocculant concentration led to an increase in the removal of the dye. At 2 ppm, 62% of mixed dyes were removed by isolates D1, R2 and A14 while at 10 ppm, 75% was removed by isolates D1, E1, R2, A22 and A14. The flocculating activity of the bacterial bioflocculants is depicted in Fig. 4.17. The best flocculating activity was achieved by isolate R2 while isolate E1 removed the least dye.

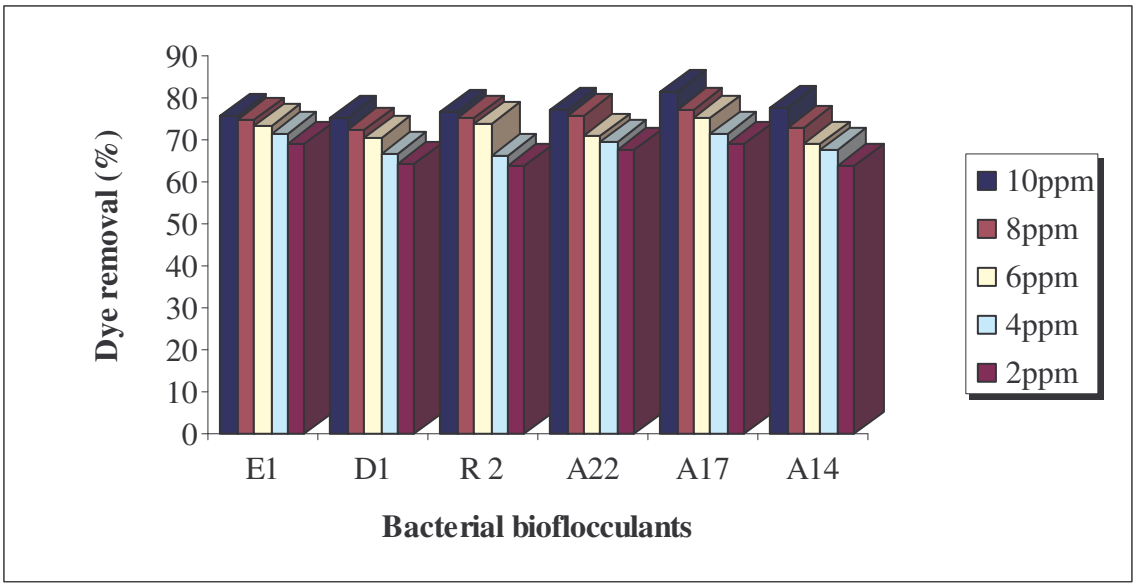

Fig. 4.16: Effect of flocculant concentration on the removal of mixed dyes.

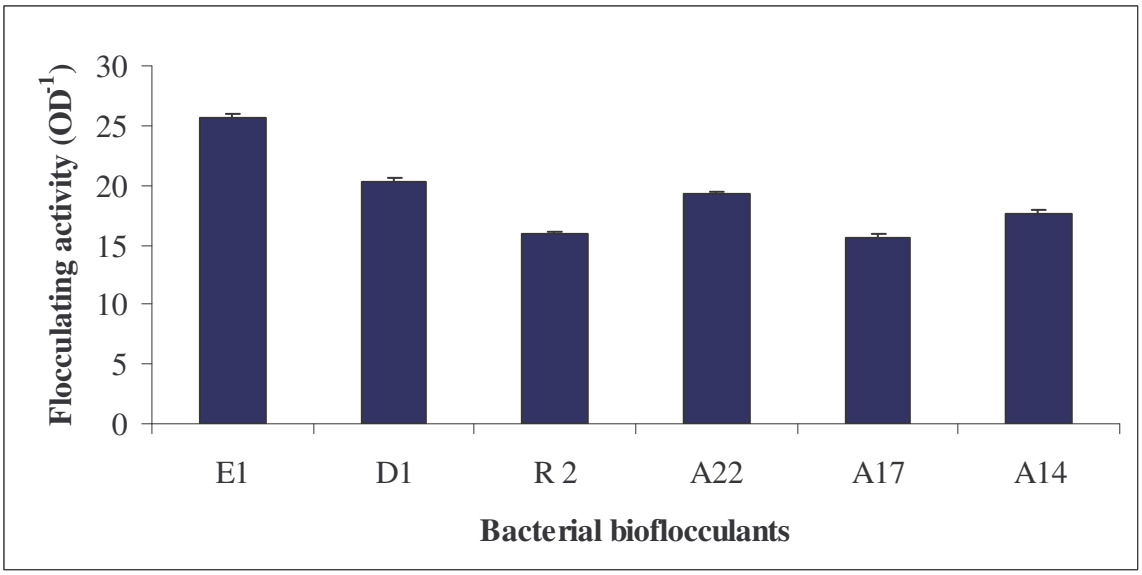

Fig. 4.17: Flocculation of mixed dyes by the bacterial bioflocculants.

4.3.8 Effect of pH, temperature, and cations on the removal of mixed dyes

The effect of pH on the removal of mixed dyes at 10 ppm bioflocculant concentration is shown in Fig. 4.18. The optimum pH for the removal of mixed dyes was 10 for all isolates. At pH 10, 97% of the dyes were removed by isolate R2; while 85% was removed by isolates D1, E1, and A17. The optimum temperature for the removal of mixed dyes was 35^{o}C for isolates E1, D1 and A14 and 40^{o}C for isolates R2, A22 and A17 (Fig. 4.19). At 35^{o}C, 99% of the dyes were removed by isolates E1, D1 and A14 while at 45^{o}C, 99.9% was removed by R2 and A17. The effect of inorganic salts on the removal of dyes is an important parameter since in textile wastewater effluents, dyes are found in solutions of high concentrations of sulphates and phosphates salts. The effect of cations on the removal of mixed dyes is depicted in Fig. 4.20. The most effective cation was $MnCl_2$ for all the isolates followed by $CaCl_2$ and $MgSO_4$. CTAB was the least effective of all the cations tested.

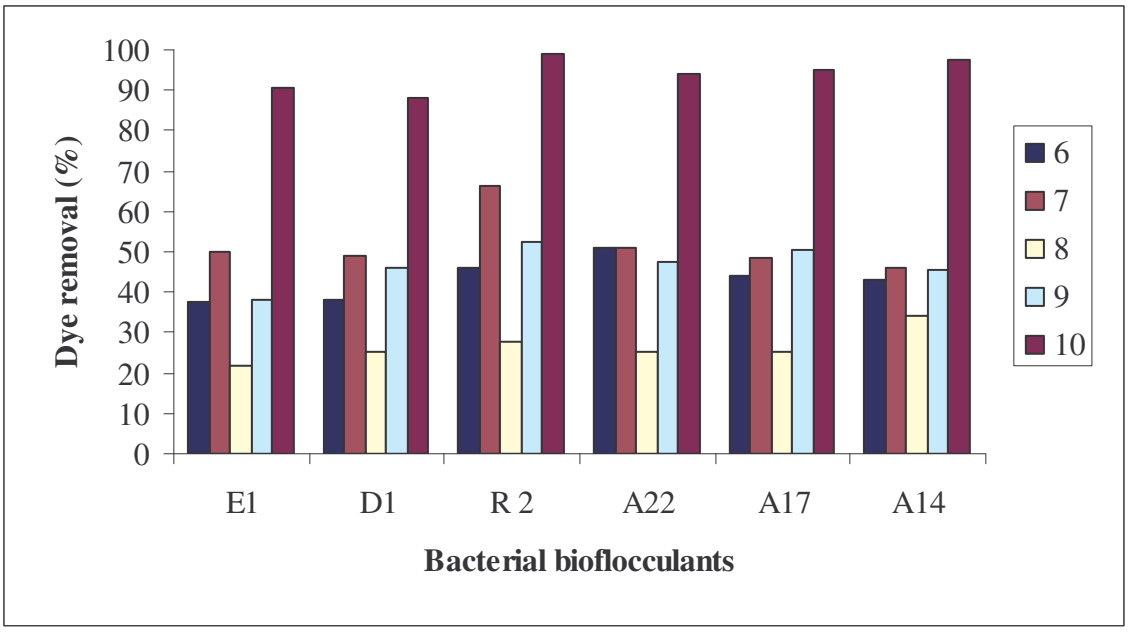

Fig. 4.18: Effect of pH on the removal of mixed dyes at 10 ppm.

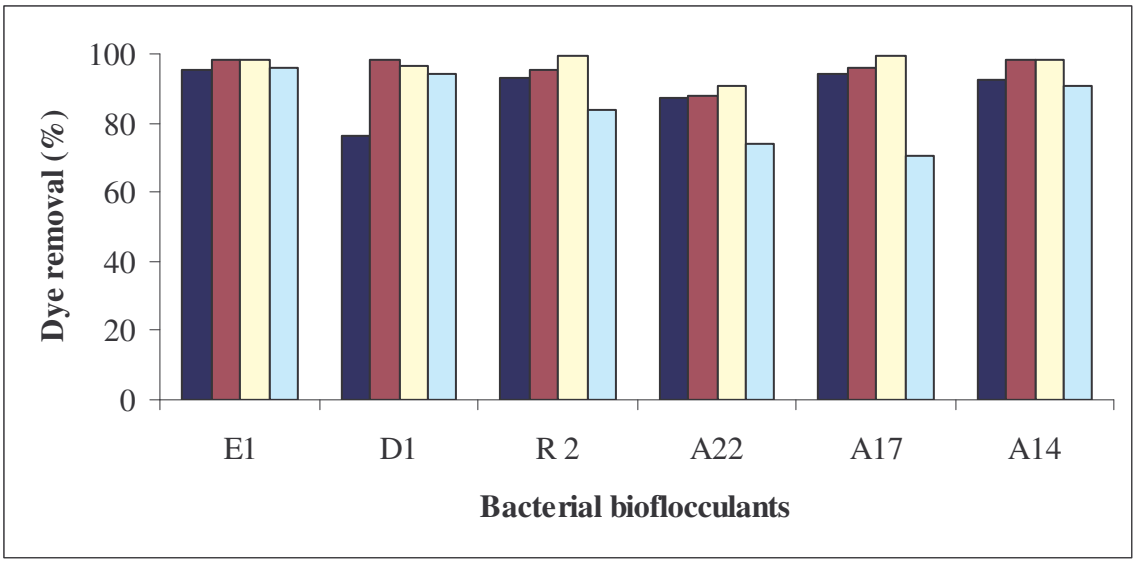

Fig. 4.19: Effect of temperature on the removal of mixed dyes at 10 ppm. Symbols: ■, 28°C; ■, 35°C; ■, 40°C; ■, 45°C.

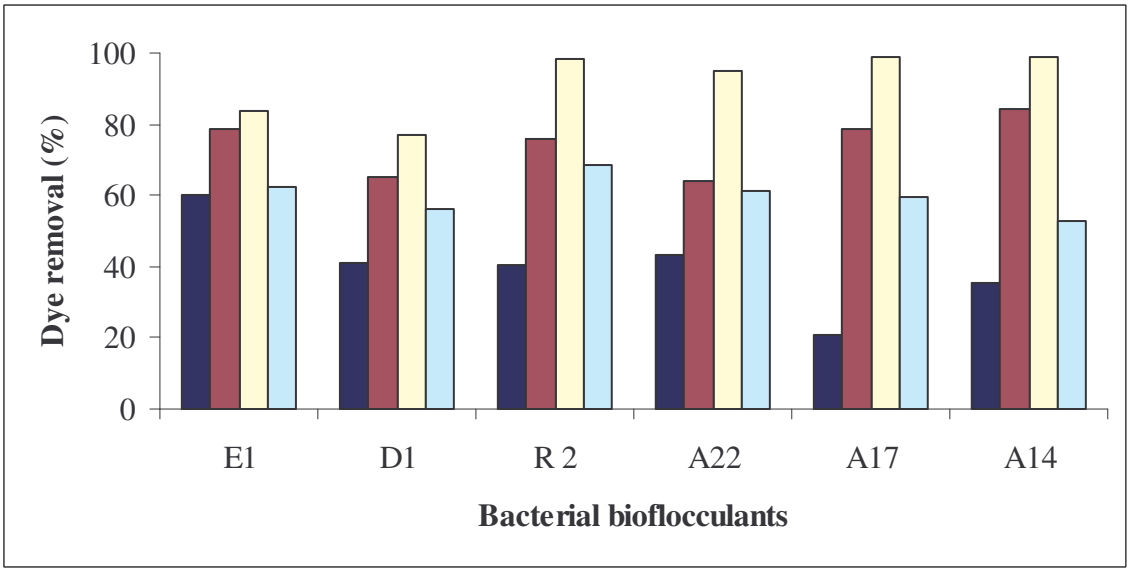

Fig. 4.20: Effect of cations on the removal of mixed dyes at 10 ppm. Symbols: ■,CTAB; ■, $CaCl_2$; ■, $MnCl_2$; ■, $MgSO_4$.

4.4 DISCUSSION

In this study, the effect of flocculant concentration on the removal of dyes was investigated. The results have shown that the removal of all the dyes tested were directly proportional to the flocculant concentration (Fig. 4.1; Fig. 4.6; Fig. 4.11; Fig. 4.16). The flocculants cause aggregation of particles and cells by bridging and charge neutralization (Salehizadeh and Sojaosadati, 2001). Bridging occurs if the flocculant extends from the particle's surface into the solution for a distance greater than the distance over which the interparticle repulsion acts. In this case, the biopolymer can adsorb to other particles to form flocs. This mechanism explains flocculation by neutral or like-charged bioflocculants (Hantula and Bamford, 1991b; Levy *et al*., 1992). Shubo *et al*. (2005) reported that higher decolourization of the dyes can be achieved by increasing the concentration of the bioflocculants.

The flocculating capacity of the bioflocculants increased with an increase in concentration and remained constant except for fawn dye where there was a slight increase in flocculating capacity with an increase in flocculant concentration (Fig. 4.1; Fig. 4.6; Fig. 4.11; Fig. 4.16). This observation may be due to a particle-polymer-particle complex formation in which polymer serves as a bridge. To be effective in destabilization, a polymer molecule must contain chemical groups, which can interact with sites on the surface of the colloidal particle. When a polymer molecule comes into contact with a colloidal particle, some of these groups adsorb at the particle surface, leaving the remainder of the molecule extending out into the solution. If a second particle with some vacant adsorption sites comes into contact with these extended segments, attachment can occur. A particle-polymer-particle complex is thus formed in which polymer serves as a bridge. If a second particle is not available, in time the extended segments may eventually adsorb on other sites on the original particle, so that the polymer is no longer capable of serving as a bridge (Salehizadeh and Sojaosadati, 2001; Salehizadeh and Sojaosadati, 2002). Mishra and Bajpai (2005) indicated that there is direct stoichiometric relationship between optimum polymer dosage and colloid concentration, and restabilization due to overdosing can occur.

In this study, the optimum pH was observed at pH 7 for whale and medi-blue dyes (Fig.4.3, Fig.4.8) while for fawn and mixed dyes it was observed at pH 10 (Fig. 4.13; Fig.4.18). According to Willmott (1997), the optimum pH for colour removal is often at a neutral pH value or a slightly alkaline pH value and the rate of colour removal tends to decrease rapidly at strongly acid or strongly alkaline pH values. As a result, the coloured wastewater is often buffered to enhance the colour removal performance. Colour removal in the alkaline pH range is presumably due to adsorption onto hydroxide flocs. Biological reduction of the azo bond of whale and fawn dye can result in an increase in the pH due to the formation of aromatic amine metabolites, which are more basic than the original azo compound (Willmott, 1997). Altering the pH within a range of 7.0 to 9.5 has very little effect on the dye reduction process. Chang *et al.* (2001) found that the dye reduction rate increased nearly 2.5-fold as the pH was raised from 5.0 to 7.0, while the rate became insensitive to pH in the range of 7.0-9.5. This is in accordance with the results obtained in this study. On the other hand Mittal and Gupta (1996), studied the effect of pH on the biosorption of three cationic dyes, Orlamar Red BG, Orlamar Blue G and Orlamar Red GTL by the fungus *Fomitopsis carnea* and their results showed that colour removal decreased with decreasing pH due to repulsive forces between coloured dye cations in solution and biosorbent surface charged positively at pH values lower than 3.0.

In many systems, the rate of colour removal increases with increasing temperature, within a defined range that depends on the system (Chang *et al*., 2001). The temperature required to produce the maximum rate of colour removal was found to range between 35-45^{o}C for all the textile dyes tested (Fig. 4.4; Fig. 4.9; Fig. 4.14; Fig. 4.19). The results are consistent with a study done by Pearce *et al*. (2003) regarding the removal of colour from textile wastewater using whole bacterial cells. The decline in colour removal activity at higher temperatures can be attributed to the loss of cell viability (Pearce *et al*., 2003). Aksu and Tezer (2000), also investigated the effect of temperature on the biosorption of Remazol Black B reactive dye by *Rhizopus arrhizus* and their results indicated that optimum adsorption temperature was 35^{o}C and adsorption decreased with further increasing temperature due to the decreased surface activity.

The addition of the divalent cations such as Mn^{2+}, Mg^{2+}, and Ca^{2+} enhanced both the flocculating activity and the decolourization of the dyes (Fig. 4.5; Fig. 4.10; Fig. 4.15; Fig. 4.20). Cations stimulate flocculating activity by neutralizing and stabilizing the residual negative charge of functional groups and by forming bridges between particles. The role of bivalent and trivalent cations is to increase the initial adsorption of biopolymers on suspended particles by decreasing the negative charge on both the polymer and the particle (Levy *et al*., 1992). The results are consistent with the findings of Kurane *et al.* (1994b) who reported that divalent cations namely Mn^{2+}, Mg^{2+}, and Ca^{2+} bind to the bioflocculants and form complexes thus stimulating flocculation. Fujita *et al.* (2000) and Yim *et al*. (2007) indicated that the flocculating activity of *Citrobacter* sp. TKF04 and *Gyrodinium impudicum* KG03 was not enhanced by the addition of any cations including Ca^{2+}. Dyeing processes consume large amounts of salts. Therefore, salt concentration in dye wastewater is one of the important factors that influence the biosorption capacity (Zhou and Banks, 1991; Zhou and Banks, 1993). Textile wastewaters may include metal ions beside dyes and salts due to metal-containing dyes used in textile industry. Metal ions would be a factor influencing biosorption rate and capacity. They might compete with dye molecules for the binding sites or stimulate the biosorption of dye onto biomass (Zhou and Banks, 1993).

Among the four dyes used in this study, whale dye was easily decolourized followed by medi-blue and mixed dyes. Fawn dye was decolourized the least. According to Pearce *et al*. (2003), a possible reason for this observation could be that fawn dye is an acidic dye. Acid dyes are the most problematic and difficult to decolourize due to their inert chemical structure and an attached phenyl group, methyl, methoxy, nitro and a sulphonate groups [PhNH; O=S=O; $(CH_2)_3$–OMe; NO_2]. Also Sanghi *et al.* (2006) suggested that removal of colour from dye solutions is complex and may be due to physicochemical mechanisms of coagulation and or chelation–complexation type reactions. The colour removal data suggests that the colour removal mechanism is predominantly physicochemical. The structure of the dyes appears to be conducive to chelation/complex formation reaction with coagulants leading to the formation of insoluble metal dye complexes, which may precipitate, from solution. In this study,

colour removal was accomplished by aggregation or precipitation and adsorption of colouring substances onto the coagulant species. Hitz *et al*. (1978), concluded that acid dyes exhibit low colour removal due to the number of sulphonate groups in the dye, direct dyes exhibit high levels of colour removal that is independent of the number of sulphonate groups in the dye and that reactive dyes exhibit low levels of colour removal. The effect of the sulphonate groups on colour removal is related to the mechanism by which the colour is removed (Pinheiro *et al*., 2004).

Water is essential in all aspects of the world. If colour could be removed from the dyeing effluent through coagulation/flocculation, wastewater could be reused several times. Decolourization of dye solutions by coagulation with bacterial bioflocculants depended largely on the type of dye, pH, temperature, and flocculants concentration. The bioflocculants were effective to varying degrees in removing the dyes in aqueous solution, in particular whale dye, medi-blue, fawn dye and mixed dyes, with a decolourization efficiency ranging between 20-99.9%. Bacterial bioflocculants may provide a promising alternative to replace or supplement present treatment processes for the removal of very high concentrations of dyes. The use of natural flocculants seems to be an economical and cleaner alternative for textile wastewater treatment, as they are biodegradable and easily available from reproducible resources (Kurane *et al*., 1986a). Undoubtedly, microbial flocculants offer many promising benefits for commercial purposes in the future and they may be very good alternatives to the chemical coagulants that are used conventionally.

CHAPTER FIVE

THE TREATMENT OF RIVER WATER BY THE BIOFLOCCULANTS

5.1 INTRODUCTION

Water is the most common and important chemical compound on earth. Only 2.6% of the global water is freshwater and consequently available as potential drinking water. Coagulation-flocculation followed by sedimentation, filtration and disinfection by chlorine, is used worldwide in the water treatment industry before the distribution of treated water to consumers (Ndabigengesere and Narasiah, 1998). The availability of drinking water has been the most critical factor for survival throughout the development of all life. In the history of humankind, cultural centres were always founded in areas with a sufficient supply of freshwater. As the population increased, the natural supply of water became limited, and all of the great cultures developed sophisticated techniques and systems to obtain access to new water reservoirs (e.g. drilling of wells and building of aqueducts) and to distribute water for irrigation and drinking (Hammerton and Sherrat, 1972). Initially, developing communities found that supplying and distributing a sufficient volume of drinking water presented major problems. However, very soon other complications of highly populated areas, such as increasing amounts of waste, wastewater, and other types of contamination, also endangered access to fresh, safe drinking water (Hunter and Quigley, 1998).

Pollutants have been transported by wind and rain to every place on earth. Today, in most industrialized countries, drinking water is ranked as food, and high standards are set for its quality and safety. The strict requirements for microbiological factors specify that bacterial content should be very low and that no pathogenic microorganisms should be detectable. These strict demands for the absence of pathogens, however, are meaningful only for the classical pathogens like *Vibrio cholerae* and *Salmonella typhi* (USEPA, 1991). The discovery of new pathogens and new insights into the microbiology of drinking water required a more detailed investigation toward the occurrence of

potentially pathogenic bacteria, viruses, and parasites. Therefore, guidelines and legislation [e.g. European Union Council Directive 98/83/EC and World Health Organization (WHO) guidelines] state that drinking water should contain pathogenic microorganisms only in such low numbers that the risk for acquiring waterborne infections is below an accepted limit. The fulfilment of these requirements demands resource protection and careful treatment of raw water, as well as accurate quality control of the treatment process. However, evaluation of the behaviour of pathogens in drinking water is also essential as a basis for further improvements of the treatment process and for new regulations (Atherton *et al*., 1995).

Faecal coliforms and enterococci have been widely used as indicators of faecal pollution (Sinton *et al*., 1998). Both microbial groups can be determined by their enumeration. Different agents can determine the proportion of faecal coliforms/enterococci, or their inactivation. Both bacterial groups include several species. For example, the genus *Enterococcus* contains 19 recognized species (Manero and Blanch, 1999). Any determination of their diversity in the environment should consider this aspect. Urban or rural wastewaters normally contain many bacterial species, each with a large number of strains. Biological treatment processes at sewage treatment plants could produce selective elimination and/or changes of proportion, in the bacterial populations (Mezrioui and Baleux, 1994). Moreover, the sewage effluent, as well as urban or industrial waste, could modify some microbial populations in the reception waters, such as rivers, lakes, or lagoons (Sinton and Donnison, 1994). This effect could become more important where policies of water re-utilization are applied in regions with poor water resources. The determination of the origin of faecal pollution in waters is important for the management and quality control of water resources. Sub-typing below the species level of bacteria could provide valuable information about the sources of pollution in surface waters (Kuhn *et al*., 1997).

In recent years, several so-called “new or emerging pathogens” have arisen as problems in drinking-water production and distribution. These include, on the one hand, newly recognized pathogens from faecal sources like *Campylobacter jejuni*, pathogenic

Escherichia coli, Yersinia enterocolitica, new enteric viruses like rotavirus, calicivirus, small round-structured virus, astrovirus, and the parasites *Giardia lambia, Cryptosporidium parvum*, and microsporidia. On the other hand, some new pathogens comprise species of environmental bacteria that are able to grow in water distribution systems and only recently were recognized as relevant pathogens, such as *Legionella* sp., *Aeromonas* sp., *Mycobacterium* sp., and *Pseudomonas aeruginosa* (Brugha *et al*., 1999).

River water is a widely used but often unappreciated source of water. In the U.S., river water supplies at least 100 million people with drinking water. In rural and suburban areas, 90-95% of the drinking water comes from river water (Prescott *et al*., 1996). Several techniques are used in the treatment of river water however; they all have their advantages and drawbacks. The retention of dissolved and dispersed organic and/or inorganic water contaminants with membrane processes for the (direct) treatment of surface waters has recently become more important. The interest in ultra-filtration (UF) and micro-filtration (MF) membranes has increased due to the extremely high water quality with respect to hygiene and microbiological safety, documented by a growing number of UF and MF membrane installations, research projects and pilot plant trials. Low-pressure membranes provide a complete barrier against microorganisms and particles. These are, for instance, the possibility of fully automatic operation, a compact system design in connection with good space utilisation and flexibility in system enlargement, modernisation, and new installations (Lerch *et al*., 2005).

Photocatalytic oxidation mediated by semi-conductor catalysts is one of the emerging advanced oxidation processes used in the treatment of river water (Meng *et al*., 2005). By applying ultraviolet (UV) radiation on photocatalysts, powerful active oxidants of hydroxyls can be formed (Ollis *et al*., 1996); therefore, photocatalytic oxidation technology is commonly considered capable of decomposing almost all types of organic contaminants. However, from a viewpoint of practical application, the feasibility of the photocatalytic process for the treatment of various river waters is of more interest. So far, the reported treatment objectives of applications or application-oriented experiments have encompassed contaminated ground waters, industrial wastewaters, and effluents of

biologically treated wastewaters and polluted river water (Dillert *et al.*, 1999; Meng *et al*., 2005). Nevertheless, traditional slurry photocatalytic oxidation systems were usually not favoured because catalyst separation and catalyst loss were too severe to warrant a long-term operation. Furthermore, the use of photocatalytic oxidation system is economically unfeasible (Rodriguez *et al*., 1996; Crittenden *et al*., 1997). Therefore, there is a great need to develop cheap and effective methods for the treatment of river water.

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

5.2 MATERIALS AND METHODS

5.2.1 Microbiological analysis of river water

The river water used in the following experiment was collected from Palmiet River close to the University of KwaZulu-Natal (Westville campus). Serial dilutions (10^{-1}–10^{-6}) of river water were carried out to enumerate the different microorganisms present. Aliquots of 0.1 ml from each dilution were plated on nutrient agar (NA); mannitol salt agar (MS); eosin-methylene blue agar (EMB) and Salmonella-Shigella agar (SS). All the

reagents used for the analysis of river water were purchased from Merck. Nutrient agar, (NA) was used to grow all the different bacteria present in river water, MS was used to isolate *Staphylococci*, while EMB and SS agar were used to isolate *E. coli*, *Salmonella* and *Shigella* species respectively. The plates were incubated overnight at 37^{o}C and colonies were then counted (Prescott *et al*., 1996).

5.2.2 Determination of flocculating activity

Different concentrations of bacterial bioflocculants (10-50 ppm) were added to 49 ml of river water spiked with 1 ml of *E. coli* (OD of 1). The tubes were left to stand for 1 hr and the results were recorded at 30 min intervals. For alum, the procedure was carried out as described above but instead of bacterial bioflocculants, different concentrations of alum (10-50 ppm) were added. Two controls were included: for the positive control 49 ml of river water was spiked with 1 ml of *E. coli* but without the bacterial bioflocculants; the negative control had only river water without the bacterial bioflocculants and *E. coli*. The turbidity and the flocculating activity of river water were measured using a HACH 2100P turbidometer in NTU and spectrophotometer (LKB ultrospec II) (OD_{550nm}), respectively. The flocculating activity was expressed as the concentration of the flocculant in parts per million (ppm) when the OD_{550} was (1/10) x OD_{550c} of the control (Nakata and Kurane, 1999). The removal rate were determined according to the method of Kurane *et al.* (1994a, b) as follows:

$$\text{Removal } (\%) = \frac{B - A}{B} \times 100$$

Where: A is the experiment with the bioflocculants and B is the control without the bioflocculants.

5.2.3 Reduction of river water turbidity and microbial load

Different concentrations of bacterial bioflocculants (10-50 ppm) were added to 49 ml of river water. The tubes were left to stand for 2 hrs. The turbidity and the flocculating activity of river water was measured using a turbidometer in NTU and spectrophotometer (OD_{550nm}), respectively. The flocculating activity and percentage removal was determined as described in Section 5.2.2. The control was conducted without the addition of bacterial bioflocculants. Aliquots of 0.1 ml from each tube was plated out on a NA and incubated at 37^{o}C for 24 hrs. The number of colonies on NA plates were counted and compared to that of the control (refer to Appendix B).

5.2.4 Effect of pH on decreasing the microbial load

The pH of the river water was adjusted to 9 and was sterilised by autoclaving and allowed to cool before spiking. Two gram-positive and two gram-negative bacteria were used to spike the river water. The four bacterial cultures used in this experiment were *Streptococcus faecalis*, *Staphylococcus aureus*, *Klebsiella oxytoca,* and *E. coli*. Serial dilutions of bacterial cultures were first carried out to enumerate bacterial population. Different concentrations of bacterial bioflocculants (10-50 ppm) were added to 49.5 ml of river water spiked with 0.5 ml of 10^5 cfu/ml of bacterial cultures. The effects of alum on decreasing the microbial load was conducted as mentioned above, but instead of bacterial bioflocculants, alum (10-50 ppm) was added. Two controls were also included; for the positive control, 49.5 ml of river water was spiked with 0.5 ml of 10^5 cfu/ml of bacterial culture but without the addition of the bacterial bioflocculants. The negative control was conducted by the addition of 0.5 ml of distilled water to 49.5 ml of river water, also without the addition of bacterial bioflocculants and the culture. The flocculating activity and percentage removal was determined according to the method of Nakata and Kurane (1999) and Kurane *et al.* (1994a, b) as described in Section 5.2.2.

5.3 RESULTS

The results in this chapter are only for the experiment using 10 ppm (lowest concentration) and 50 ppm, which is the highest concentration of bacterial bioflocculants. However, data for the experiment using 20 and 30 ppm are included in Appendix B. These concentrations of bioflocculants were chosen in order to compare the reduction of turbidity and microbial load of river water at low and high concentrations.

5.3.1 Microbiological analysis of river water

The river water used in the current study was found to be contaminated with various Gram-positive and Gram-negative bacteria. The most predominant genera were *Staphylococci, Salmonella, Shigella, Bacillus, E. coli*, and *Proteus* species. The presence of *E. coli* in river water indicated that it was contaminated by faecal matter.

5.3.2 The reduction of river water turbidity by the bacterial bioflocculants and the flocculating activities

The effect of bacterial bioflocculants and alum on the turbidity of river water at 10 and 50 ppm is depicted in Fig. 5.1 - 5.2. Sixty eight percent of river water turbidity was removed by isolate A17 at 120 min. With an increase in the bioflocculant concentration (50 ppm), there was a further reduction in turbidity. There was 81% reduction by isolate A22 at 50 ppm. The reduction of river water turbidity upon the addition of alum was similar for isolates D1, E1, and A14 at 10 and 50 ppm. Figure 5.3 illustrates the flocculating activity of the bacterial bioflocculants at the different time intervals. Isolate D1 showed the best flocculating activity at 120 min while E1 showed the least. The flocculating activity of alum was similar to that of isolate A14.

The pH of the river water before and after the addition of bacterial bioflocculants and alum is shown in Fig. 5.4. Before the addition of the bacterial bioflocculants, the pH of the river water was 7.38, which is close to neutral. After the addition of the bacterial

bioflocculants, the pH ranged from 6.55-6.92, which is slightly acidic. The pH of river water dropped from 7.38 to 4.14 upon the addition of alum. Therefore, the pH shifted from neutral to acidic.

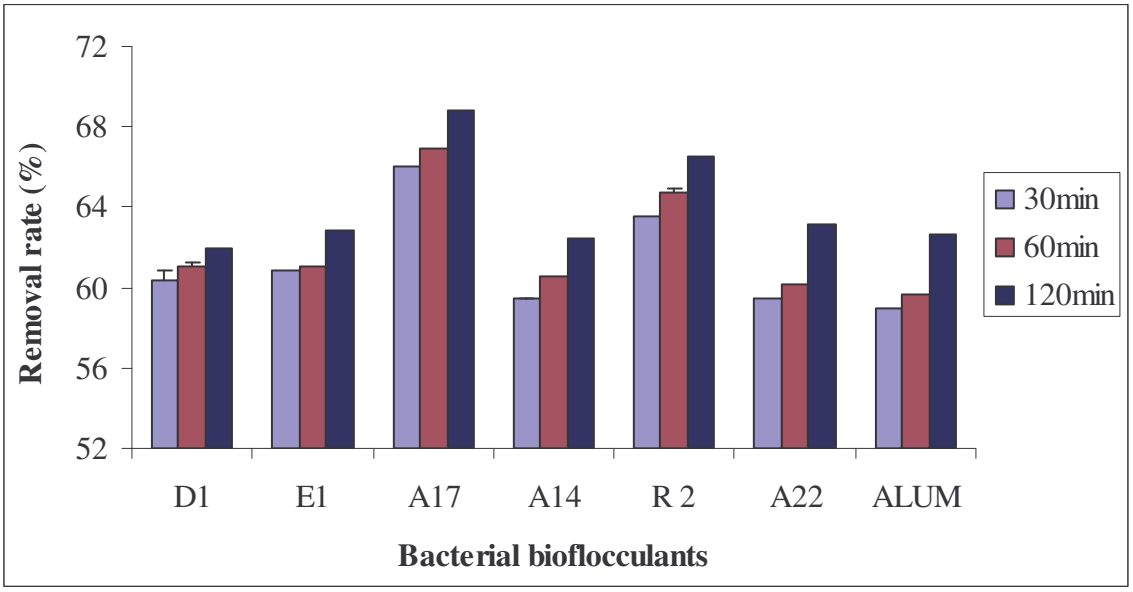

Fig. 5.1: Effect of bacterial biofflocculants and alum on the turbidity of river water at 10 ppm.

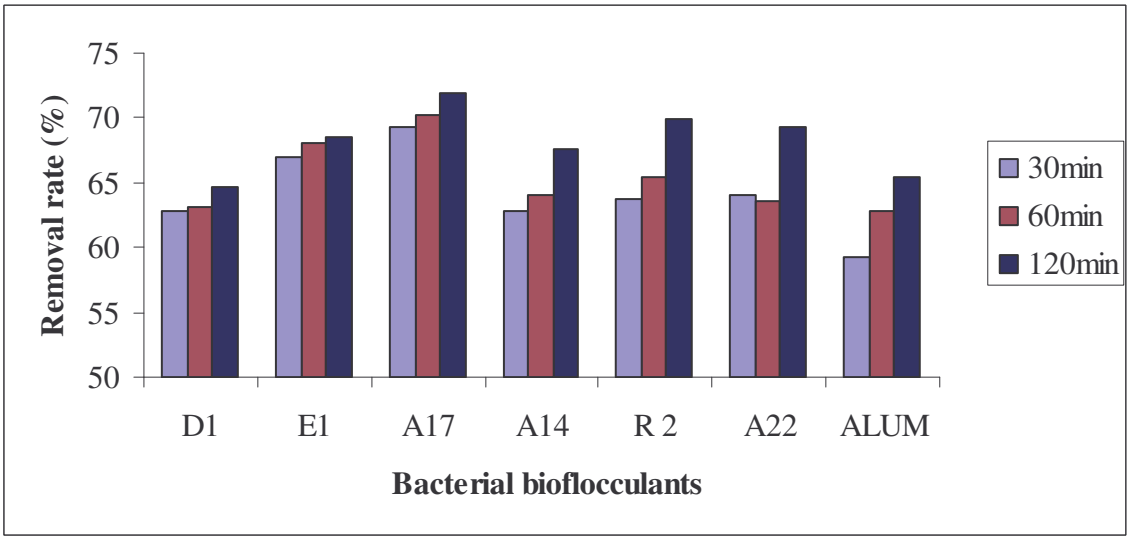

Fig. 5.2: Effect of bacterial biofflocculants and alum on the turbidity of river water at 50 ppm.

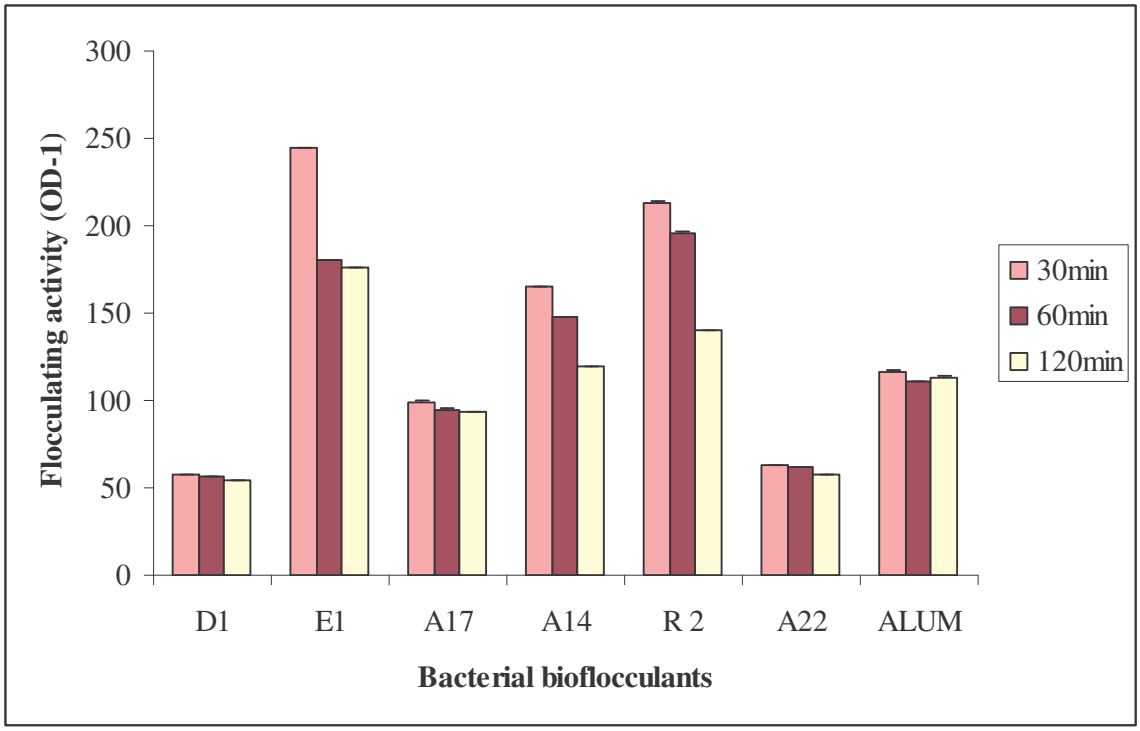

Fig. 5.3: Flocculating activity of the bacterial bioflocculants at different time intervals with *E. coli.*

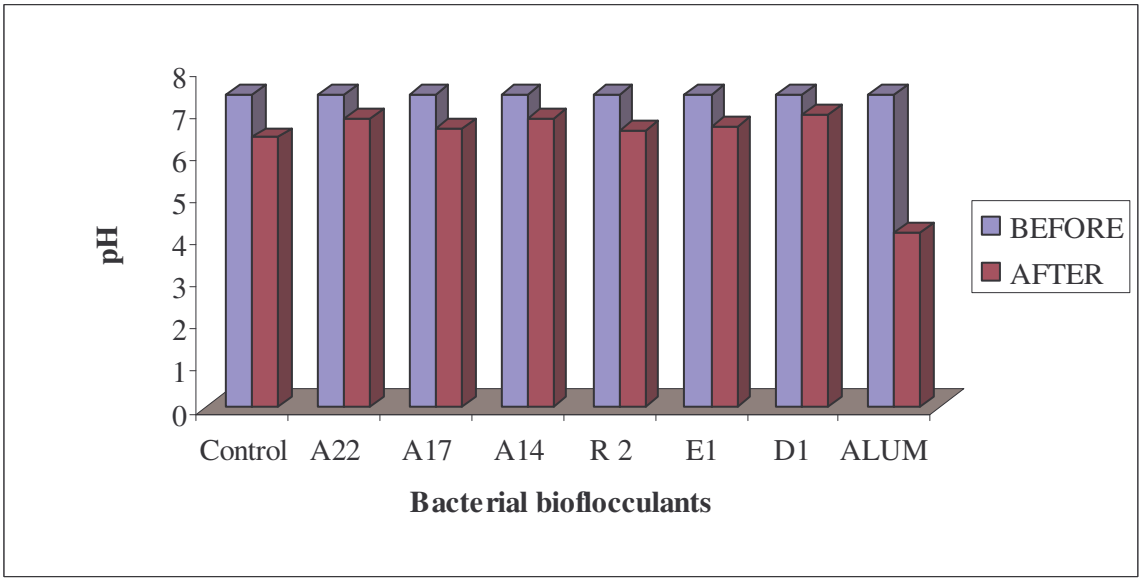

Fig. 5.4: pH of the river water before and after the addition of the bacterial bioflocculants and alum.

5.3.3 Effect of bioflocculants on river water turbidity and microbial load

Isolates A22 and D1 were very effective in reducing the river water turbidity at both low and high concentrations; up to 96% of the turbidity was reduced (Fig. 5.5). The reduction of river water turbidity by isolates E1, A17, A14, and alum were similar; up to 90% of the turbidity was reduced at 50 ppm. At 10 ppm the percentage removal was the least for isolate R2, but as the concentration of the bioflocculants increased, there was also an increase in the reduction of river water turbidity.

The flocculating activity of the bacterial bioflocculants at the different time intervals is depicted in Fig. 5.6. The best flocculating activity was achieved for isolate D1 followed by A22, R2, and A17 while alum and isolate E1 had the least activity. Figure 5.7 shows the reduction of the microbial load by the bioflocculants at different concentrations compared to alum. Up to 92% of the microbial load was reduced by the isolates D1, E1, A17, A22, and alum at 50 ppm. Isolates A14, and R2 reduced the microbial load by 87%.

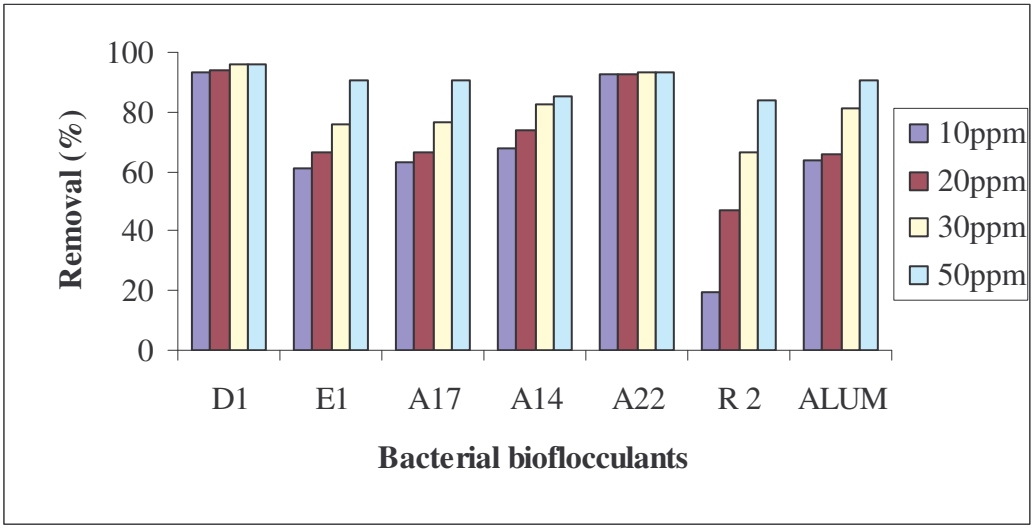

Fig. 5.5: Effect of bioflocculant concentration and alum on the turbidity of river water.

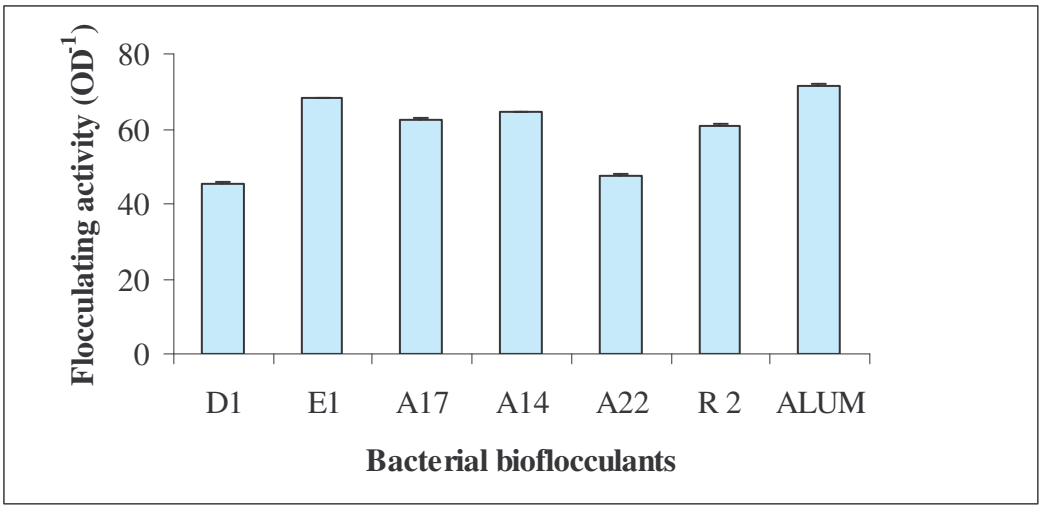

Fig. 5.6: Flocculating activity of bioflocculants compared to alum using river water.

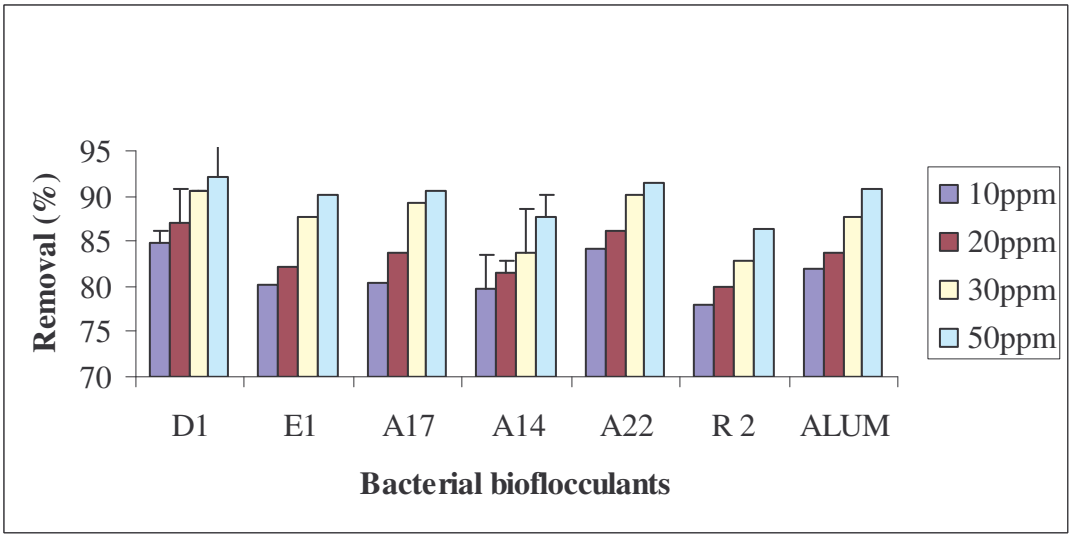

Fig. 5.7: Effect of bioflocculant concentration and alum on the microbial load in river water.

5.3.4 Effect of pH on decreasing the microbial load

Figure 5.8 illustrates the effect of pH on the reduction of river water turbidity by the bacterial bioflocculants at different concentrations compared to alum. Ninety-six percent of river water turbidity was reduced by isolate A22 at 50 ppm, while 95% of the river water turbidity was reduced by the isolates D1, E1, A17, A14, and alum at 50 ppm. The reduction of river water turbidity was directly proportional to the bioflocculant concentration. The best flocculating activity was achieved by isolate A14 followed by A22, E1, and alum, while for R2 was only 25 OD^{-1}. The flocculating activity of A17 and D1 ranged from 17-20 OD^{-1}, respectively (Fig. 5.9). The effect of pH on the reduction of the microbial load by the bioflocculants at different concentrations compared to alum is shown in Fig. 5.10. Ninety-eight percent of the microbial load was reduced by alum at 50 ppm whereas 96% was removed by isolates A14 and R2. There was a 95% reduction of the microbial load by isolate D1 whereas 94% was removed by isolates E1, A17, and A22 at 50 ppm.

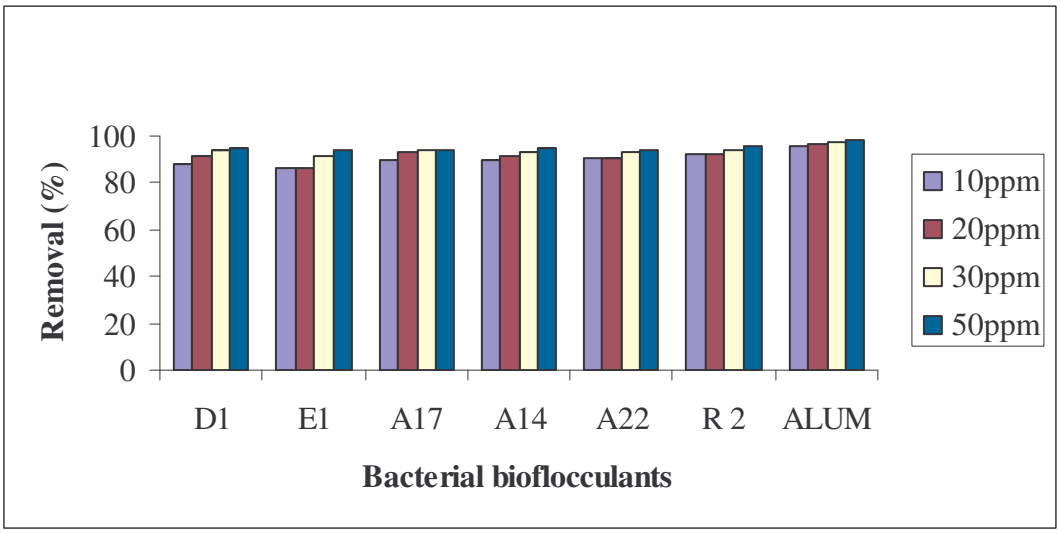

Fig. 5.8: The effect of pH on the reduction of river water turbidity by the bacterial bioflocculants at different concentrations compared to alum.

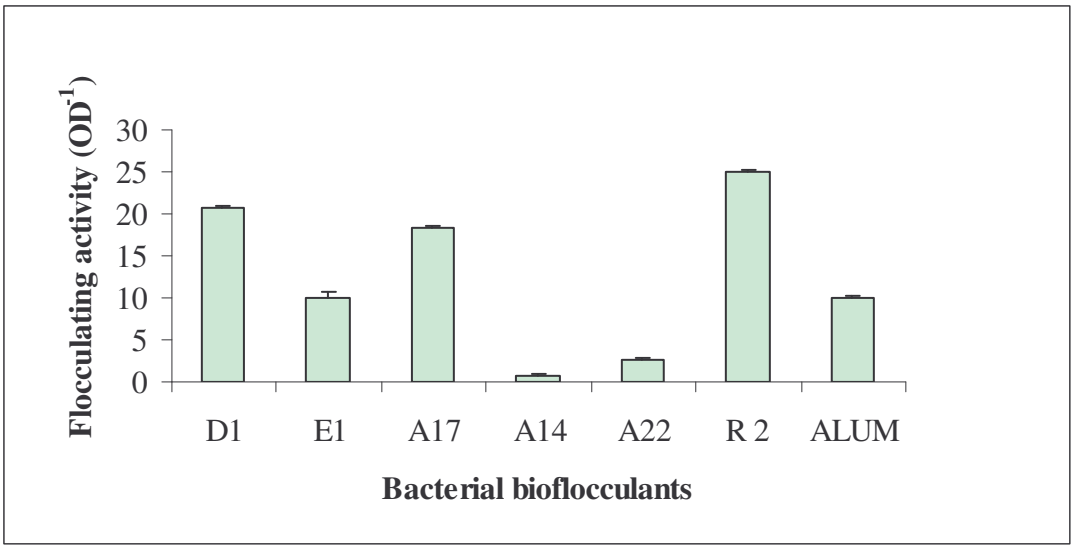

Fig. 5.9: The effect of pH on the flocculating activity of the bacterial bioflocculants compared to alum.

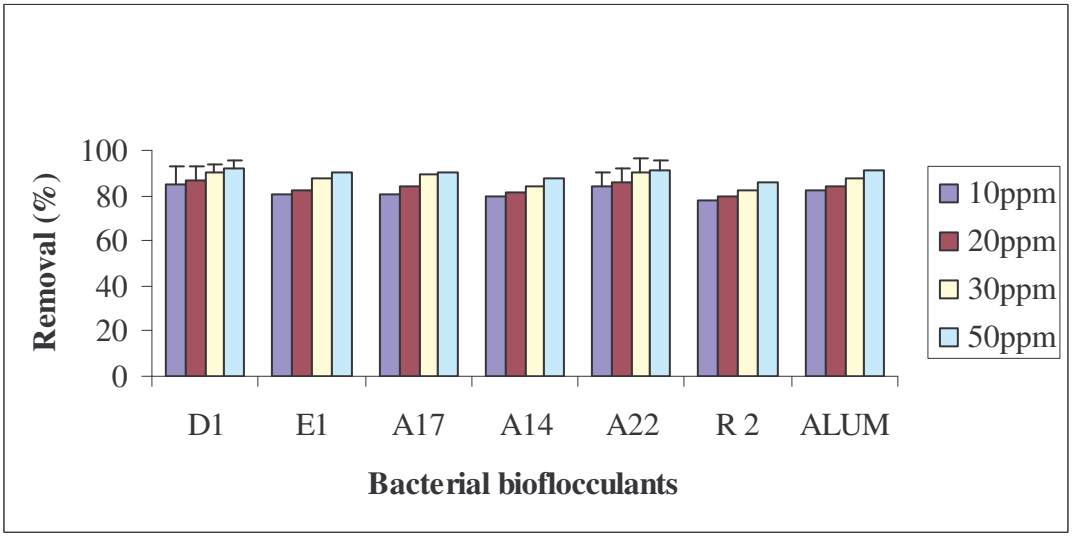

Fig. 5.10: The effect of pH on the reduction of the microbial load by the bioflocculants at different concentrations compared to alum.

5.3.5 Effect of bioflocculants on river water spiked with bacteria

The reduction of river water turbidity spiked with two gram-positive and two gram-negative bacteria by the bacterial bioflocculants compared to alum at different concentrations is depicted in Fig. 5.11 - 5.12. was by all The bacterial bioflocculants decreased the number of *K. oxytoca* at different concentrations followed by *E. coli*. The turbidity was reduced up to 100%. The same pattern was observed for the reduction of *S. faecalis* and *S. aureus* where there was up to 94% reduction of turbidity. The increase in concentration of the bacterial bioflocculants had no significant effect on the reduction of the river water turbidity when compared to alum. Alum, reduced river water turbidity the least at 10 ppm but with an increase in concentration of alum (50 ppm) there was an increase in the removal of river water turbidity. There was 85% reduction of *S. faecalis* followed by *K. oxytoca* and *S. aureus* where 75% and 65% were reduced, respectively on river water spiked with these bacteria. The turbidity of *E. coli* was reduced the least (45%) from spiked river water.

The flocculating activity of bacterial bioflocculants compared to alum is depicted in Fig. 5.13. The best flocculating activity was achieved by all the isolates when the river water was spiked with *S. faecalis*. When the river water was spiked with *E. coli,* the flocculating activity was very weak. The reduction of the microbial load by the bioflocculants at different concentrations compared to alum is shown in Fig. 5.14 - 5.15. The reduction of *S. faecalis* by isolates D1 and A14 was the least at 10 ppm, where there was only 20% reduction by D1 and 24% by isolate A14. With an increase in concentration of these bioflocculants, there was an increase in the removal of the microbial load. One hundred percent of the microbial load was removed by isolates A22, A17, R2, A14, E1, and alum at 50 ppm.

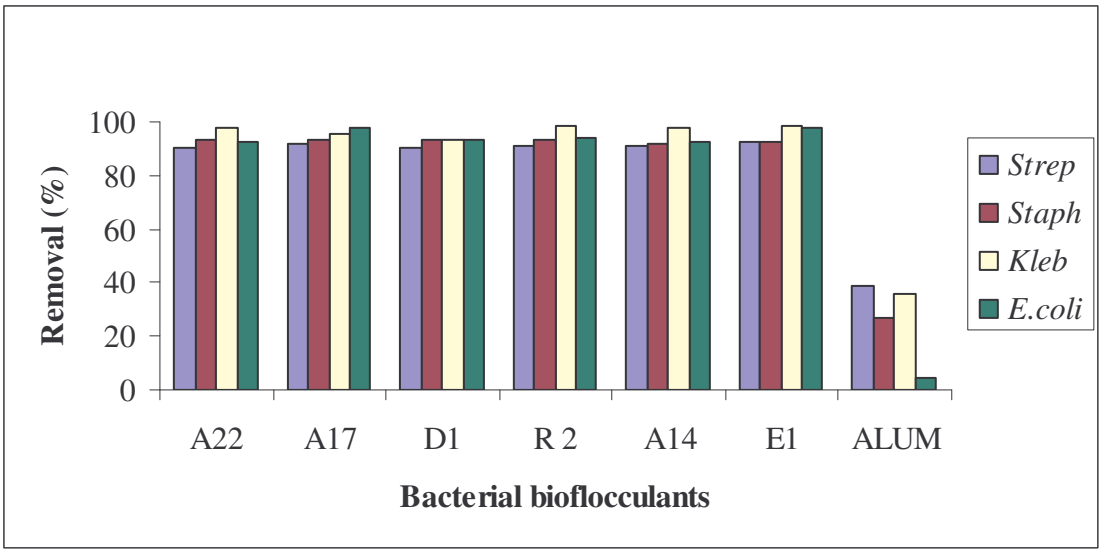

Fig. 5.11: The reduction of river water turbidity spiked with two Gram-positive and Gram negative bacteria by the bacterial bioflocculants compared to alum at 10 ppm (*Strep* = *S. faecalis*, *Staph* = *S. aureus, Kleb* = *K. oxytoca, E. coli* = *E. coli*).

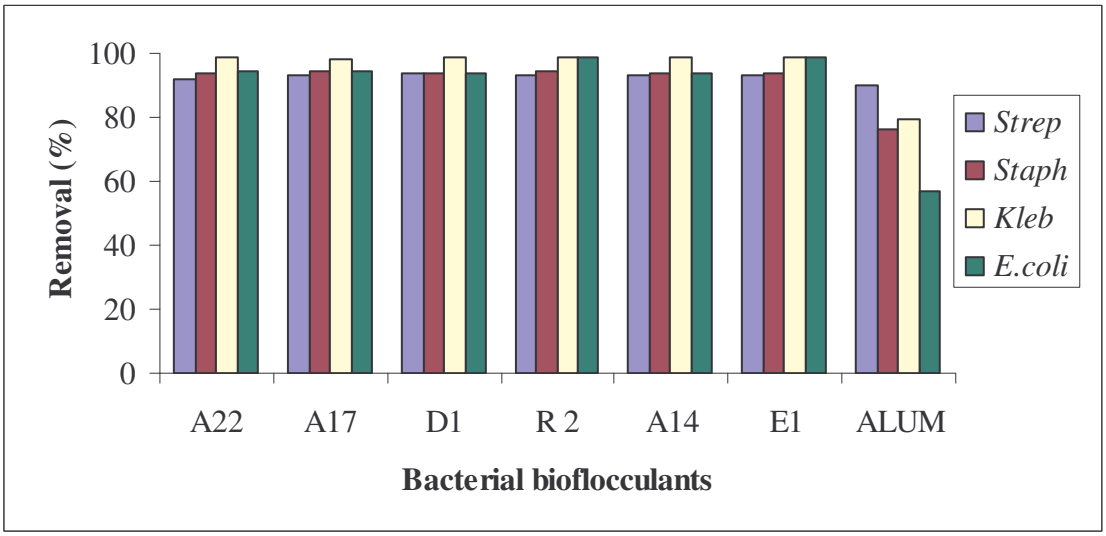

Fig. 5.12: The reduction of river water turbidity spiked with two Gram-positive and Gram-negative bacteria by the bacterial bioflocculants compared to alum at 50 ppm. (*Strep* = *S. faecalis*, *Staph* = *S. aureus, Kleb* = *K. oxytoca, E. coli* = *E. coli*).

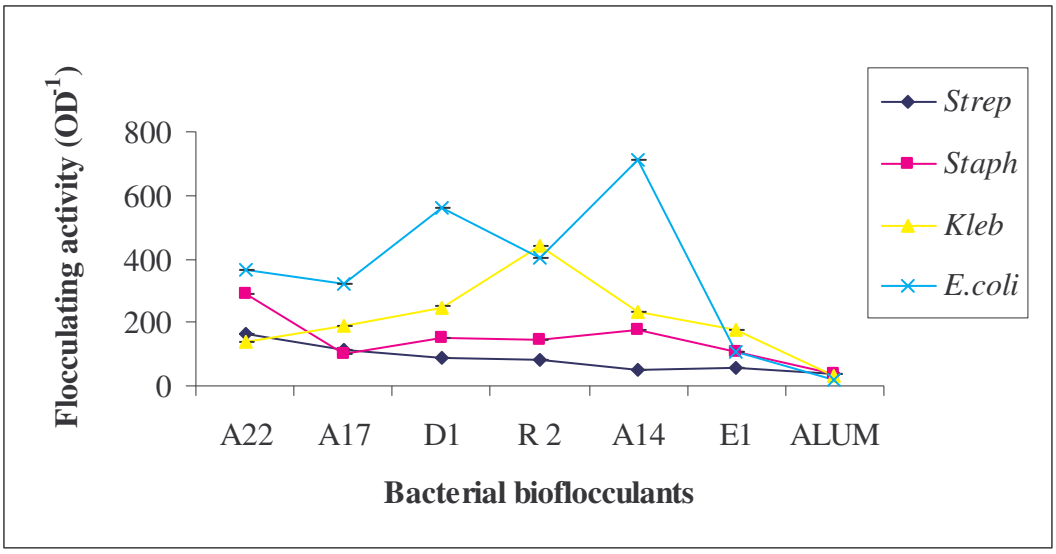

Fig. 5.13: Flocculating activity of bioflocculants compared to alum using river water spiked with two Gram-positive and Gram-negative bacteria. (*Strep* = *S. faecalis*, *Staph* = *S. aureus, Kleb* = *K. oxytoca, E. coli* = *E. coli*).

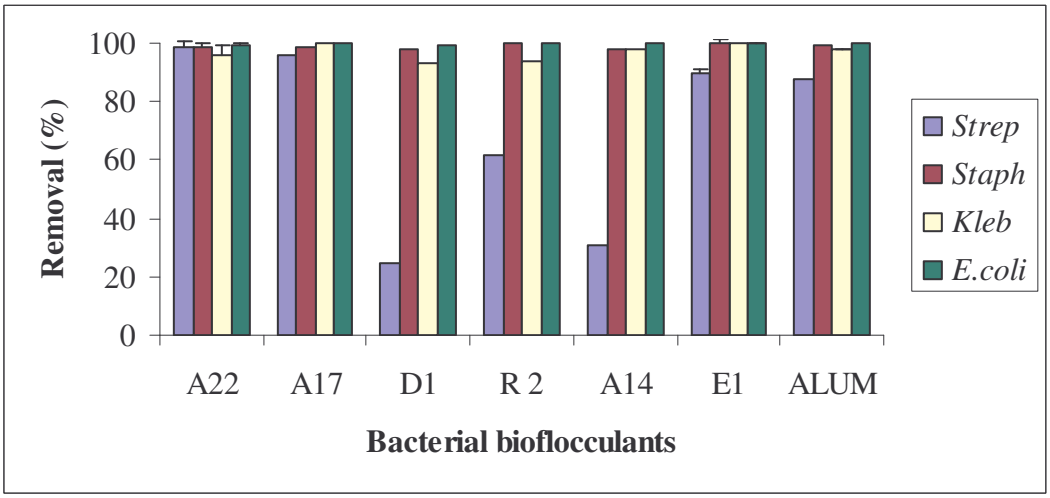

Fig. 5.14: The reduction of the microbial load by the bioflocculants at different concentrations compared to alum at 10 ppm. (*Strep* = *S. faecalis*, *Staph* = *S. aureus, Kleb* = *K. oxytoca, E. coli* = *E. coli*).

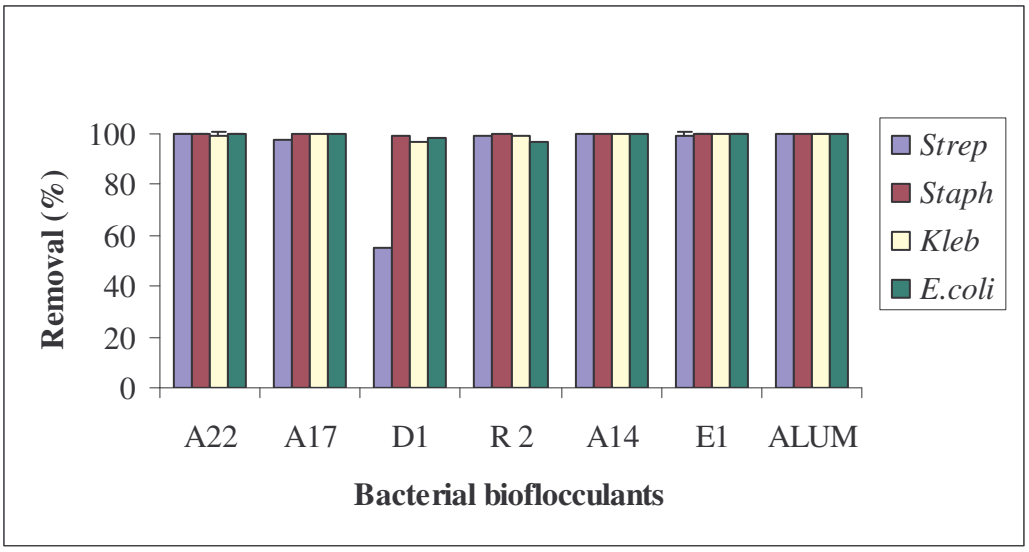

Fig. 5.15: The reduction of the microbial load by the bioflocculants at different concentrations compared to alum at 50 ppm. (*Strep* = *S. faecalis*, *Staph* = *S. aureus, Kleb* = *K. oxytoca, E. coli* = *E. coli*).

5.4 DISCUSSION

River water turbidity decreased with an increase in flocculant concentration of 50 ppm, and then remained constant with a further increase in flocculant level. This occurs because the optimum amount of flocculants in the suspension causes the microorganisms and the fine particles to aggregate and settle. However, when the optimum concentration of flocculant is exceeded it is known to cause the aggregated particles to redisperse and this disturbs particle-settling (Chan and Chiang, 1995). Mishra *et al*. (2004) explained that this behaviour could be based on an increase in the repulsive energy between the flocculants and the microorganisms, which causes hindrance in floc formation.

The pH of the river water prior to the addition of the bacterial bioflocculants and alum was neutral, with a subsequent decrease in pH upon the addition of bacterial bioflocculants and alum. The addition of alum caused the pH to drop to 4.14, which is more acidic, which means that in practical terms, further chemical addition is necessary

in order to correct the pH of the finished water to values between 6.5 and 8.5 (USEPA, 1991). Stumm and Morgan (1981) and Nordstrom and May (1989), reported that the reduction in pH is attributed to alum hydrolysis and production of H^+; alum dissociates and dissolved alum undergoes a series of hydrolysis reactions that result in the generation of acidity and a decrease in pH. The magnitude of the pH shift is related to the pH of the water and alum dosage. Coagulation/flocculation was improved by using coagulant aids such as alum. Alum helps form large flocs that settle out rapidly. Their concentration is an important parameter since excessive dosage can inhibit flocculation. Coagulation merely transfers pathogenic microorganisms from water to the flocculated material (Stumm and Morgan, 1981; Faust and Aly, 1998).

The pH of the water is probably the most significant factor affecting coagulation/flocculation. In this study the pH of river water was maintained at 9. Other factors include turbidity, temperature, and mixing regime. Coagulation is the most important process used in water treatment plants for clarification of coloured and turbid waters (Bitton, 1994). There was further reduction of river water turbidity as well as the microbial load at pH 9 compared to when the pH was not adjusted (Fig. 5.5 and 5.7). Turbid water contains, in addition to dissolved and settleable solids, colloids, which are electrically charged (Hammerton and Sherratt, 1972). Immediately after the addition of a flocculating agent such as alum to the water, reaction with the water and other ions occurs, resulting in the production of multi-positive hydroxo and polynuclear species of compounds. The coagulant species are rapidly adsorbed onto the surface of the turbidity particles, which ultimately become “coated” with coagulant. The electrostatic attraction between the negatively charged particle and the positively charged hydrolysis products enhances the deposition. The net result is that the electric charges on the particles are reduced. Depending on pH and coagulant dose, the charge on the particle may vary from slightly negative to neutral to slightly positive. The suspension becomes destabilized and the process of flocculation, where the particle can agglomerate to a settleable size, can precede unhindered (Hannah *et al*., 1967; Ndabigengesere and Narasiah, 1998).

Bacterial bioflocculants were very effective in reducing both the turbidity and the microbial load of river water. Different bacterial isolates used to spike river water were flocculated randomly by the bioflocculants (Fig. 5.11 – 5.15). In this study, there was a maximum removal of microbial load and turbidity of river water by both the bacterial bioflocculants and alum. Bitton (1994) indicated that removal of bacteria, although variable may exceed 90% during the flocculation process, furthermore, coagulation removes 74-99.4% of *E. coli* and coliforms. Kurane *et al.* (1986a) reported that the flocculant produced by *Rhodococcus erythropolis* could efficiently flocculate all suspended solids in aqueous solutions tested and had a wide flocculating activity against both organic and inorganic materials. Among those effectively tested were microrganisms such as *E. coli*, and alcohol yeast, activated sludge, *Microcystis aeruginosa* (AOKO), kaolin clay, muddy water, river dredging water, river bottom sediment (HEDORO), ash from a stream-power station and charcoal. Takagi and Kadowaki (1985) reported that the *Paecilomyces* flocculant also had the ability to flocculate all suspended solids from organic materials such as microorganisms to inorganic materials such as aluminium oxide and that *Paecilomyces* flocculant was a polysaccharide composed of galactosamine.

The application of bioflocculants for the reduction of microbial load and turbidity from river water was examined in comparison with a conventional coagulant agent, alum (Fig. 5.11 - 5.15). The results indicated that the bioflocculants were efficient for the reduction of these bacteria as well as the turbidity. Furthermore, the bioflocculants produced similar results as alum did, but at a slightly lower dosage. The use of alum produced the best results in the removal of the microbial load (Fig. 5.15). Higher concentrations of alum were required to reduce the river water turbidity compared to the use of the bacterial bioflocculants (Fig. 5.11 and 5.13). This can be explained by the production of aluminium hydroxide as a precipitate. In the case of bacterial bioflocculants, only initial suspended particles are agglomerated into larger and settleable flocs, but no additional precipitate is formed. Besides being voluminous, the alum sludges are gelatinous, acidic, and difficult to dewater and dispose in the environment (Degremont, 1989 – cited by Ndabigengesere and Narasiah, 1998). Faust and Aly (1998)

showed that alum was not effective in removing bacteria within the range of 5–10 mg/l. A removal of 99.7% was achieved with 50 mg/l when 14 nephelometric units (NTU) of turbidity was present.

Alum is a widely used coagulant in wastewater treatment. However, medical reports indicated that aluminum might induce Alzheimer's disease, while residual aluminum concentrations in treated water can also impose health problems apart from the production of high amounts of sludge (Letterman and Driscoll, 1988). The maximum contaminant level of aluminum in drinking water was set to 200 µg/l (Zouboulisa *et al*., 2004). Therefore, the use of high concentrations of alum in the treatment of river water must be avoided.

High turbidity and/or colour impart an aesthetically displeasing appearance to water. Apart from a displeasing appearance, turbidity provides adsorption sites for biological organisms and interferes with disinfection. Only a turbidity of 1 NTU is allowed in drinking water (Bitton, 1994). The river water used in this study is not suitable for human consumption because the turbidity was 11.7 NTU. Compared with USEPA Standards (1991), which state that the turbidity of drinking water should be less than 1 ntu, the value of 11.7 NTU is quite excessive. Although turbidity has no serious health effects, it can interfere with disinfection and provide a medium for microbial growth. Turbidity has been shown through scientific studies to be correlated with the contamination of water supplies (typically surface water supplies) with *Giardia* and *Cryptosporidium* (USEPA, 1991). Excessive turbidity is often associated with unacceptable tastes, odours, and colour in water and may represent a health concern where heavy metal ions, pesticides or waterborne disease causing organisms may attach to the suspended particles. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhoea, and associated headaches (Vigneswaran and Visvanathan, 1995).

It is anticipated that bacterial bioflocculants will be utilized in the areas of wastewater treatment. Due to their relative harmlessness towards humans and the

environment, they can also be used in drinking water, downstream processing, in food and fermentation industry. Using natural coagulants in developing countries could effectively alleviate their economic situation and allow further extension of water supply in rural areas. The application of bacterial bioflocculants in the treatment of river water is a promising alternative to using alum. To the best of our knowledge, this is the first study demonstrating the use of bacterial bioflocculants for removal of microorganisms from contaminated water and as such, it was difficult to compare the results with other publications. The removal of organic and inorganic matter from river water using bioflocculants is still in the research stages. However, more studies are required to develop practical applications.

CHAPTER SIX

CONCLUDING REMARKS

6.1 THE RESEARCH IN PERSPECTIVE

The main objectives of this study were to characterize the properties of the bioflocculants from bacteria, to evaluate the efficacy of the bioflocculant as an alternative to alum in decreasing the microbial load of river water, and to evaluate the ability of the bacterial bioflocculants to remove dyes and chemicals from the textile industries. Six bioflocculant producing bacteria were isolated from Northern Wastewater Treatment Plant. The bacterial isolates were identified and their bioflocculants were purified and analyzed. The bioflocculants were found to be composed of carbohydrates, proteins, uronic acid and amino sugars in varying quantities.

The bioflocculants produced by these bacteria were capable of flocculating kaolin clay, dyes and microbes in river water. The results of the experiments described here demonstrate that these flocculants could efficiently flocculate all suspended solids in aqueous solution and had a wide flocculating activity against both inorganic and organic materials. Flocculant concentration, temperature, pH, time, and cations or salts influenced flocculation by increasing or decreasing the flocculating activity and percentage removal.

The bioflocculants produced by these bacterial strains had a satisfactory level of flocculating activity. These results suggest that these bioflocculants can be successfully applied for the clarification of river water or wastewaters under various environmental conditions. These bioflocculants exhibited flocculating activity comparable or superior to that of existing inorganic flocculants (alum), where up to 99% microbial load and turbidity were reduced.

Coloured-dye-wastewater treatment and decolourization presents an arduous task. Wide ranges of pH, salt concentrations, and chemical structures often add to the

complication (Banat *et al*., 1996; Pinheiro *et al*., 2004). This study revealed that the bacterial isolates were capable of producing bioflocculants with very good flocculating activity. The bioflocculants were effective to varying degrees in removing the dyes in aqueous solution, in particular whale dye, medi-blue, fawn dye and mixed dyes, with a decolourization efficiency ranging between 20-99.9%. Thus, these bioflocculants may be used in the coagulation process instead of alum. However, more research still needs to be conducted to optimize the conditions for maximum flocculation. Among the most economically viable choices available for effluent treatment/decolourization, and the most practical in terms of labour requirements and running expenses to adopt and develop, appear to be the biological systems. At present, biological systems are known to be capable of dealing with BOD and COD reduction or removal through conventional aerobic biodegradation. They have however, an inherent problem in their inability to remove colour (Banat *et al*., 1996).

Although decolourization is a challenging process to both the textile industry and the wastewater-treatment facilities that must treat them, the literature suggests a great potential for microbial decolourizing systems for achieving total colour removal and (occasionally) with only a few hours of exposure. Such biological processes could be adopted as a pre-treatment decolourization step, combined with the conventional treatment system (e.g. activated sludge) to reduce the BOD and COD, as an effective alternative for use by the textile-dyeing industries (Pereira *et al*., 2003). Concerted efforts are still required to establish biological decolourization systems. The techniques by which decolourization occurs vary and among them adsorption seems of great significance for future development in bio-removal or bio-recovery of dye substances. It is important to develop a novel biodegradable and eco-friendly organic coagulant without secondary pollution for wastewater treatment. Unlike some synthetic flocculants, bioflocculants are generally non-toxic and benign to the environment. They have a potential to improve productivities and product quality in bioprocessing, wastewater treatment, and many other industrial operations (Sanghi *et al*., 2006). Bioflocculants can be produced relatively inexpensively from a variety of microorganisms. Microbial flocculation is a

promising alternative to replace or supplement present treatment processes for river water and the removal of very high concentrations of dyes.

6.2 POTENTIAL FOR FUTURE DEVELOPMENT OF THIS WORK

Undoubtedly, low-cost bacterial bioflocculants offer many promising benefits for commercial purposes in the future. However, despite a number of papers published on low-cost flocculants, there is little information containing a full study of comparison between flocculants. Although a lot has been accomplished in the area of flocculants, more work is necessary (i) to predict the performance of the adsorption processes for dye removal from industrial effluents under a range of operating conditions, (ii) to better understand the flocculation or adsorption mechanisms and (iii) to demonstrate the use of inexpensive flocculants at an industrial scale (Crini, 2006).

One of the routes still to be explored is the use of thermotolerant or thermophilic microorganisms in decolourization systems. This would be of advantage as many textile and other dye effluents are produced at relatively high temperatures (50-60°C), even after a cooling or heat-exchange step. The availability of thermotolerant organisms may consequently reduce cost significantly, through removing the need for further removal of low-grade heat and through allowing more immediate treatment (Banat *et al*., 1996).

Techniques used in studies of polysaccharides, including chemical composition, linkage pattern, and higher order structures are in constant development. They provide information necessary for understanding of the polysaccharide properties and functions. Recent advancements in studies of the polysaccharides at the single-molecule level are essential. These techniques can be used to investigate properties of single molecules close to physiological conditions. This field is expected to have increasing impact on the further advancement of the molecular understanding of the role of polysaccharides in various biological processes such as recognition and cell adhesion. Since the primary structure of polysaccharides is not coded directly in the genetic sequence, the advancement in primary structure determination has not evolved as rapidly within the

polysaccharide field as it has for proteins (Sletmoen *et al*., 2003). This, together with the fact that the experimental procedures for determination of the relative contents and arrangement of the monosaccharides are technically more demanding, makes the description of the polysaccharide primary structure not an easy task.

The development of sensitive detection methods will provide very useful information that will aid the structural elucidation of these extracellular polysaccharides. The use of accurate equipment such as Fourier transform infrared, X-ray photoelectron spectroscopy, and High performance liquid chromatography etc. in the future will aid in the analysis of the chemical compositions of the extracellular polysaccharides. The role of molecular biology has yet to feature prominently in this vital area of environmental protection (Shubo *et al*., 2005). More research on these areas mentioned will offer a better understanding of the function and properties of the bacterial bioflocculants.

REFERENCES

Aksu, Z. 2003. Reactive dye bioaccumulation by *Saccharomyces cerevisiae*. *Process Biochemistry.* **38:**1437-1444.

Aksu, Z. 2005. Application of biosorption for the removal of organic pollutants: a review. *Process Biochemistry*. **40:**997-1026.

Aksu, Z., and S. Tezer. 2000. Equilibrium and kinetic modelling of biosorption of Remazol Black B by *Rhizopus arrhizus* in a batch system: effect of temperature. *Process Biochemistry.* **36:**431-439.

Atherton, F., C. P.C. Newman, and D. P. Casemore.1995. An outbreak of waterborne cryptospondiosis associated with a public water supply in the UK. *Epidemiology of Infections.* **115:**123-131.

Banat, I M., P. Nigam, D. Singh, and R. Marchant. 1996. Microbial decolourization of textile-dye-containing effluents. *Bioresource Technology.* **58:**217-227.

Bar-Or, Y., and M. Shilo. 1987. Characterization of macromolecular flocculants produced by *Phormidium* sp. J-1 and by *Anabaenopsis circularis* PCC 6720. *Applied and Environmental Microbiology.* **53:**2226-2230.

Baughman, G. L., and T. A. Perenich. 1988. Fate of dyes in aquatic systems: I. Solubility and partitioning of some hydrophobic dyes and related compounds. *Environmental Toxicological Chemistry.* **7:**183-199.

Bender, J., E. R. Archibold, V. Ibeanusi, and J. P. Gould. 1989. Lead removal from contaminated water by a mixed microbial ecosystem. *Water Science Technology.* **21:**1661-1665.

Bhattacharyya, K. G., and A. Sarma. 2003. Adsorption characteristics of the dye, brilliant green, on neem leaf powder. *Dyes Pigments.* **57:**211-222.

Bitton, G. 1994. Wastewater microbiology, p. 265-266. *In* R. Mitchell (ed.), Division of applied sciences. Harvard University. John Wiley and Sons Ltd., New York.

Black, A. P., F. B. Birkner, and J. J. Morgan. 1966. The effect of polymer adsorption on the electrokinetic stability of dilute clay suspension. *Journal of Colloidal and Interface Science.* **21:**626.

Bragger, J. L., A. W. Lloyd, S. H. Soozandehfar, S. F. Bloomfield, C. Marriott, and G. P. Martin. 1997. Investigations into the azo reducing activity of a common colonic microorganism. *International Journal of Pharmaceutics.* **157:**61-71.

Bras, R., I. A. Ferra, H. M. Pinheiro, and I. C. Goncalves. 2001. Batch tests for assessing decolourisation of azo dyes by methanogenic and mixed cultures. *Journal of Biotechnology.* **89:**155-162.

Brugha, A., I. B. Vipond, and M. R. Evans. 1999. A community outbreak of food borne small round-structured virus gastroenteritis caused by a contaminated water supply. *Epidemiology of Infections.* **122:**145-154.

Brosius, J., J. L. Palmer, H. P. Kennedy, and H. F. Nuller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proceedings of the National Academy of Sciences.* **75**: 4801-4805.

Buchhammer, H. M., M. Oelmann, and G. Petzold. 2001. Flocculation of disperse dyes in effluents with polyelectrolyte complexes. *Melliand English*. **82:** 104-105.

Butterfield, C. T. 1935. A *Zoogloea*-forming bacterium isolated from activated sludge, studies of sewage purification (II). *Public Health Report.* **50:**671-681.

Chan, W. C., and C. Y. Chiang. 1995. Flocculation of clay suspensions with water-insoluble starch grafting acrylamide/sodium allylsulfonated copolymer powder. *Applied Polymer Science*. **58:**1721-1726.

Chandrasekhar, S., and S. Ramaswamy. 2006. Iron minerals and their influence on the optical properties of two Indian kaolins. *Applied Clay Science*. **33:**269-277.

Chang, J. S., C. Chou, Y. C. Lin, P. J. Lin, J. Y. Ho, and T. L. Hu. 2001. Kinetic characteristics of bacterial azo-dye decolourization by *Pseudomonas luteola. Water Research.* **35:**2841-2850.

Chang, J. S., and Y. C. Lin. 2000. Fed-batch bioreactor strategies for microbial decolourization of azo dye using a *Pseudomonas luteola* strain. *Biotechnology Progress.* **16:**979–85.

Chaplin, M. F. 1994. Monosaccharides, p. 2-6. *In* M. F. Chaplin & J. F. Kennedy (ed.), Carbohydrate analysis: a practical approach, Oxford University Press, New York.

Cheng, X., and L. A. Kaplan. 2003. Simultaneous analyses of neutral carbohydrates and amino sugars in freshwaters with HPLC-PAD. *Journal of Chromatographic Science*. **41:**1-5.

Choy, J. H., W. S. Shin, S. H. Lee, D. J. Joo, and J. D. Lee. 2001. Application of synthetic polyamine flocculants for dye wastewater treatment. *Science and Technology*. **36(13):**2945–2958.

Chu, H.C., and K. M. Chen. 2002. Reuse of activated sludge biomass: removal of basic dyes from wastewater by biomass. *Process Biochemistry.* **37:**595-600.

Clarke, E. A., and R. Anliker. 1980. Organic dyes and pigments, p. 181-215. *In* O. Hutzinger (ed.), Handbook of environmental chemistry, anthropogenic compounds, vol. 3, part A. Springer-Verlag, New York.

Cohen, J. M., G. A. Rourke, and R. L. Woodward. 1972. Natural and synthetic polyelectrolyte as coagulant aids. *Journal of Agriculture of Western Australia.* **50:**463-471.

Cowan, R. M., N. G. Love, S. M. Sock, and K. White. 1995. Activated sludge and other aerobic suspended culture processes. *Water Environment Resource.* **67:**433-450.

Cowan, M. K., and K. P. Talaro. 2006. Microbiology: A systems approach 1st ed., p. 785-786. The McGraw-Hill, Inc., New York.

Crini, G. 2006. Non-conventional low-cost adsorbents for dye removal. *Bioresource Technology*. **97:**1061-1085.

Crittenden, J. C., R. P. S. Suri, D. L. Perram, and D. W. Hand. 1997. Decontamination of water using adsorption and photocatalysis. *Water Resource*. **31:**411-418.

Dabrowski, A. 2001. Adsorption, from theory to practice. *Advances in Colloid Interface Science.* **93:**135-224.

Deng, S. B., R. B. Bai, X. M. Hu, and Q. Luo. 2003. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch and wastewater treatment. *Applied Microbiology and Biotechnology.* **60:**588-593.

Decker, K. C., and B. W. Long. 1992. Canada's cooperative approach to drinking water regulations. *Journal of Wastewater Analysis*. **84:**120-127.

Dermlim, W., P. Prasertsan, and H. Doelle. 1999. Screening and characterization of bioflocculant produced by isolated *Klebsiella* sp. *Applied Microbiology and Biotechnology*. **52:**698-703.

De Vuyst, L., F. De Vin, F. Vaningelgem, and B. Degeest. 2001. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal* **11:** 687-707.

Dhodapkar, R., N. N. Rao, S. P. Pande, and S. N. Kaul. 2006. Removal of basic dyes from aqueous medium a novel polymer: Jalshakti. *Bioresource Technology*. **97:**877-885

Dillert, R., S. Vollmer, M. Schober, J. Theurieh, D. Bahnemann, H. J. Amtz, K. Pahlmann, and J. Wienefeld. 1999. Photocatalytic treatment of an industrial wastewater in the double-skin sheet reactor. *Chemical Engineering and Technology*. **22:**931-934.

DiTerlizzi, S., and B. Fall. 1994. Coagulation and flocculation of wastewater, p. 414-415. John Wiley and Sons Ltd., New York.

Dönmez, G. 2002. Bioaccumulation of the reactive textile dyes by *Candida tropicalis* growing in molasses medium. *Enzyme and Microbial Technology.* **30:**363-366.

Dos Santos, A. B., F. J. Cervantes, and J. B. van Lier. 2007. Current technologies for decolourization of textile wastewaters: perspectives for anaerobic biotechnology. *Bioresource Technology.* doi:10.1016/j.biortech.2006.11.013.

Dos Santos, A. B., M. P. Madrid, F. A. M. de Bok, A. J. M. Stams, J. B. Van Lier, and F. J. Cervantes. 2006. The contribution of fermentative bacteria and methanogenic archaea to azo dye reduction by thermophilic anaerobic consortium. *Enzyme and Microbial Technology*. **39:**38-46.

Dube, J. 1992. Microorganisms as chemical reagents. *Minerals Engineering.* **5:**547-556.

Dudman, W. F. 1977. The role of surface polysaccharides in natural environments, p. 357-414. *In* I. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press Inc., New York.

Egorov, A. I., A. A. Tereschenko, L. M. Altshul, T. Vartiainen, D. Samsonov, B. LaBrecque, J. Mäki-Paakkanen, N. L. Drizhd, and T. E. Ford. 2003. Exposures to drinking water chlorinated by-product in a Russian city. *International Journal of Hygiene and Environmental Health.* **206:**539-551.

El-Geundi, M. S. 1991. Colour removal from textile effluents by adsorption techniques. *Water Resource.* **25:**271-273.

Elwood, D. C., and D. W. Tempest. 1972. Effect of environment on bacterial wall content and composition. *Advances in Microbial Physiology.* **7:**83-117.

Eriksson, L., and B. Alm. 1991. Study of flocculation mechanisms by observing effects of a complexing agent on activated sludge properties. *Water Science and Technology.* **24:**21-25.

Faust, S. D., and O. M. Aly. 1998. Chemistry of water treatment 2nd ed., p. 581 CRC Press LLC, Lewis Publishers, New York.

Fazio, S. A., D. J. Uhlinger, J. H. Parker, and D. C. White. 1982. Estimations of uronic acids as quantitative measures of extracellular and cell wall polysaccharide polymers from environmental samples. *Applied and Environmental Microbiology.* **43:**1151-1159.

Fewson, C. A. 1988. Biodegradation of xenobiotic and other persistent compounds: the causes of recalcitrance. *Trends in Biotechnology.* **6:**148-153.

Ford, T. E. 1993. Aquatic microbiology: an ecological approach, p. 9-13. *In* T. E. Ford (ed.), Combridge, Mass Blackwell Scientific publications, Inc., New York.

Foster, C. F. 1983. Bound water in sewage sludges and its relationship to sludge surfaces and sludge viscosities. *Journal of Chemical Technology and Biotechnology.* **33:**76-80.

Fu, Y., and T. Viraraghavan. 2001. Fungal decolourization of wastewaters. *Bioresource Technology.* **79:**251-262.

Fu, Y., and T. Viraraghavan. 2002. Removal of Congo red from an aqueous solution by fungus *Aspergillus niger*. *Advances in Environmental Resource.* **7:**239-247.

Fumio, Y. 1991. Flocculation of yeast cells by *Lactobacillus fermentum*. *Resource Microbiology.* **22:**12-16.

Fujita, M., M. Ike, S. Tachibana, G. Kitada, S. M. Kim, and Z. Inoue. 2000. Characterization of a bioflocculant produced by *Citrobacter* sp. TKF04 from acetic and propionic acids. *Journal of Bioscience and Bioengineering*. **89:**40-46.

Garrity, G M. 2005. Bergey's Manual of Systematic Bacteriology (Vol. 2, Part A,B,C), p. 35-127. Springer, New York.

Ghoreishi, S. M., and R. Haghighi. 2003. Chemical catalytic reaction and biological oxidation for treatment of non-biodegradable textile effluent. *Journal of Chemical Engineering.* **95:**163-169.

Gingell, R., and R. Walker. 1971. Mechanisms of azo reduction by *Streptococcus faecalis*: the role of soluble flavins. *Xenobiotica.* **1:**231-239.

Glenn, J. K., and M. H. Gold. 1983. Decolourization of several polymeric dyes by the lignin degrading basidiomycete, *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology.* **45:**1741-1747.

Guirand, J. 1992. Isolation and characterization of a flocculating mutant of *Saccharomyces diastaticus*. *Resource Microbiology.* **1433:**81-91.

Gupta, G. S., G. Prasad, and V. H. Singh. 1990. Removal of chrome dye from aqueous solutions by mixed adsorbents: fly ash and coal. *Water Resource.* **24:**45-50.

Hammerton, C., and J. G. Sherratt. 1972. Analysis of raw, potable and wastewater, p. 304. Great Britain, Department of the Environment, London. H.M.S.O.

Hannah, S. A., J. M. Cohen, and G. C. Robeck. 1967. Control techniques for coagulation-filtration. *Journal of Agriculture of Western Australia.* **59:**1149-1152.

Hantula, J., and D. H. Bamford. 1991a. The efficiency of the protein dependent flocculation of *Flavobacterium* sp. *Applied Microbiology and Biotechnology.* **36:**100-104.

Hantula, J., and D. H. Bamford. 1991b. Bacterial phage resistance and flocculation deficiency of *Flavobacterium* sp. are phenotypically interrelated. *Applied Microbiology and Biotechnology.* **36:**105-108.

Higgins, M. J. 1995. The roles and interactions of metal salts, proteins, and polysaccharides in the settling and dewatering of activated sludge. Ph.D. thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Hitz, H. R., W. Huber, and R. H. Reed. 1978. The absorption of dyes on activated sludge. *Journal of the Society of Dyers and Colourists.* **94:**71-76.

Ho, Y. S., and G. McKay. 1999. Comparative sorption kinetic studies of dye and aromatic compounds onto fly ash. *Journal of Environmental Science Health.* **34:**1179-1204.

http://web.deu.edu.tr/atiksu/toprak/summary4.html

http://www.usgcrp.gov/usgcrp/images/ocp2003/WaterCycle-optimized.jpg

Huang, L. 1990. Studies on culture condition for production of flocculant by bacterial strain S-4K. *International Journal of Mineral Processing*. **129:**11-19.

Huang, C., and H. Shui. 1996. Interaction between alum and organics in coagulation, p.113-163. John Wiley and Sons Ltd., New York.

Hu, T. L. 1992. Sorption of reactive dyes by *Aeromonas* biomass. *Water Science Technology*. **26:**357-366.

Hu, Y., and X. Liu. 2003. Chemical composition and surface property of kaolins. *Minerals Engineering*. **16:**1279-1284.

Hunter, P. R., and C. Quigley. 1998. Investigation of an outbreak of cryptospondiosis associated with treated surface water finds limits to the value of case control studies. *Communicable disease and public health*. **1(4):**234-238.

Jansson, P. E., J. Lindberg, K. M. Wimalsiri, and M. A. Dankert. 1993. Structural studies of acetan, an exopolysaccharide elaborated by *Acetobacter xylinum. Carbohydrate Resource.* **245:**303-310.

Jin, Y., and A. Speers. 1998. Flocculation of *Saccharomyces cerevisiae*. *Food Research International.* **31:**421-440.

Jones, M. N. 1998. Colloidal properties of humic substances. *Advances in Colloid Interface Science.* **78:**1-48.

Johnson, S. B., G. V. Franks, P. J. Scales, D. V. Boger, and T. W. Healy. 2000. Surface chemistry–rheology relationships in concentrated mineral suspensions. *International Journal of Mineral Processing*. **58:**267-304.

Juang, R. S., R. L. Tseng, F. C. Wu, and S. H. Lee. 1997. Adsorption behaviour of reactive dyes from aqueous solutions on chitosan. *Journal of Chemistry, Technology and Biotechnology.* **70:**391-399.

Kakii, K., M. Hasumi, T. Shirakashi, and M. Kuriyama. 1990. Involvement of Ca^{2+} in the flocculation of *Kluyvera cryocrescens* KA-103. *Journal of Fermentation and Bioengineering*. **69:**224-227.

Kapdan, K., I. Kargi, G. McMullan, and R. Marchant. 2000. Effect of environmental conditions on biological decolourization of textile dyestuff by *Coriolus versicolor*. *Enzyme and Microbial Technology.* **26:**381-387.

Karcher, S., A. Kornmuller, and M. Jekel. 1999. Removal of reactive dyes by sorption/complexation with cucurbituril. *Water Science Technology.* **40:**425-433.

Keck, A., J. Klein, M. Kudlich, A. Stolz, H. J. Knackmuss, and R. Mattes. 1997. Reduction of azo dyes by redox mediators originating in the naphthalenesulfonic acid degradation pathway of *Sphingomonas* sp. strain BN6. *Applied and Environmental Microbiology*. **63:**3684-3690.

Khan, A. A., and Q. Husain. 2007. Decolorization and removal of textile and non-textile dyes from polluted wastewater and dyeing effluent by using potato (Solanum tuberosum) soluble and immobilized polyphenol oxidase. *Bioresource Technology*. **98:**1012-1019.

Kim, Y. 1993. The production and properties of exo-polysaccharides (POL-IIs) by *Bacillus* sp. LK-1. *Korean Journal of Applied Microbiology and Bioengineering*. **21:**478-485.

Kiss, J. 1974. Beta-elimination degradations of carbohydrates containing uronic acid residues. *Advances in Carbohydrates, Chemistry and Biochemistry*. **29:**229-303.

Knackmuss, H. J. 1996. Basic knowledge and perspectives of bioelimination of xenobiotic compounds. *Journal of Biotechnology*. **51:**287-295.

Knapp, J. S., and P. S. Newby. 1999. The decolourization of a chemical industry effluent by white rot fungi. *Water Resource.* **33:**575-577.

Konan, K. L., C. Peyratout, J. Bonnet, A. Smith, A. Jacquet, P. Magnoux, and P. Ayrault. 2006. Surface properties of kaolin and illite suspensions in concentrated calcium hydroxide medium. *Journal of Colloid and Interface Science.* doi:10.1016/j.jcis.2006.10.085

Kudlich, M., A. Keck, J. Klein, and A. Stolz. 1997. Localization of the enzyme system involved in anaerobic reduction of azo dyes by *Sphingomonas* sp. strain BN6 and effect of artificial redox mediators on the rate of azo dye reduction. *Applied and Environmental Microbiology.* **63:**3691-3694.

Kuhn, I., G. Allestam, M. Engdahl, and A. A. Stenstrom. 1997. Biochemical fingerprinting of coliform bacterial populations comparisons between polluted river water and factory effluents. *Water Science and Technology*. **35:**343-350.

Kurane, R., K. Hatamochi, T. Kakuno, M. Kiyohara, K. Kawaguchi, Y. Mizuno, M. Hirano, and Y. Taniguchi. 1994a. Production of a bioflocculant by *Rhodococcus erythropolis* S-l grown on alcohols. *Bioscience, Biotechnology and Biochemistry.* **58:**428-429.

Kurane, R., K. Hatamochi, T. Kakuno, M. Kiyohara, K. Kawaguchi, Y. Mizuno, M. Hirano, and Y. Taniguchi. 1994b. Purification and characterization of lipid bioflocculant produced *Rhodococcus erythropolis. Bioscience, Biotechnology and Biochemistry.* **58:**1977-1982.

Kurane, R., K. Hatamochi, T. Kakuno, M. Kiyohara, T. Tajima, M. Hirano, and Y. Taniguchi. 1995. Chemical structure of lipid bioflocculant produced by *Rhodococcus erythropolis*. *Journal of Agricultural and Biological Chemistry.* **59:**1652-1656.

Kurane, R., S. Hatakeyama, and H. Tsugeno. 1991 Correlation between flocculation production and morphological changes in *Rhodococcus erythropolis* S-1. *Journal of Fermention and Bioengineering.* **72:**498-500.

Kurane, R., and H. Matsuyama. 1994. Production of bioflocculant by mixed culture. *Bioscience, Biotechnology and Biochemistry.* **58:**1589-1594.

Kurane, R., and Y. Nohata. 1991. Microbial flocculation of waste liquids and oil emulsion by a bioflocculant from *Alcaligenes latus*. *Journal of Agricultural and Biological Chemistry.* **56:**1127-1129.

Kurane, R., K. Toeda, K. Takeda, and T. Suzuki. 1986a. Screening for and characteristics of microbial flocculants. *Journal of Agricultural and Biological Chemistry.* **50(9):**2301-2307.

Kurane, R., K. Toeda, K. Takeda, and T. Suzuki. 1986b. Culture conditions for production of microbial flocculant by *Rhodococcus erythropolis*. *Journal of Agricultural and Biological Chemistry.* **50:**2309-2313.

La-Mer, V. K., and T. W. Healy. 1963. Adsorption-flocculation reactions of macromolecules at the solid-liquid interface. *Reviews in Pure and Applied Chemistry* **13:**112.

Lambert, S. D., N. J. D. Graham, C. J. Sollars, and G. D. Fowler. 1997. Evaluation of inorganic adsorbents for the removal of problematic textile dyes and pesticides. *Water Science Technology.* **36:**173-180.

Lee, S. H., S. O. Lee, K. L. Jang, and T. H. Lee. 1995. Microbial flocculant from *Arcuadendron* sp. TS-49. *Biotechnology Letters.* **17:**95-199.

Lee, C. K., K. S. Low, and P. Y. Gan. 1999. Removal of some organic dyes by acid-treated spent bleaching earth. *Process Biochemistry.* **34:**45-465.

Lerch, A., S. Panglisch, P. Buchta, Y. Tomita, H. Yonekawa, K. Hattori, and R. Gimbel. 2005. Direct river water treatment using coagulation/ceramic membrane microfiltration. *Desalination*. **179:**41-50.

Letterman, R. D, and C. T. Driscoll. 1988. Survey of residual aluminum in filtered water. *Journal of Water Works Association*. **80:**154-158.

Lian, B., Y. Chen, J. Zhao, H. H. Teng, L. Zhu, and S. Yuan. 2007. Microbial flocculation by *Bacillus mucilaginosus*: applications and mechanisms. *Bioresource technology*. doi:10.1016/j.biortech.2007.09.045.

Li, J., M. Li, J. Li, and H. Sun. 2007. Decolourization of azo dye direct scarlet 4BS solution using exfoliated graphite under ultrasonic irradiation. *Ultrasonics Sonochemistry*. **14:**241-245.

Lopez, E., I. Ramos, and M. A. Sanroman. 2003. Extracellular polysaccharides production by *Arthrobacter viscosus*. *Journal of Food Engineering*. **60:**463-467.

Lourenco, N. D, J. M. Novais, and H. M. Pinheiro. 2000. Reactive textile dye colour removal in a sequencing batch reactor. *Water Science and Technology.* **42:**321-328.

Low, K. S., and C. K. Lee. 1997. Quaternized rice husk as sorbent for reactive dyes. *Bioresource Technology.* **61:**121-125.

Manu, B., and S. Chaudhari. 2001. Anaerobic decolourization of simulated textile wastewater containing azo dyes. *Bioresource Technology.* **82:**225-231.

Manero, A., and A. R. Blanch. 1999. Identification of *Enterococcus* sp. with a biochemical key. *Applied and Environmental Microbiology*. **65:**4425-4430.

Marchesi, J. R., T. Sato, A. J. Weightman, T. A. Martin, J. C. Fry, S. J. Hiom, D. Dymock, and W. G. Wade. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for 16S rRNA. *Applied and Environmental Microbiology.* **64**: 795-799.

Marco, A., S. Esplugas, and G. Saum. 1997. How and why to combine chemical and biological processes for wastewater treatment. *Water Science Technology.* **35:**231-327.

McCurdy, M. W., G. D. Boardman, D. L. Michelsen, and B. M. Woodby. 1992. Chemical reduction and oxidation combined with biodegradation for the treatment of a textile dye, p. 229-234. *In* 46th Proc. Purdue Industrial Waste Conf. Lewis Publishers, Florida.

McKinney, **R. E.** 1956. Biological flocculation. *Biological treatment of sewage and industrial wastes*. **1:**88-117.

McKinney, R. E. 1957. Activity of microorganisms in organic waste disposal aerobic processes. *Applied Microbiology.* **5:**167-174.

McMullan, G., C. Meehan, A. Conneely, N. Kirby, T. Robinson, P. Nigam, I. M. Banat, R. Marchant, and W. F. Smyth. 2001. Microbial decolourization and degradation of textile dyes. *Applied Microbiology Biotechnology.* **56:**81-87.

Meehan, C., I. M. Banat, G. McMullan, P. Nigam, F. Smyth, and R. Marchant. 2000. Decolourization of remazol-black using a thermotolerant yeast, *Kluyveromyces marxianus* IMB3. *Environmental Biotechnology.* **26:**75-79.

Meng, Y, X. Huang, Q. Yang, Y. Qian, N. Kubota, and S. Fukunaga. 2005. Treatment of polluted river water with a photocatalytic slurry reactor using low-pressure mercury lamps coupled with a membrane. *Desalination.* **181:**121-133.

Meyer, U. 1981. Biodegradation of synthetic organic colorants, p. 371-385. *In* T. Leisinger, A. M. Cook, R. Hutter and J. Nuesch. (ed.), Microbial degradation of xenobiotic and recalcitrant compounds, FEMS Symposium 12th ed. Academic Press, London.

Mezrioui, N., and B. Baleux. 1994. Resistance patterns of *E. coli* strains isolated from domestic sewage before and after treatment in both aerobic lagoon and activated sludge. *Water Research*. **28:**2394-2406.

Mishra, G., and M. Tripathy. 1993. A critical review of the treatment for decolourization of textile effluent. *Colourage.* **40:**35-38.

Mishra, A., M. Yadav, B. Agarwal, and S. Rajani. 2004. Use of polyacrylamide-grafted *Plantago psyllium* mucilage as a flocculant for treatment of textile wastewater. *Colloid Polymer Science.* **282:**722-727.

Mishra, A., and M. Bajpai. 2005. Flocculation behaviour of model textile wastewater treated with a food grade polysaccharide. *Journal of Hazardous Material*. **B118:**213-217.

Misra, M. 2002. Selective flocculation of fine coal with hydrophobic *Mycobacterium phlei*. *Minerals and Metallurgical Processing.* **10:**20-23.

Mittal, A. K., and S. K. Gupta. 1996. Biosorption of cationic dyes by dead macro-fungus *Fomitopsis carnea*: batch studies. *Water Science Technology.* **34:**157-181.

Morais, L. C., O. M. Freitas, E. P. Gonçalves, L. T. Vasconcelos, and C. G. Gonzalez Beça. 1999. Reactive dyes removal from wastewaters by adsorption on eucalyptus bark: variables that define the process. *Water Resource.* **33:**979-988.

Moran, C., M. E. Hall, and R. C. Howell. 1997. Effects of sewage treatment on textile effluent. *Journal of Social Dyers Colour*. **113:**272-274.

Morgan, J., C. Foster, and L. Evison. 1990. A comparative study of the nature of biopolymers extracted from activated sludges. *Water Resource.* **24:**743-750.

Muller, J. P., A. Manceau, G. Calas, T. Allard, P. Ildefone, and J. L. Hazemann. 1995. Crystal chemistry of kaolinite and Fe–Mn oxides: relation with formation condition of low-temperature systems. *American Journal of Science.* **295:**1115–1155.

Myers, C. R., and J. M. Myers. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *Journal of Bacteriology.* **174:**3429-3438.

Nakamura, J., S. Miyashiro, and Y. Hirose. 1976a. Screening, isolation, and some properties of microbial cell flocculants. *Agricultural and Biological Chemistry.* **40:**377-383.

Nakamura, J., S. Miyashiro, and Y. Hirose. 1976b. Purification and chemical analysis of microbial cell flocculant produced by *Aspergillus sojae* AJ7002. *Agricultural and Biological Chemistry.* **40:**619-624.

Nakamura, J., S. Miyashiro, and Y. Hirose. 1976c. Conditions of production of microbial cell flocculant by *Aspergillus sojae* AJ-7002. *Agricultural and Biological Chemistry*. **40:**1341-1347.

Nakata, K., and R. Kurane. 1999. Production of extracellular polysaccharide bioflocculants by *Klebsiella pneumoniae*. *Journal of Bioscience, Biotechnology and Biochemistry*. **63:**2064-068.

Napoli, C., F. Dazzo, and D. Hubbell. 1975. Production of cellulose microfibrils by *Rhizobium*. *Applied Microbiology*. **30:**123-131.

Navin, K., C. Twarakavi, and J. Kaluarachchi. 2006. Sustainability of ground water quality considering land use changes and public health risks. *Journal of Environmental Management*. **81:**405-419.

Ndabigengesere, A., and K. S. Narasiah. 1998. Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water Resources*. **32(3):**781-791.

Nester, E. W., D. G. Anderson, C. E. Roberts, N. N. Pearcal, and M. T. Nester. 2001. Microbiology: a human perspective third edition, p. 787-799. The McGraw-Hill, Inc., New York.

Nguyen, A. L., S. Grothe, and J. H. T. Loung. 1988. Lactose hydrolysis in aqueous two -phase system by whole-cell ß- galactosidase of *Kluyveromyces marxianus*. *Applied Microbiology and Biotechnology*. **27:**341-346.

Nigam, P., I. M. Banat, D. Singh, and R. Marchant. 1996. Microbial process for the decolourization of textile effluent containing azo, diazo, and reactive dyes. *Process Biochemistry*. **31(5):**435-42.

Norberg, A. B., and S. O. Enfors. 1982. Production of extracellular polysaccharide by *Zoogloea ramigera*. *Applied and Environmental Microbiology.* **44:**1231-1237.

Nordstrom, D. K., and H. M. May. 1989. Aqueous equilibrium data for mononuclear aluminum species, p. 29-55. *In* G. Sposito, (ed.), The environmental chemistry of aluminum, CRC Press, Boca Raton. Florida.

Ogawa, T., I. Fuji, K. Kawal, C. Yatome, and E. Idaka. 1989. Growth inhibition of *Bacillus subtilis* upon interaction between basic dyes and DNA. *Bulletin of Environmental Contamination and Toxicology.* **42:**402-408.

Ogawa, T., M. Shibata, C. Yatome, and E. Idaka. 1988. Growth inhibition of *Bacillus subtilis* by basic dyes. *Bulletin of Environmental Contamination and Toxicology.* **40:**545-552.

Ollis, D. F., E. Pelizzetti, and N. Serpone. 1996. Destruction of water contaminants. *Environmental Science and Technology*. **25:**1523-1529.

O'Mahony, T., E. Guibal, and J. M. Tobin. 2002. Reactive dye biosorption by *Rhizopus arrhizus* biomass. *Enzyme and Microbial Technology.* **31:**456-463.

Otero, M., F. Rozada, L. F. Calvo, A. I. Garcia, and A. Moran. 2003. Kinetic and equilibrium modelling of the methylene blue removal from solution by adsorbent materials produced from sewage sludge. *Journal of Biochemical Engineering.* **15:**59-68.

Pagga, U., and D. Brown. 1986. The degradation of dyestuffs. *Chemosphere.* **15:**479-491.

Pala, A., and E. Tokat. 2002. Colour removal from cotton textile industry wastewater in an activated sludge system with various additives. *Water Resource.* **36:**2920-2925.

Pearce, C. I., J. R. Lloyd, and J. T. Guthrie. 2003. The removal of colour from textile wastewater using whole bacterial cells. *Dyes Pigments*. **58:**179-196.

Pereira, M. F. R., S. F. Soares, J. J. M. Orfao, and J. L. Figueiredo. 2003. Adsorption of dyes on activated carbons: influence of surface chemical groups. *Carbon*. **41:**811-821.

Peter, H. A. S., S. M. Nicholas, M. E. Sharpe, and J. G. Holt. 1986. Bergey's Manual of Systematic Bacteriology, vol. 2, p. 1120-1136. Williams and Wilkin, Baltimore, MD.

Petzold, G., U. Geissler, S. Smolka, and S. Schwarz. 2004. Influence of humic acid on the flocculation of clay. *Colloid Polymer Science*. **282:**670–676.

Petzold, G., M. Mende, K. Lunkwitz, S. Schwarz, and H. M. Buchhammer. 2003a. Higher efficiency in the flocculation of clay suspensions by using combinations of oppositely charged polyelectrolytes. *Colloids Surface. A: Physicochemical Engineering. Aspects.* **218:**47–57.

Petzold, G., A. Nebel, H. M. Buchhammer, and K. Lunkwitz. 1998. Preparation and characterization of different polyelectrolyte complexes and their application as flocculants. *Colloid Polymer Science*. **276:**125-130.

Petzold, G., S. Schwarz, and K. Lunkwitz. 2003b. Higher efficiency in particle flocculation by using combinations of oppositely charged polyelectrolytes. *Chemical Engineering and Technology.* **26:**48-53.

Pinheiro, H. M., E. Touranud, and O. Thomas. 2004. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes and Pigments*. **61:**121-139.

Plumb, J. J., J. Bell, and D. C. Stuckey. 2001. Microbial populations associated with treatment of an industrial dye effluent in an anaerobic baffled reactor. *Applied and Environmental Microbiology.* **67:**3226-3235.

Plummer, D. T. 1978. An introduction to practical biochemistry 2nd ed., p. 158-159. McGraw–Hill Press, London.

Pollock, T. J. 1993. Gellan related polysaccharides and the genus *Sphingomonas. General Microbiology*. **139:**1939-1945.

Prasad, M. S., K. J. Reid, and H. H. Murray. 1991. Kaolin: processing and applications. *Applied Clay Science*. **6:**87-119.

Prescott, M., J. P. Harley, and D. A. Klein. 1996. Microbiology 3rd ed., p. 935. W. M. C. Brow Publishers, New York.

Ramakrishna, K. R., and T. Viraraghavan. 1997. Dye removal using low cost adsorbents. *Water Science Technology.* **36:**189-196.

Ramalho, P. A., H. Scholze, M. H. Cardoso, M. T. Ramalho, and A. M. Oliveira-Campos. 2002. Improved conditions for the aerobic reductive decolourization of azo dyes by *Candida zeylanoides*. *Enzyme and Microbial Technology.* **31:**848-854.

Rauf, M. A., I. A. Shehadi, and W. W. Hassan. 2006. Studies on the removal of neutral red on sand from aqueous solution and its kinetic behavior. *Dyes and Pigments.* doi:10.1016/j.dyepig.2006.07.024.

Ravi-Kumar, M. N. V., T. R. Sridhari, K. D. Bhavani, and P. K. Dutta. 1998. Trends in colour removal from textile mill effluents. *Colorage.* **40:**25-34.

Razo-Flores, E., M. Luijten, B. Donlon, G. Lettinga, and J. Field. 1997. Biodegradation of selected azo dyes under methanogenic conditions. *Water Science Technology.* **36:**65-72.

Robinson, T., B. Chandran, and P. Nigam. 2002. Removal of dyes from a synthetic textile dye effluent by adsorption on apple pomace and wheat straw. *Water Resource.* **36:**2824-2830.

Robinson, T., G. McMullan, R. Marchant, and P. Nigam. 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource Technology.* **77:**247-255.

Rodriguez, S. M., C. Richter, J. B. Galvez, and M. Vincent. 1996. Photocatalytic degradation of industrial residual waters. *Solar Energy*. **56:**401-410.

Russ, R., J. Rau, and A. Stolz. 2000. The function of cytoplasmic flavin reductases in the reduction of azodyes by bacteria. *Applied and Environmental Microbiology.* **66:**1429-1434.

Sakka, K., and H. Takahashi. 1981. DNA as a flocculation factor in *Pseudomonas* sp. *Journal of Agricultural and Biological Chemistry*. **45:**2869-2876.

Salehizadeh, H., and S. A. Shojaosadati. 2001. Extracellular biopolymeric flocculants: recent trends and biotechnological importance. *Biotechnology Advances.* **19:**371-385.

Salehizadeh, H., and S. A. Shojaosadati. 2002. Isolation and characterization of the bioflocculant produced by *Bacillus firmus*. *Biotechnology Letters.* **24:**35-40.

Salehizadeh, H., M. Vossoughi, and I. Alemzadeh. 1998. Bioflocculant productions by *Bacillus* sp. AS-101. *Fourth Annual Congress of the Society of Chemical Engineering of Iran.* **3:**104-108.

Salehizadeh, H., M. Vossoughi, and I. Alemzadeh. 2000. Some investigations on bioflocculant producing bacteria. *Biochemical Engineering*. **5:**39-44.

Sanghi, R., B. Bhattacharya, A. Dixit, and V. Singh. 2006. *Ipomoea dasysperma* seed gum: An effective natural coagulant for the decolourization of textile dye solutions. *Journal of Environmental Management*. **81:**36-41.

Sani, R. K., and U. C. Banerjee. 1999. Decolourization of triphenylmethane dyes and textile and dye-stuff effluent by *Kurthia* sp. *Enzyme and Microbial Technology.* **24:**433-437.

Seo, H. 1993. Bioflocculant production from *Bacillus* sp. A56. *Sanop Misaengmul Hakhoechi.* **21:**486-493.

Seshadri, S., P. L. Bishop, and A. M. Agha. 1994. Anaerobic/aerobic treatment of selected azo dyes in wastewater. *Waste Management.* **15:**127-137.

Shih, I. L., L. C. Van, H. G. Lin, and Y. N. Chang. 2001. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. *Bioresource Technology*. **78:**267-272.

Shimofuruya, H., A. Koide, K. Shirota, T. Tsuji, M. Nakamura, and J. Suzuki. 1996. The production of flocculating substance(s) by *Streptomyces griseus*. *Journal of Bioscience, Biotechnology and Biochemistry.* **60:**498-500.

Shubo, D., G. Yu, and T. Yen Peng. 2005. Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal. *Colloids and Surfaces B.* **44:**179-186.

Singh, R. P., T. Tripathy, G. P. Karmakar, S. K. Rath, N. C. Karmakar, S. R. Pandey, K. Kannan, S. K. Jain, and N. T. Lan. 2000. Novel biodegradable flocculants based on polysaccharides. *Current Science*. **78:**798-803.

Sinton, L. W., and A. M. Donnison. 1994. Characterization of faecal streptococci from some New Zealand effluents and receiving water. *New Zealand Journal of Marine Freshwater Research*. **28:**145-158.

Sinton, L. W., R. K. Finlay, and D. J Hannah. 1998. Distinguishing human from animal faecal contamination in water. *New Zealand Journal of Marine Freshwater Research*. **32:**323-348.

Sirianuntapiboon, S., and P. Srisornsak. 2007. Removal of disperse dyes from textile wastewater using bio-sludge. *Bioresource Technology*. **98:**1057-1066.

Sletmoen, M., G. Maurstad, P. Sikorski, B. S. Paulsen, and B. T. Stokke. 2003. Characterization of bacterial polysaccharides: steps towards single-molecular studies**.** *Carbohydrate Research*. **14:**2459-2475.

Slokar, Y. M., and A. M. Le-Marechal. 1997. Methods of decolouration of textile wastewaters. *Dyes Pigments.* **37:**335-356.

Song, S. H., Ying, Z. He, and J. Chen. 2006. Mechanism of decolorization and degradation of CI Direct Red 23 by ozonation combined with sonolysis. *Chemosphere*. doi:10.1016/j.chemosphere.2006.07.090

Sousa, M., J. Teixeira, and M. Mota. 1992. Difference in flocculation mechanism of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. *Biotechnology Letters.* **14:**213-218.

Southern, T. G. 1995. Technical solutions to the colour problem: a critical review, p. 75. *In* P. Cooper, (ed.), Colour in dyehouse effluent, Society of Dyers and Colourists, Bradford.

Srivastava, P. N., and A. Prakash. 1991. Bioaccumulation of heavy metals by algae and wheat plants fed by textile effluents. *Journal of Pollution Control Federation.* **7:**25-30.

Stolz, A. 2001. Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbiology and Biotechnology.* **56:**9-80.

Stumm, W., and J. Morgan. 1981. Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters, p 123-157. Wiley, New York.

Suh, H., G. Kwon, C. Lee, H. Kim, H. Oh, and B. Yoon. 1997. Characterization of bioflocculant produced by *Bacillus* sp. DP-152. *Journal of Fermentation and Bioengineering.* **84:**108-112.

Sumathi, S., and B. S. Manju. 2000. Uptake of reactive textile dyes by *Aspergillus foetidus*. *Enzyme and Microbial Technology.* **27:**347-352.

Sutherland, I. W. 1990. Microbial extracellular polymeric substances: characterization of structure and function, p 26. *In* J. Wingender, T. R. Neu and H. C Flemming (ed.), Microbial extracellular polymeric substances. Comridge University Press, Inc., England.

Sutherland, I. W. 1995. Biotechnology of exopolysaccharides. *Biotechnology Advances*. **12:**393-448.

Sutherland, I. W. 1998. Novel and established applications of microbial polysaccharides. *Trends in Biotechnology*. **16:**41-46.

Tago, Y, and K. O. Aida. 1977. Exocellular mucopolysaccharide closely related to bacterial floc formation. *Applied and Environmental Microbiology***. 34:**108-112.

Takagi, H., and K. Kodowaki. 1985. Floccculant production by *Paecilomyces* sp. taxonomic studies and culture conditions for production. *Journal of Agricultural and Biological Chemistry.* **49:**3151-3159.

Takeda, M., J. Koizumi, H. Matsuoka, and M. Hikuma. 1992. Factors affecting the activity of a protein bioflocculant produced by *Nocardia amarae*. *Journal of Fermentation and Bioengineering.* **74:**408-409.

Takeda, M., R. Kurane, J. Koizumi, and I. Nakamura. 1991. A protein bioflocculant produced by *Rhodococcus erythropolis*. *Journal of Agricultural and Biological Chemistry.* **55:**2663-2664.

Tenny, M. W., and W. Stumm. 1965. Chemical flocculation of microorganisms in biological waste treatment. *Journal of Water Pollution Control Federation.* **37:**1370-1388.

Toeda, K., and R. Kurane. 1991. Microbial flocculant from *AIcaligenes cupidus* KT201. *Journal of Agricultural and Biological Chemistry.* **55:**2793-2799.

Tong, Z., L. Zhe, and Z. Huai-lan. 1999. Microbial flocculant and its application in environmental protection. *Journal of Environmental Science.* **11:**1-12.

Twarakavi, N. K. C., and J. J. Kaluarachchi. 2006. Sustainability of ground water quality considering land use changes and public health risks. *Journal of Environmental Management.* **81:**405-419.

Unz, R. F., and S. R. Farrah. 1976. Exopolymer production and flocculation by *Zoogloea* MP6. *Applied and Environmental Microbiology.* **31:**623-626.

USEPA. 1991. Is your drinking water safe? Office of water (WH-550), EPA 570/9-91-005, Washington DC.

Van-Kranenburg, R., I. C. Boels, M. Kleerebezem, and W. M. de-Vos. 1999. Genetics and engineering of microbial exopolysaccharides for food: approaches for the production of existing and novel polysaccharides. *Current Opinions in Biotechnology*. **10:**498-504.

Van der Zee, F. P., G. Lettinga, and J. A. Field. 2000. The role of (auto) catalysis in the mechanism of an anaerobic azo reduction. *Water Science and Technology*. **42:**301-308.

Vigneswaran, S., and C. Visvanathan. 1995. Water treatment processes: simple options, p.215. CPR Press, Inc., New York.

Vossoughl, S., and C. S. Buller. 1991. Permeability modification by *in-situ* gelation with a newly discovered biopolymer. *Journal of Reservoir Engineering.* **5:**485-489.

Weber, E. J., and N. L. Wolfe. 1987. Kinetics studies of reduction of aromatic azo compounds in anaerobic sediment/water systems. *Environmental Toxicological Chemistry.* **6:**911-920.

Wiley, B. J., D. H. Ball, S. M. Arcidiacono, S. Sous, J. M. Mayer, and D. L. Kaplan. 1993. Control of molecular weight distribution of the biopolymer pullulan produced by *Aereobasidium pullulans. Journal of Polymers and the Environment.* **1:**3-9.

Willmott, N. J. 1997. The use of bacteria–polymer composites for the removal of colour from reactive dye effluents. Ph.D. thesis, UK: University of Leeds.

Willmott, N. J., J. T. Guthrie, and G. Nelson. 1998. The biotechnology approach to colour removal from textile effluent. *Journal of the Society of Dyers and Colourists.* **114:**38-41.

Yan, Y. D., S. M. Glover, G. J. Jameson, and S. Biggs. 2004. The flocculation efficiency of polydisperse polymer flocculants. *International Journal of Mineral Processing.* **73:**161-175.

Yavuz, O., and A. H. Aydin. 2002. The removal of acid dye from aqueous solution by different adsorbents. *Fresenius Environmental Bulletin*. **11(7):**377-383.

Yilmaz, A., E. Yilmaz, M. Yilmaz, and R. A. Bartsch. 2007. Removal of azo dyes from aqueous solutions using calix[4]arene and β-cyclodextrin. *Dyes and Pigments*. **74:** 54-59.

Yim, J. H., S. J. Kim, S. H. Ahn, and H. K. Lee. .2007. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. *Bioresource Technology*. **98:**361-367.

Yokoi, H., T. Arima, S. Hayashi, and Y. Takasaki. 1996a. Flocculation properties of poly(γ-glutamic acid) produced by *Bacillus subtilis*. *Journal of Fermentation and Bioengineering.* **82:**84-87.

Yokoi, H., O. Natsuda, J. Hirose, S. Hayashi, and Y. Takasaki. 1995. Characteristics of biopolymer flocculant produced by *Bacillus* sp. PY-90. *Journal of Fermentation and Bioengineering.* **79:**378-380.

Yokoi, H., T. Yoshida, S. Mori, J. Hirose, S. Hayashi, and Y. Takasaki. 1997. Biopolymer flocculant produced by an *Enterobacter* sp. *Biotechnology Letters.* **19:**569-573.

Yoo, E. S., J. Libra, and L. Adrian. 2001. Mechanism of decolorization of azo dyes in anaerobic mixed culture. *Journal of Environmental Engineering.* **127:**844-849.

Yoo, E. S., J. Libra, and U. Wiesmann. 2000. Reduction of azo dyes by *Desulfovibrio desulfuricans*. *Water Science and Technology.* **41:**15-22.

Yoshinaga, F., N. Tonouchi, and K. Watanabe. 1997. Research project in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. *Bioscience, Biotechnology and Biochemistry*. **61:**219-224.

Zhou, J. L., and C. J. Banks. 1991. Removal of humic acid fraction by *Rhizopus arrhizus*: uptake and kinetic studies. *Environmental Technology*. **12:**859-869.

Zhou, J. L., and C. J. Banks. 1993. Mechanism of humic acid colour removal from natural waters by fungal biomass biosorption. *Chemosphere.* **27:**607-620.

Zouboulis, A. I., X. Chaib, and I. A. Katsoyiannis. 2004. The application of bioflocculant for the removal of humic acids from stabilized landfill leachates. *Journal of Environmental Management*. **70:**35-41.

APPENDIX A: 16S rDNA sequences

16S rDNA sequence of *Bacillus subtilis* (E1)

AGATAGCTCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGG
GAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAGACATAAAAGGTGGCTTT
GGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA
GGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG
CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAACAAGTGCCGTTC
AAATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCAGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTGTCCGAATTATTGGGCGTAAAGGGCTCGCAGGCGGT
TTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACT
TGAGTGCAGAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGGGAAGG
AACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAGCGTGGGGA
GCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTGAGTGCTAAGTGTAGGGGGT
CG

16S rDNA sequence of *Pseudomonas pseudoalcaligenes* (A17)

TATTTAGCGTCTCTGGACGGGTGAGTAATGCCTTAGGAATCTGCCTGGTAGTGGGGGATAACG
TTCCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCT
TGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGA
CGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACCTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCG
CGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTA
ATACGTTAGTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGC
GGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
CGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCT
AGAGTACGGTAGAGGGTAGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGG
AACACCAGTGGCGAAGGCGACTACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGG
AGCAAACAGGATTAAGATACCCTGGTAGTCCACGCCGTAAACGTGTCAACTAGCCGTTGGAA
TCCTTGAGATTTTAGTGGCGCAGCTAACGCATTTG

16S rDNA sequence of *Exiguobacterium acetylicum* D1

TGGCATGAGCGGCGGACGGGTGAGTAACACGTAAGGAACCTGCCTCAAGGATTGGGATAACT
CCGAGAAATCGGAGCTAATACCGGATAGTTCAACGGACCGCATGGTCCGCTGATGAAAGGCG
CTCCGGCGTCACCTTGAGATGGCCTTGCGGTGCATTAGCTAGTTGGTGGGGTAACGGCCCACC
AAGGCGACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC
CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCA
ACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTAAGGGAAGAACACGTA
CGAGAGGGAATGCTCGTACCTTGACGGTACCTTACGAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGC
AGGCGGCCTTTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGCCATTGGAAACT
GGAAGGCTTGAGTACAGAAGAGAAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGA
TGTGGAGGAACACCAGTGGCGAAGGCGACTCTTTGGTCTGTAACTGACGCTGAGGCGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTATG

16S rDNA sequence of *Staphylococcus aureus* (A22)

CGACGGGTATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCAATACCGGATA
ATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCG
CGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGA
GAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTC
GGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGG
TACCTAATCAAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGTGGCAA
GCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAG
CCCACGGCTCAACCCGTGGAGGGTCATTTGGAAACTGGAAAACTTGAGTGCATAATGAAGGA
AAGTGGAATTCCATGTGTAGCCGGTGAAATGCGCAGAGATTTTGGAGGAACACCAGGTGGCG
AGGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAAGGAT
TAGATACCCTGGTAGTCCACGCCGTATACGATGAGTGCTAATGTGTAATGGGGTTTCCGCCCT
CG

16S rDNA sequence of *Klebsiella terrigena* (R2)

CAGGGTGCAGTAATGTCTGGGTAAACTGCCCGATGGAAGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCACACCATCGGATG
TGCCCAGATGGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCCTAGCTGG
TCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGG
CCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCATTAAGGTTAATAACCTTAGTGATT
GACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGG
TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAG
GGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA
AGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGTCTATTTGAAGGTGTTCCCTTGAGGAGTGCTTTC
GGAGC

16S rDNA sequence of *Pseudomonas plecoglossicida* (A14)

CATCAGCGCGTGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTT
CGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGAGGGGACCTTCGGGCCTTGCG
CTATCAGATGAGCCTAGGTCGGATTAGCTAGTGGTGGGGTAATGGCTCACCAAGGCGACGAT
CCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGT
GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATAC
CTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA
ATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTT
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TACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAAGGAAC
ACAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTT
GAGATTTAGTG

Table 6.1: Identity of bacterial isolates based on the API (percentage probability)

Isolate code	percentage probability
A22	78% *Staphylococcus*
A14	83% *Pseudomonas*
A17	77% *Pseudomonas*
D1	70% *Corynebacterium*
E1	93% *Bacillus*
R2	88% *Klebsiella*

APPENDIX B: Data

Figures in parenthesis appear in the text

Table 7.1: Production of bioflocculants by different bacterial isolates (Fig. 2.2)

Isolate	Amount of polysaccharide produced (g\l)
A11	3.000
A12	6.333
A13	3.667
A14*	8.333
A15	2.333
A16	7.333
A17*	15.167
A18	9.333
A21	1.000
A22*	10.833
A23	7.667
A24	8.667
D1*	10.167
D2	5.000
E1*	6.333
E2	0.667
E3	4.333
E4	2.667
E5	6.667
R1	4.667
R2*	27.660
R3	4.333
R4	1.333
R5	5.000
R6	7.667

***Bacteria selected for further study**

A1 = activated sludge (aerobic)
A2 =activated sludge (anaerobic)
D1 = digested sludge
E1 = effluent clarifier

(a) *Phenol-sulfuric acid assay* for the determination of carbohydrates

Sensitivity: glucose (0.05–2mM)

Final volume: 1.4 ml

Reagents:

(a) phenol dissolved in water (5% w/v).

(b) Concentrated sulphuric acid.

Method

(i) Mix samples, standards and control solutions (200 µl containing up to 100 µg carbohydrate) with 200 µl of reagent A.

(ii) Add 1.0 ml of reagent B rapidly and directly to the solution surface without touching the sides of the tube.

(iii) Leave the solutions undisturbed for 10 min before shaking vigorously.

(iv) Determine the absorbance at 490 nm after a further 30 min.

(b) *Folin-Lowry method* for the determination of protein

Sensitivity: Bovine serum albumin (0.002–2.000mM)

Final volume: 6.5 ml

Reagents:

(a) 2% Na_2CO_3 in 0.1 N NaOH

(b) 1% NaK Tartrate in H_2O

(c) 0.5% $CuSO_4$.5 H_2O in H_2O

(d) 48 ml of A, 1 ml of B, 1 ml C

(e) Phenol Reagent – 1 part Folin-Phenol (2 N): 1 part water

Method

(i) Add 5 ml of reagent D to 1 ml of the test solution.

(ii) Mix thoroughly and allow to stand for 10 min or longer.

(iii) Add 0.5 ml of reagent E rapidly with immediate mixing.

(iv) After 30 min read the extinction against the appropriate blank at 750 nm

(v) Estimate the protein concentration of the unknown solution after preparing a standard curve.

(c) *Carbazole assay* for the determination of uronic acid

Sensitivity: D-glucurono-6,3-lactone (0.001–0.050mM)

Final volume: 1.8 ml

Reagents:

(a) Dissolve 0.95 g of sodium tetraborate decahydrates in 2.0 ml of hot water and add 98 ml of ice-cold concentrated sulphuric acid carefully with stirring.

(b) Dissolve 125 mg of carbazole (recrystallized from ethanol) in 100 ml of absolute ethanol to give a stable reagent.

Method

(i) Cool the samples, standards and controls (250 µl) in an ice bath.

(ii) Add ice-cold reagent A (1.5 ml) with mixing and cooling in the ice bath.

(iii) Heat the mixtures at 100^{o}C for 10 min.

(iv) Cool rapidly in the ice-bath.

(v) Add 50 µl of reagent B and mix well.

(vi) Reheat at 100^{o}C for 15 min.

(vii) Cool rapidly at room temperature and determine the absorbance at 525 nm.

(d) *Morgan–Elson assay* for the determination of hexosamine

Sensitivity: 2-acetamido-2-deoxy-D-glucose(0.001–0.110 mM)

Final volume: 1.8 ml

Reagents:

(a) Dissolve 6.1 g of di-potassium tetraborate tetrahydrate in 80 ml of water and make up to 100 ml with water.

(b) Add 1.5 ml of water to 11 ml of concentrated hydrochloric acid. Add a further 87.5 ml of glacial acetic acid and dissolve 10 g of 4-(*N,N*-dimethylamino)-

benzaldehyde in this mixture. Dilute 10 ml to 100 ml with glacial acetic acid immediately prior to use.

Method

(i) Add samples, standards and controls (250 µl) to 50 µl of reagent A

(ii) Heat each mixture at 100^{o}C for 3 min.

(iii) After cooling rapidly to room temperature, add 1.5 ml of reagent B, washing down any condensate formed.

(iv) Incubate the samples at 37^{o}C for 20 min.

(v) After cooling to room temperature, determine the absorbance at 585 nm.

Table 7.2: Determination of the total sugar content using Phenol Sulphuric Acid assay at OD_{490nm}

Conc (mM)	OD_{490nm}	Average	Standard deviation
0.002	0.054	**0.057**	0.006
	0.066		
	0.051		
0.004	0.138	**0.123**	0.012
	0.108		
	0.123		
0.006	0.141	**0.132**	0.560
	1.320		
	0.123		
0.008	0.270	**0.180**	0.037
	0.180		
	0.210		
0.01	0.195	**0.187**	0.006
	0.180		
	0.189		
0.050	0.231	**0.219**	0.010
	0.219		
	0.207		
0.100	0.399	**0.395**	0.004
	0.390		
	0.396		
0.300	0.633	**0.642**	0.006
	0.648		
	0.645		
0.500	0.984	**0.985**	0.004
	0.981		
	0.990		
1	1.293	**1.302**	0.007
	1.302		
	1.311		
1.500	2.067	**2.064**	0.009
	2.073		
	2.052		
2	2.856	**2.853**	0.002
	2.850		

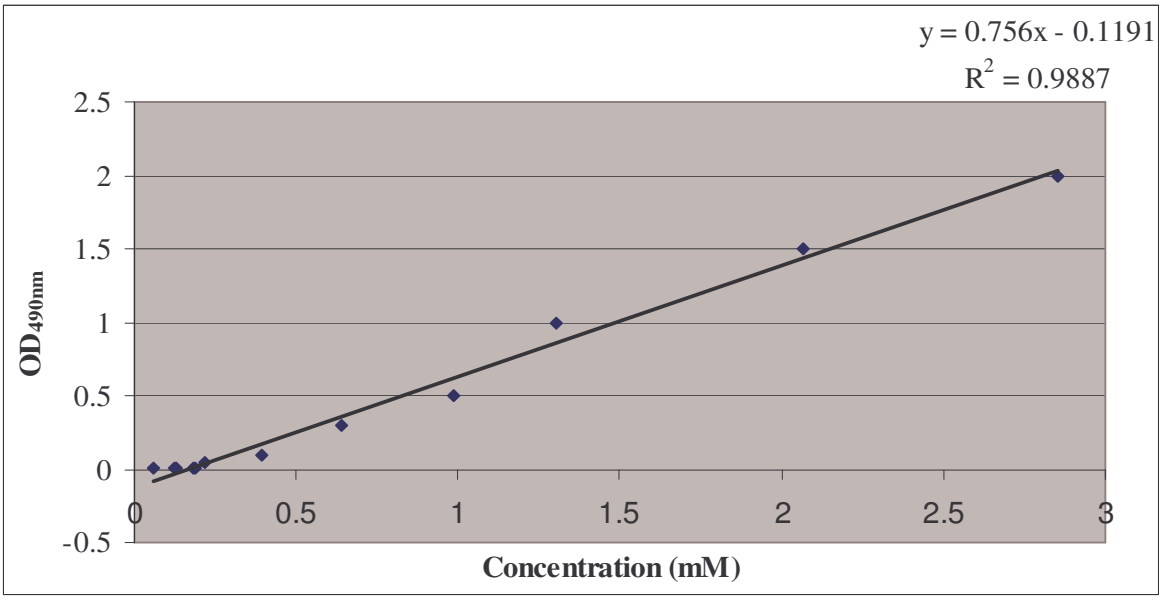

Fig.7.1: Standard curve for the determination of D-Glucose concentration at OD_{490nm}.

Table 7.3: Determination of the total protein content using the Folin-Lowry method at OD_{750nm}

Conc (mM)	OD_{750nm}	Average	Standard deviation
0.002	0.021	**0.021**	0.003
	0.015		
	0.027		
0.004	0.03	**0.038**	0.006
	0.039		
	0.045		
0.006	0.039	**0.049**	0.007
	0.051		
	0.057		
0.008	0.057	**0.055**	0.007
	0.063		
	0.045		
0.010	0.099	**0.088**	0.009
	0.087		
	0.078		
0.050	0.216	**0.209**	0.006
	0.201		
	0.210		
0.100	0.378	**0.370**	0.006
	0.369		
	0.363		
0.500	1.410	**1.404**	0.004
	1.401		
	1.401		
1.500	2.559	**2.562**	0.009
	2.574		
	2.553		
2	2.610	**2.616**	0.005
	2.616		
	2.622		

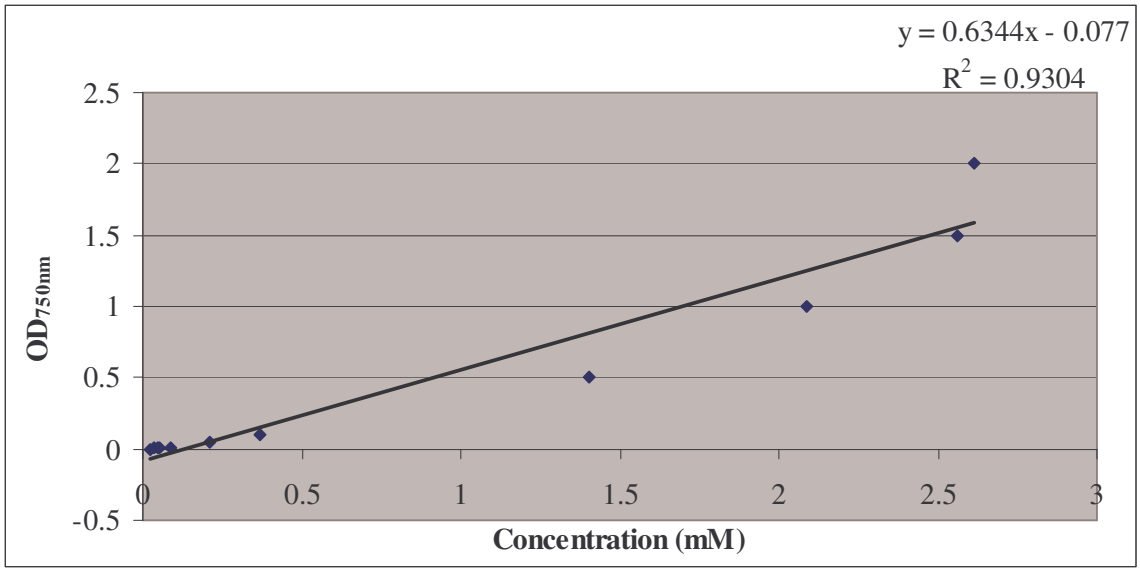

Fig. 7.2: Standard curve for the determination of Bovine serum album (BSA) concentration at OD_{750nm}.

Table 7.4: Determination of the uronic acid content using the Carbazole assay at OD_{750nm}

Conc (mM)	OD_{750nm}	Average	Standard deviation
0.001	0.630	**0.540**	0.073
	0.540		
	0.450		
0.003	0.57	**0.570**	0.073
	0.660		
	0.480		
0.005	0.999	**1.002**	0.003
	1.005		
	1.002		
0.010	2.613	**2.628**	0.012
	2.628		
	2.643		
0.050	2.790	**3**	0.218
	2.910		
	3.300		

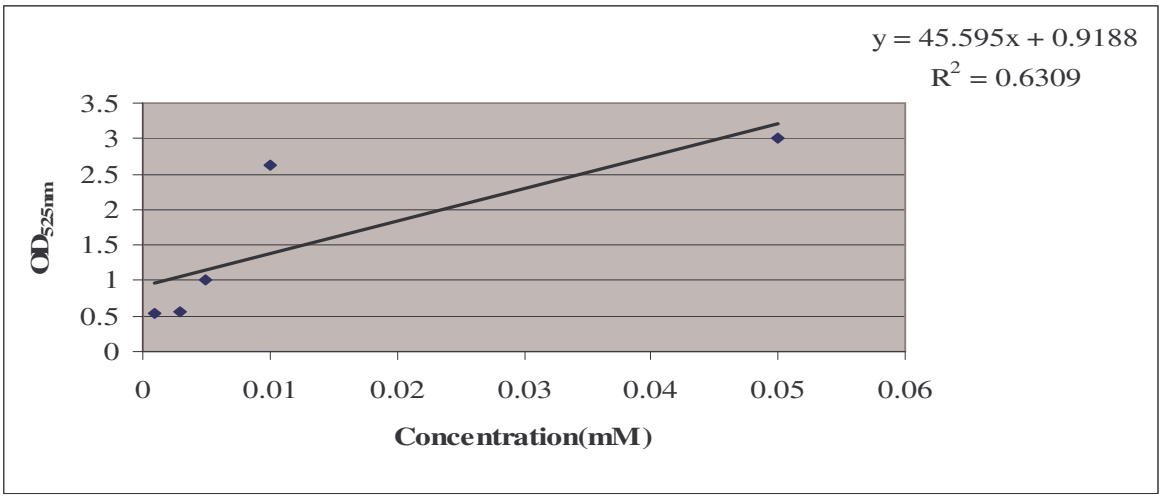

Fig. 7.3: Standard curve for the determination of D- Glucurono-6,3-lactone concentration at OD_{750nm}.

Table 7.5: Determination of the hexosamine (amino sugars) content using the Morgan-Elson assay at OD_{585nm}

Conc (mM)	$OD7_{585nm}$	Average	Standard deviation
0.001	0.003	**0.002**	0.001
	0		
	0.003		
0.005	0.006	**0.014**	0.007
	0.021		
	0.015		
0.010	0.030	**0.026**	0.004
	0.027		
	0.021		
0.030	0.048	**0.055**	0.006
	0.054		
	0.063		
0.050	0.111	**0.123**	0.009
	0.126		
	0.132		
0.070	0.198	**0.199**	0.009
	0.210		
	0.189		
0.090	0.249	**0.242**	0.008
	0.231		
	0.246		
0.110	0.300	**0.298**	0.001
	0.297		
	0.297		

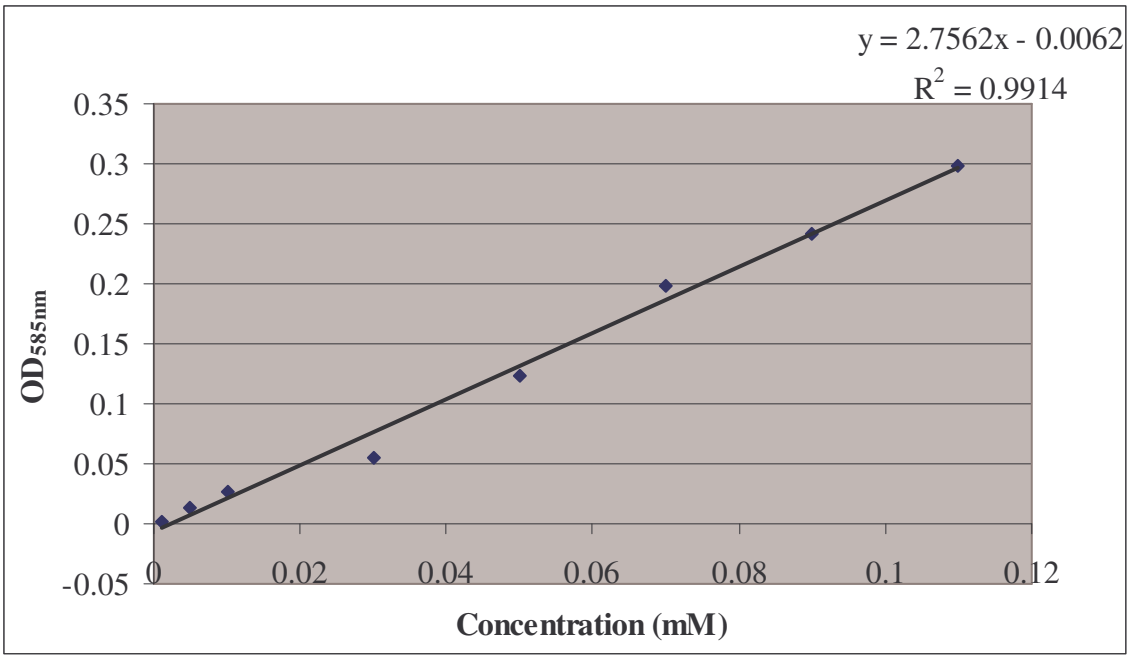

Fig.7.4: Standard curve for the determination of 2-acetamido-2deoxy-D- Glucuse concentration at OD_{585nm}.

Table 7.6: OD_{490nm} values of various dilutions of bioflocculants used for the determination of total sugar concentration

Isolates	Undiluted	01:05	01:10	01:15	01:20
R 2	0.624	0.120	0.114	0.039	0.048
	0.630	0.132	0.093	0.051	0.036
	0.612	0.141	0.105	0.045	0.039
Average	**0.622**	**0.134**	**0.104**	**0.045**	**0.041**
Standard deviation	0.007	0.009	0.009	0.005	0.005
E1	0.309	0.048	0.063	0.033	0.018
	0.291	0.066	0.051	0.039	0.009
	0.297	0.072	0.045	0.030	0.015
Average	**0.299**	**0.062**	**0.053**	**0.034**	**0.014**
Standard deviation	0.007	0.010	0.007	0.004	0.004
A14	1.092	0.066	0.057	0.030	0.033
	1.107	0.084	0.048	0.042	0.027
	1.077	0.096	0.063	0.033	0.024
Average	**1.092**	**0.082**	**0.056**	**0.035**	**0.028**
Standard deviation	0.012	0.012	0.006	0.005	0.004
A17	0.225	0.186	0.165	0.105	0.090
	0.231	0.192	0.183	0.090	0.099
	0.219	0.177	0.177	0.114	0.111
Average	**0.225**	**0.185**	**0.175**	**0.103**	**0.100**
Standard deviation	0.005	0.006	0.007	0.010	0.009
D1	0.174	0.099	0.153	0.075	0.051
	0.183	0.117	0.126	0.084	0.093
	0.195	0.099	0.144	0.066	0.072
Average	**0.184**	**0.105**	**0.141**	**0.075**	**0.072**
Standard deviation	0.009	0.008	0.011	0.007	0.017
A22	0.789	0.186	0.135	0.069	0.039
	0.777	0.207	0.159	0.093	0.030
	0.771	0.219	0.111	0.060	0.063
Average	**0.779**	**0.204**	**0.135**	**0.074**	**0.044**
Standard deviation	0.007	0.013	0.020	0.014	0.014

Table 7.7: OD_{750nm} values of various dilutions of bioflocculants used for the determination of protein concentration

Isolates	Undiluted	01:05	01:10	01:15	01:20
R 2	0.117	0.090	0.033	0.039	0.051
	0.126	0.081	0.048	0.045	0.042
	0.138	0.066	0.063	0.048	0.027
Average	**0.127**	**0.079**	**0.048**	**0.044**	**0.04**
Standard deviation	0.009	0.011	0.012	0.004	0.010
E1	0.300	0.141	0.081	0.063	0.051
	0.297	0.132	0.060	0.057	0.045
	0.291	0.129	0.039	0.045	0.033
Average	**0.296**	**0.134**	**0.060**	**0.055**	**0.043**
Standard deviation	0.004	0.005	0.018	0.007	0.007
A14	0.162	0.138	0.126	0.081	0.069
	0.189	0.123	0.105	0.066	0.057
	0.201	0.111	0.087	0.051	0.045
Average	**0.184**	**0.124**	**0.106**	**0.066**	**0.057**
Standard deviation	0.016	0.011	0.016	0.012	0.010
A17	0.231	0.186	0.153	0.144	0.15
	0.249	0.177	0.132	0.135	0.135
	0.216	0.168	0.120	0.120	0.108
Average	**0.232**	**0.177**	**0.135**	**0.133**	**0.131**
Standard deviation	0.013	0.007	0.013	0.010	0.017
D1	0.129	0.123	0.135	0.057	0.066
	0.156	0.108	0.099	0.075	0.054
	0.183	0.096	0.087	0.084	0.039
Average	**0.156**	**0.109**	**0.107**	**0.072**	**0.053**
Standard deviation	0.022	0.011	0.020	0.011	0.011
A22	0.135	0.081	0.072	0.093	0.084
	0.123	0.096	0.081	0.048	0.054
	0.147	0.111	0.069	0.057	0.057
Average	**0.135**	**0.096**	**0.074**	**0.066**	**0.065**
Standard deviation	0.011	0.012	0.005	0.019	0.013

Table 7.8: Final concentrations (mM) of the unknown carbohydrates and proteins in undiluted bioflocculants as calculated from the standard curves (Fig. 7.1 and 7.2) and OD values (Table 7.2, 7.3, 7.6 and 7.7) (Fig. 2.3)

Bioflocculants	Carbohydrates (mM)	Proteins (mM)
R2	0.356	0.004
E1	0.107	0.111
A14	0.706	0.040
A17	0.056	0.070
D1	0.020	0.022
A22	0.470	0.009

Table 7.9: OD_{525nm} values of various dilutions of bioflocculants used for the determination of uronic acid concentration

Isolates	Undiluted	01:05	01:10	01:15	01:20
R 2	0.987	0.597	0.297	0.264	0.207
	0.972	0.591	0.303	0.255	0.183
	0.963	0.585	0.303	0.246	0.189
Average	**0.974**	**0.591**	**0.301**	**0.255**	**0.193**
Standard deviation	0.010	0.005	0.003	0.007	0.010
E1	0.597	0.297	0.183	0.159	0.075
	0.579	0.282	0.171	0.144	0.066
	0.561	0.273	0.159	0.132	0.054
Average	**0.579**	**0.284**	**0.171**	**0.145**	**0.065**
Standard deviation	0.015	0.012	0.010	0.011	0.009
A14	0.591	0.351	0.315	0.177	0.129
	0.567	0.363	0.303	0.162	0.141
	0.546	0.339	0.297	0.144	0.111
Average	**0.568**	**0.351**	**0.305**	**0.161**	**0.127**
Standard deviation	0.018	0.009	0.008	0.013	0.012
A17	0.579	0.366	0.213	0.135	0.204
	0.573	0.381	0.207	0.129	0.195
	0.561	0.351	0.177	0.117	0.183
Average	**0.571**	**0.366**	**0.199**	**0.127**	**0.194**
Standard deviation	0.007	0.012	0.016	0.007	0.009
D1	0.567	0.360	0.270	0.210	0.069
	0.558	0.354	0.255	0.201	0.054
	0.549	0.345	0.237	0.192	0.042
Average	**0.558**	**0.353**	**0.254**	**0.201**	**0.055**
Standard deviation	0.007	0.006	0.013	0.007	0.011
A22	0.564	0.327	0.273	0.141	0.045
	0.555	0.312	0.267	0.129	0.033
	0.543	0.294	0.261	0.111	0.039
Average	**0.554**	**0.311**	**0.267**	**0.127**	**0.039**
Standard deviation	0.009	0.013	0.005	0.012	0.005

Table 7.10: Final concentration of the unknown uronic acid (mM) in undiluted bioflocculants as calculated from the standard curve (Fig.7.3) and OD values (Table 7.4 and 7.9) (Fig. 2.4)

Bioflocculants	Concentration (mM)
R2	45.330
E1	27.318
A14	26.817
A17	26.954
D1	26.361
A22	26.178

Table 7.11: OD_{585nm} values of various dilutions of bioflocculants used for the determination of hexose amine

Isolates	Undiluted	01:05	01:10	01:15	01:20
A17	0.018	0.012	0.006	0.003	0
	0.021	0.015	0.006	0.006	0.003
	0.027	0.006	0.003	0.003	0.003
Average	**0.022**	**0.011**	**0.005**	**0.004**	**0.002**
Standard deviation	0.003	0.004	0.001	0.001	0.001
A14	0.003	0	0	0	0
	0.003	0	0	0	0
	0	0	0	0	0
Average	**0.002**	**0**	**0**	**0**	**0**
Standard deviation	0.001	0	0	0	0
A22	0.003	0.003	0	0	0
	0.006	0.003	0	0	0
	0.003	0	0	0	0
Average	**0.004**	**0.002**	**0**	**0**	**0**
Standard deviation	0.001	0.001	0	0	0
D1	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
Average	**0**	**0**	**0**	**0**	**0**
Standard deviation	0	0	0	0	0
E1	0.021	0.003	0	0	0
	0.012	0.006	0	0	0
	0.018	0.003	0	0	0
Average	**0.017**	**0.004**	**0**	**0**	**0**
Standard deviation	0.00374	0.00141	0	0	0
R 2	0.003	0	0	0	0
	0.003	0.003	0	0	0
	0.003	0	0	0	0
Average	**0.003**	**0.001**	**0**	**0**	**0**
Standard deviation	0	0.00141	0	0	0
A17	0.021	0.012	0.006	0.003	0
	0.018	0.015	0.006	0.006	0.003
	0.027	0.006	0.003	0.003	0.003
Average	**0.022**	**0.011**	**0.005**	**0.004**	**0.002**
Standard deviation	0.004	0.004	0.001	0.001	0.001

Table 7.12: Final concentration of the unknown hexosamine (mM) in undiluted bioflocculants as calculated from the standard curve (Fig.7.4) and OD values (Table 7.5 and 7.11) (Fig. 2.5)

Bioflocculants	Concentration (mM)
A17	0.054
E1	0.041
A22	0.005
R2	0.002
A14	0.002
D1	0.000

Table 7.13: Growth patterns of bacterial isolates with time at OD_{550nm} (Fig. 3.1)

Isolates	0	12	24	36	48	60	72	84
E1	0.147	1.302	1.737	1.773	1.848	1.773	1.752	1.749
	0.141	1.293	1.728	1.755	1.842	1.767	1.734	1.734
	0.156	1.308	1.746	1.788	1.854	1.782	1.767	1.767
Average	**0.148**	**1.301**	**1.737**	**1.772**	**1.848**	**1.774**	**1.751**	**1.75**
Standard deviation	0.006	0.006	0.007	0.013	0.005	0.006	0.013	0.013
R 2	0.147	1.125	1.419	1.485	1.527	1.572	1.521	1.761
	0.141	1.113	1.401	1.482	1.521	1.566	1.503	1.746
	0.156	1.134	1.437	1.491	1.536	1.578	1.536	1.779
Average	**0.148**	**1.126**	**1.419**	**1.486**	**1.528**	**1.572**	**1.52**	**1.762**
Standard deviation	0.006	0.009	0.014	0.004	0.006	0.005	0.013	0.013
A17	0.147	0.768	1.35	1.383	1.644	1.632	1.683	1.44
	0.141	0.765	1.2	1.365	1.635	1.614	1.671	1.35
	0.156	0.774	1.5	1.401	1.653	1.65	1.698	1.53
Average	**0.148**	**0.769**	**1.35**	**1.383**	**1.644**	**1.632**	**1.684**	**1.44**
Standard deviation	0.006	0.004	0.122	0.015	0.007	0.015	0.011	0.073
A14	0.147	1.029	1.617	1.632	1.797	1.803	1.869	1.53
	0.141	1.008	1.602	1.62	1.8	1.785	1.86	1.515
	0.156	1.047	1.632	1.641	1.794	1.821	1.878	1.548
Average	**0.148**	**1.028**	**1.617**	**1.631**	**1.797**	**1.803**	**1.869**	**1.531**
Standard deviation	0.003	0.016	0.012	0.009	0.00245	0.0147	0.00735	0.01349
A22	0.147	0.498	1.941	1.659	1.608	1.566	1.557	1.554
	0.141	0.492	1.923	1.644	1.602	1.557	1.542	1.557
	0.156	0.504	1.959	1.674	1.617	1.572	1.572	1.554
Average	**0.148**	**0.498**	**1.941**	**1.659**	**1.609**	**1.565**	**1.557**	**1.555**
Standard deviation	0.006	0.005	0.015	0.012	0.006	0.006	0.012	0.001
D1	0.147	1.368	1.539	1.554	1.569	1.551	1.539	1.539
	0.141	1.356	1.527	1.545	1.566	1.533	1.527	1.527
	0.156	1.377	1.554	1.563	1.575	1.569	1.554	1.554
Average	**0.148**	**1.367**	**1.54**	**1.554**	**1.57**	**1.551**	**1.54**	**1.54**
Standard deviation	0.006	0.009	0.011	0.007	0.004	0.015	0.011	0.011
Control	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147
	0.141	0.141	0.141	0.141	0.141	0.141	0.141	0.141
	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156
Average	**0.148**	**0.148**	**0.148**	**0.148**	**0.148**	**0.148**	**0.148**	**0.148**
Standard deviation	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006

Table 7.14: OD_{550nm} values at 12 hr time intervals for the determination of flocculating activity

Isolates	0	12	24	36	48	60	72	84
E1	0.654	0.378	0.180	0.258	0.249	0.225	0.219	0.219
	0.648	0.375	0.270	0.240	0.237	0.219	0.204	0.204
	0.663	0.384	0.360	0.276	0.258	0.231	0.237	0.237
Average	**0.655**	**0.379**	**0.270**	**0.258**	**0.248**	**0.225**	**0.220**	**0.220**
Standard deviation	0.006	0.004	0.073	0.015	0.009	0.005	0.013	0.013
R 2	0.654	0.357	0.327	0.258	0.237	0.294	0.330	0.330
	0.648	0.354	0.315	0.255	0.225	0.3	0.315	0.315
	0.663	0.363	0.342	0.264	0.246	0.285	0.348	0.348
Average	**0.655**	**0.358**	**0.328**	**0.259**	**0.236**	**0.293**	**0.331**	**0.331**
Standard deviation	0.006	0.004	0.011	0.004	0.009	0.006	0.013	0.013
A17	0.654	0.480	0.192	0.273	0.309	0.315	0.324	0.324
	0.648	0.390	0.177	0.252	0.291	0.306	0.318	0.318
	0.663	0.570	0.104	0.291	0.324	0.321	0.333	0.333
Average	**0.655**	**0.48**	**0.191**	**0.272**	**0.308**	**0.314**	**0.325**	**0.325**
Standard deviation	0.006	0.073	0.038	0.016	0.013	0.006	0.006	0.006
A14	0.654	0.357	0.297	0.279	0.225	0.315	0.354	0.354
	0.648	0.354	0.306	0.258	0.216	0.306	0.339	0.339
	0.663	0.363	0.288	0.297	0.234	0.321	0.372	0.372
Average	**0.655**	**0.538**	**0.297**	**0.278**	**0.225**	**0.314**	**0.355**	**0.355**
Standard deviation	0.006	0.004	0.007	0.016	0.007	0.006	0.013	0.013
A22	0.654	0.576	0.273	0.219	0.219	0.330	0.333	0.333
	0.648	0.567	0.252	0.204	0.201	0.330	0.321	0.321
	0.663	0.585	0.291	0.237	0.234	0.360	0.348	0.348
Average	**0.655**	**0.576**	**0.272**	**0.22**	**0.218**	**0.33**	**0.334**	**0.334**
Standard deviation	0.006	0.007	0.015	0.013	0.013	0.014	0.011	0.011
D1	0.654	0.369	0.318	0.282	0.231	0.285	0.342	0.342
	0.648	0.360	0.306	0.297	0.213	0.279	0.336	0.336
	0.663	0.375	0.327	0.273	0.249	0.294	0.348	0.348
Average	**0.655**	**0.368**	**0.317**	**0.284**	**0.231**	**0.286**	**0.342**	**0.342**
Standard deviation	0.006	0.006	0.009	0.010	0.015	0.006	0.005	0.005
Control	0.654	0.654	0.654	0.654	0.654	0.654	0.654	0.654
	0.648	0.648	0.648	0.648	0.648	0.648	0.648	0.648
	0.663	0.663	0.663	0.663	0.663	0.663	0.663	0.663
Average	**0.655**	**0.655**	**0.655**	**0.655**	**0.655**	**0.655**	**0.655**	**0.655**
Standard deviation	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006

Table 7.15: Flocculating activity of the culture broth with time as derived from Table 7.14 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.2)

Isolate	0	12hrs	24hrs	36hrs	48hrs	60hrs	72hrs	84hrs
E1	0	1.112	2.177	2.349	2.505	2.917	3.018	3.018
R2	0	1.267	1.522	2.334	2.710	1.886	1.494	1.494
A17	0	0.556	3.709	2.149	1.720	1.658	1.550	1.550
A14	0	0.332	1.840	2.070	2.395	1.658	1.290	1.290
A22	0	0.371	2.149	3.018	3.060	1.503	1.467	1.467
D1	0	1.190	1.628	1.994	2.802	1.970	1.397	1.397
Control	0	0	0	0	0	0	0	0

Table 7.16: Percentage removal (B−A/B x 100) of kaolin clay with time by the culture broth as derived from Table 7.14 (Fig. 3.3)

Isolate	0	12	24	36	48	60	72	84
E1	0	42.137	58.779	60.611	62.137	65.649	66.412	66.412
R2	0	45.344	49.924	60.458	63.969	55.267	49.466	49.466
A17	0	26.718	70.840	58.473	52.977	52.061	50.382	50.382
A14	0	17.863	54.656	57.557	61.069	52.061	45.802	45.802
A22	0	12.672	58.473	66.412	66.718	49.618	49.008	49.008
D1	0	43.817	51.603	56.641	64.733	56.336	47.786	47.786
Control	0	0	0	0	0	0	0	0

Table 7.17: OD_{550nm} values for the determination of flocculating activity assay of the bacterial bioflocculants

Isolates	10	8	6	4	2
A17	1.308	0.948	0.750	0.570	0.324
	1.302	0.939	0.600	0.600	0.315
	1.317	0.954	0.900	0.540	0.330
Average	**1.309**	**0.947**	**0.75**	**0.570**	**0.323**
Standard deviation	0.006	0.006	0.122	0.024	0.006
A14	1.572	1.539	0.876	0.549	0.411
	1.554	1.530	0.861	0.531	0.339
	1.590	1.548	0.894	0.567	0.420
Average	**1.572**	**1.539**	**0.877**	**0.549**	**0.410**
Standard deviation	0.015	0.007	0.013	0.015	0.036
D1	1.617	0.948	0.747	0.534	0.312
	1.602	0.936	0.741	0.522	0.300
	1.635	0.957	0.753	0.549	0.321
Average	**1.618**	**0.947**	**0.747**	**0.535**	**0.311**
Standard deviation	0.013	0.009	0.005	0.011	0.009
R 2	1.317	1.185	0.594	0.465	0.300
	1.341	1.197	0.609	0.459	0.297
	1.299	1.206	0.579	0.471	0.300
Average	**1.319**	**1.197**	**0.594**	**0.465**	**0.299**
Standard deviation	0.017	0.009	0.012	0.005	0.001
E1	1.995	1.563	0.630	0.405	0.210
	2.013	1.545	0.618	0.396	0.207
	1.974	1.581	0.645	0.414	0.216
Average	**1.994**	**1.563**	**0.631**	**0.405**	**0.211**
Standard deviation	0.015	0.015	0.011	0.007	0.004
A22	1.374	1.218	0.846	0.579	0.378
	1.362	1.209	0.828	0.561	0.360
	1.383	1.224	0.864	0.597	0.396
Average	**1.373**	**1.217**	**0.846**	**0.579**	**0.378**
Standard deviation	0.009				
Control	2.883				
	2.862				
	2.892				
Average	**2.879**				
Standard deviation	0.013				

Table 7.18: Flocculating activity assay of the bacterial bioflocculants as derived from Table 7.17 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.4)

Isolate	Flocculating activity	Standard deviation
E1	3.362	0.159
A14	2.072	0.213
A17	1.885	0.407
D1	2.701	0.215
R2	2.746	0.383
A22	1.567	0.411

Table 7.19: OD_{550nm} values for the determination of flocculation of bacterial cells

Isolates	OD_{550nm}	Average	Standard deviation
Control	0.231	**2.332**	0
	0.231		
	0.231		
A14	1.929	**1.929**	0.012
	1.944		
	1.914		
A17	2.004	**2.005**	0.011
	2.019		
	1.992		
A22	1.917	**1.919**	0.010
	1.932		
	1.908		
D1	1.416	**1.417**	0.011
	1.431		
	1.404		
E1	1.200	**1.201**	0.011
	1.215		
	1.188		
R 2	2.073	**2.073**	0.007
	2.082		
	2.064		

Table 7.20: Flocculation of the bacterial cells as derived from Table 7.19 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.5)

Isolate	Flocculating activity	Standard deviation
A14	2.072	0.145
A17	1.885	0.099
A22	1.567	0.100
D1	2.701	0.152
E1	3.362	0.127
R2	1.103	0.119

Fig. 7.21: OD_{550nm} values for the determination of flocculation of microbial cultures (Fig. 3.6)

	A17	A14	D1	E1	R 2	A22	Control
P. aeruginosa	1.674	1.713	1.644	1.707	1.734	1.599	1.74
	1.689	1.725	1.659	1.722	1.731	1.632	1.728
	1.653	1.704	1.626	1.737	1.692	1.653	1.719
Average	**1.672**	**1.714**	**1.643**	**1.722**	**1.719**	**1.628**	**1.729**
Standard deviation	0.015	0.009	0.013	0.012	0.019	0.022	0.009
P. maribilis	1.395	1.344	1.362	1.299	1.344	1.344	1.398
	1.407	1.362	1.401	1.317	1.374	1.353	1.416
	1.383	1.323	1.383	1.308	1.359	1.347	1.407
Average	**1.394**	**1.343**	**1.384**	**1.308**	**1.360**	**1.348**	**1.407**
Standard deviation	0.099	0.016	0.016	0.007	0.012	0.004	0.007
S. marcescens	1.794	1.794	1.827	1.809	1.803	1.647	1.839
	1.821	1.821	1.827	1.830	1.827	1.626	1.863
	1.809	1.809	1.833	1.818	1.815	1.635	1.857
Average	**1.808**	**1.808**	**1.829**	**1.819**	**1.815**	**1.636**	**1.853**
Standard deviation	0.011	0.011	0.003	0.009	0.010	0.009	0.010
A. faecalis	0.573	0.645	0.576	0.621	0.627	0.588	0.708
	0.552	0.657	0.561	0.585	0.618	0.606	0.717
	0.564	0.651	0.567	0.606	0.621	0.6	0.696
Average	**0.563**	**0.651**	**0.568**	**0.604**	**0.622**	**0.598**	**0.707**
Standard deviation	0.009	0.005	0.006	0.015	0.004	0.007	0.009
C. fruendii	1.386	1.407	1.296	1.359	1.374	1.389	1.401
	1.365	1.374	1.32	1.335	1.341	1.371	1.389
	1.377	1.392	1.308	1.347	1.359	1.38	1.413
Average	**1.376**	**1.391**	**1.308**	**1.347**	**1.358**	**1.381**	**1.4**
Standard deviation	0.009	0.013	0.010	0.010	0.013	0.007	0.0010
B. coagulans	1.656	1.653	1.659	1.677	1.683	1.665	1.677
	1.629	1.629	1.650	1.656	1.668	1.638	1.692
	1.644	1.626	1.671	1.668	1.677	1.650	1.665
Average	**1.643**	**1.642**	**1.660**	**1.667**	**1.676**	**1.651**	**1.678**
Standard deviation	0.011	0.012	0.009	0.009	0.006	0.011	0.011
B. cereus	1.455	1.383	1.443	1.437	1.443	1.401	1.455
	1.443	1.404	1.431	1.404	1.416	1.386	1.44
	1.449	1392	1.437	1.422	1.428	1.392	1.47
Average	**1.449**	**1.393**	**1.437**	**1.421**	**1.429**	**1.393**	**1.455**
Standard deviation	0.005	0.065	0.005	0.013	0.011	0.006	0.012
C. hystolyticum	0.033	0.069	0.081	0.072	0.051	0.051	0.105
	0.024	0.036	0.048	0.051	0.036	0.069	0.078
	0.027	0.054	0.066	0.078	0.042	0.060	0.093
Average	**0.028**	**0.053**	**0.065**	**0.067**	**0.043**	**0.06**	**0.092**
Standard deviation	0.003	0.013	0.013	0.011	0.006	0.007	0.011
S. pyogenes	0.693	0.69	0.759	0.702	0.741	0.681	0.768
	0.690	0.717	0.741	0.687	0.735	0.666	0.759
	0.699	0.702	0.750	0.693	0.738	0.672	0.774
Average	**0.694**	**0.703**	**0.750**	**0.694**	**0.738**	**0.673**	**0.767**
Standard deviation	0.004	0.011	0.007	0.006	0.002	0.006	0.006
S. cerevisiae	0.087	0.075	0.099	0.093	0.054	0.003	0.111
	0.081	0.063	0.066	0.066	0.051	0	0.093
	0.093	0.069	0.084	0.081	0.06	0.003	0.102
Average	**0.087**	**0.069**	**0.083**	**0.08**	**0.055**	**0.002**	**0.102**
Standard deviation	0.005	0.005	0.013	0.011	0.004	0.001	0.007

Table 7.22: OD_{550nm} values for the determination of comparison between flocculating activity of alum and bacterial bioflocculants

Isolates	10	20	30	50
Control	0.084	0.084	0.084	0.084
	0.075	0.075	0.075	0.075
	0.069	0.069	0.069	0.069
Average	**0.076**	**0.076**	**0.076**	**0.076**
Standard deviation	0.006	0.006	0.006	0.006
Alum	0.015	0.015	0.015	0.015
	0.006	0.006	0.006	0.006
	0.021	0.021	0.021	0.021
Average	**0.014**	**0.014**	**0.014**	**0.014**
Standard deviation	0.006	0.006	0.006	0.006
D1	0.027	0.024	0.015	0.006
	0.027	0.030	0.003	0.015
	0.030	0.015	0.021	0.012
Average	**0.028**	**0.023**	**0.022**	**0.011**
Standard deviation	0.001	0.006	0.007	0.004
A14	0.063	0.054	0.033	0.024
	0.048	0.042	0.018	0.009
	0.057	0.048	0.027	0.015
Average	**0.056**	**0.048**	**0.0026**	**0.016**
Standard deviation	0.006	0.005	0.006	0.006
A17	0.024	0.015	0.006	0.009
	0.033	0.027	0.015	0.003
	0.027	0.036	0.012	0.006
Average	**0.028**	**0.026**	**0.011**	**0.006**
Standard deviation	0.004	0.009	0.004	0.002
A22	0.033	0.027	0.009	0.006
	0.027	0.009	0.006	0.003
	0.039	0.018	0.006	0.006
Average	**0.033**	**0.018**	**0.007**	**0.005**
Standard deviation	0.005	0.007	0.001	0.00
R 2	0.03	0.027	0.024	0.123
	0.015	0.012	0.006	0.111
	0.021	0.018	0.015	0.096
Average	**0.022**	**0.019**	**0.015**	**0.011**
Standard deviation	0.006	0.006	0.007	0.011

Table 7.23: Comparison between flocculating activity of alum and bacterial bioflocculants as derived from Table 7.22 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.7)

Conc (ppm)	Control	Alum	D1	A14	A17	A22	R2	E1	Standard deviation
10	0	58.271	22.556	4.699	22.556	17.145	32.297	13.869	1.407
20	0	0	30.32	7.675	25.304	42.398	39.474	15.413	1.853
30	0	0	32.197	36.842	77.751	129.699	53.509	17.145	0.707
50	0	0	77.751	49.342	153.589	186.842	77.751	42.397	1.400

Table 7.24: OD_{550nm} values for the determination of effect of pH on the flocculating activity

pH6	Isolates	10	8	6	4	2
	A22	0.387	0.375	0.261	0.261	0.198
		0.393	0.387	0.300	0.264	0.213
		0.390	0.381	0.297	0.261	0.207
	Average	**0.390**	**0.381**	**0.297**	**0.262**	**0.206**
	Standard deviation	0.002	0.005	0.018	0.001	0.006
	D1	0.306	0.285	0.261	0.243	0.204
		0.318	0.300	0.279	0.246	0.219
		0.312	0.294	0.276	0.243	0.213
	Average	**0.312**	**0.293**	**0.272**	**0.244**	**0.212**
	Standard deviation	0.005	0.006	0.008	0.001	0.006
	A17	0.369	0.300	0.273	0.267	0.195
		0.381	0.315	0.297	0.273	0.210
		0.375	0.309	0.291	0.267	0.204
	Average	**0.375**	**0.308**	**0.287**	**0.269**	**0.203**
	Standard deviation	0.005	0.006	0.010	0.003	0.006
	A14	0.336	0.240	0.222	0.204	0.147
		0.339	0.258	0.228	0.219	0.159
		0.336	0.255	0.222	0.210	0.153
	Average	**0.337**	**0.251**	**0.224**	**0.211**	**0.153**
	Standard deviation	0.001	0.008	0.003	0.006	0.005
	E1	0.324	0.270	0.249	0.243	0.180
		0.333	0.279	0.258	0.252	0.186
		0.327	0.273	0.252	0.246	0.183
	Average	**0.328**	**0.274**	**0.253**	**0.247**	**0.183**
	Standard deviation	0.004	0.004	0.004	0.004	0.00245
	R 2	0.387	0.246	0.195	0.192	0.111
		0.393	0.255	0.213	0.201	0.126
		0.387	0.249	0.204	0.195	0.120
	Average	**0.389**	**0.250**	**0.204**	**0.196**	**0.119**
	Standard deviation	0.003	0.004	0.007	0.004	0.006
	Control	2.043				
		2.160				
		2.100				
	Standard deviation	0.048				

pH7	Isolates	10	8	6	4	2
	A22	0.366	0.297	0.261	0.249	0.201
		0.375	0.285	0.267	0.252	0.207
		0.357	0.27	0.258	0.246	0.198
	Average	**0.366**	**0.284**	**0.262**	**0.249**	**0.202**
	Standard deviation	0.007	0.011	0.004	0.002	0.004
	D1	0.330	0.249	0.237	0.225	0.126
		0.342	0.252	0.246	0.228	0.123
		0.321	0.246	0.228	0.222	0.132
	Average	**0.331**	**0.249**	**0.237**	**0.225**	**0.127**
	Standard deviation	0.009	0.002	0.007	0.002	0.004
	A17	0.357	0.339	0.261	0.213	0.171
		0.354	0.33	0.255	0.195	0.162
		0.366	0.333	0.270	0.228	0.177
	Average	**0.359**	**0.334**	**0.262**	**0.212**	**0.17**
	Standard deviation	0.005	0.004	0.006	0.013	0.006
	A14	0.357	0.333	0.261	0.213	0.171
		0.354	0.318	0.255	0.195	0.162
		0.366	0.351	0.270	0.228	0.177
	Average	**0.359**	**0.334**	**0.262**	**0.212**	**0.170**
	Standard deviation	0.005	0.014	0.006	0.013	0.006
	E1	0.327	0.318	0.294	0.228	0.216
		0.309	0.306	0.291	0.216	0.201
		0.342	0.33	0.297	0.237	0.231
	Average	**0.326**	**0.318**	**0.294**	**0.227**	**0.216**
	Standard deviation	0.013	0.010	0.002	0.009	0.012
	R 2	0.345	0.339	0.237	0.213	0.192
		0.336	0.327	0.225	0.201	0.186
		0.357	0.354	0.246	0.225	0.198
	Average	**0.346**	**0.34**	**0.236**	**0.213**	**0.192**
	Standard deviation	0.009	0.011	0.009	0.010	0.005
	Control	2.256				
		2.256				
		2.259				
	Average	**2.257**				
	Standard deviation	0.001				

pH8	Isolates	10	8	6	4	2
	A22	0.615	0.507	0.438	0.216	0.153
		0.621	0.516	0.447	0.228	0.138
		0.612	0.495	0.432	0.207	0.165
	Average	**0.616**	**0.506**	**0.439**	**0.217**	**0.152**
	Standard deviation	0.004	0.009	0.006	0.009	0.011
	D1	0.825	0.726	0.591	0.315	0.138
		0.828	0.714	0.588	0.321	0.150
		0.819	0.735	0.597	0.309	0.129
	Average	**0.824**	**0.725**	**0.592**	**0.315**	**0.139**
	Standard deviation	0.004	0.009	0.004	0.005	0.009
	A17	0.759	0.561	0.426	0.318	0.156
		0.771	0.555	0.435	0.312	0.156
		0.744	0.567	0.420	0.324	0.159
	Average	**0.758**	**0.561**	**0.427**	**0.318**	**0.157**
	Standard deviation	0.011	0.005	0.006	0.005	0.001
	A14	0.690	0.552	0.339	0.207	0.102
		0.681	0.543	0.333	0.201	0.090
		0.702	0.558	0.342	0.216	0.111
	Average	**0.691**	**0.551**	**0.338**	**0.208**	**0.101**
	Standard deviation	0.009	0.006	0.004	0.006	0.009
	E1	0.834	0.726	0.585	0.372	0.201
		0.822	0.717	0.573	0.366	0.192
		0.849	0.738	0.594	0.375	0.207
	Average	**0.835**	**0.727**	**0.584**	**0.371**	**0.200**
	Standard deviation	0.011	0.009	0.009	0.004	0.006
	R 2	0.450	0.546	0.486	0.309	0.138
		0.450	0.540	0.474	0.306	0.123
		0.450	0.555	0.495	0.312	0.150
	Average	**0.600**	**0.547**	**0.485**	**0.309**	**0.137**
	Standard deviation	0	0.006	0.0089	0.002	0.011
	Control	1.809				
		1.794				
		1.821				
	Average	**1.808**				
	Standard deviation	0.011				

pH9	Isolates	10	8	6	4	2
	A22	0.891	0.684	0.513	0.423	0.255
		0.885	0.675	0.504	0.411	0.252
		0.897	0.690	0.519	0.435	0.261
	Average	**0.891**	**0.683**	**0.512**	**0.423**	**0.256**
	Standard deviation	0.005	0.006	0.006	0.010	0.004
	D1	0.714	0.627	0.588	0.414	0.222
		0.711	0.621	0.582	0.408	0.219
		0.741	0.633	0.597	0.423	0.228
	Average	**0.715**	**0.627**	**0.589**	**0.415**	**0.223**
	Standard deviation	0.01	0.005	0.006	0.006	0.004
	A17	0.873	0.723	0.618	0.405	0.210
		0.855	0.717	0.612	0.399	0.201
		0.888	0.729	0.627	0.408	0.222
	Average	**0.872**	**0.723**	**0.619**	**0.404**	**0.211**
	Standard deviation	0.013	0.005	0.006	0.004	0.009
	A14	0.918	0.783	0.630	0.573	0.234
		0.912	0.783	0.624	0.561	0.222
		0.927	0.780	0.639	0.582	0.249
	Average	**0.919**	**0.782**	**0.631**	**0.572**	**0.235**
	Standard deviation	0.006	0.001	0.006	0.009	0.011
	E1	0.657	0.567	0.405	0.216	0.138
		0.648	0.564	0.408	0.207	0.129
		0.69	0.576	0.399	0.225	0.147
	Average	**0.658**	**0.569**	**0.404**	**0.216**	**0.138**
	Standard deviation	0.018	0.005	0.004	0.007	0.007
	R 2	0.831	0.78	0.528	0.303	0.219
		0.816	0.771	0.519	0.294	0.213
		0.849	0.792	0.537	0.312	0.225
	Average	**0.832**	**0.781**	**0.528**	**0.303**	**0.219**
	Standard deviation	0.014	0.009	0.007	0.007	0.005
	Control	2.313				
		2.304				
		2.322				
	Average	**2.313**				
	Standard deviation	0.007				

pH10	Isolates	10	8	6	4	2
	A22	0.825	0.684	0.438	0.216	0.159
		0.813	0.675	0.432	0.204	0.156
		0.834	0.690	0.447	0.225	0.156
	Average	**0.824**	**0.683**	**0.439**	**0.215**	**0.158**
	Standard deviation	0.009	0.006	0.006	0.009	0.001
	D1	0.765	0.675	0.531	0.357	0.210
		0.753	0.669	0.525	0.348	0.195
		0.777	0.678	0.540	0.366	0.228
	Average	**0.765**	**0.674**	**0.532**	**0.357**	**0.211**
	Standard deviation	0.010	0.004	0.006	0.007	0.013
	A17	0.951	0.732	0.618	0.504	0.231
		0.945	0.720	0.606	0.492	0.222
		0.957	0.741	0.627	0.519	0.243
	Average	**0.951**	**0.731**	**0.617**	**0.505**	**0.232**
	Standard deviation	0.005	0.0086	0.008602325	0.01105	0.0086
	A14	0.738	0.663	0.519	0.378	0.201
		0.732	0.651	0.516	0.369	0.192
		0.744	0.678	0.522	0.384	0.207
	Average	**0.738**	**0.664**	**0.519**	**0.377**	**0.2**
	Standard deviation	0.005	0.011	0.002	0.006	0.006
	E1	0.618	0.582	0.426	0.222	0.162
		0.612	0.570	0.429	0.222	0.153
		0.627	0.591	0.426	0.225	0.168
	Average	**0.619**	**0.581**	**0.427**	**0.223**	**0.161**
	Standard deviation	0.006	0.009	0.001	0.001	0.006
	R 2	0.594	0.402	0.357	0.255	0.138
		0.597	0.393	0.345	0.243	0.132
		0.588	0.414	0.369	0.267	0.147
	Average	**0.593**	**0.403**	**0.357**	**0.255**	**0.139**
	Standard deviation	0.004	0.009	0.010	0.010	0.006
	Control	1.473				
		1.464				
		1.479				
	Average	**1.472**				
	Standard deviation	0.006				

Table 7.25: Effect of pH on the flocculating activity as derived from Table 7.24 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.8)

pH	A22	D1	A17	A14	E1	R2	Standard deviation
6	2.169	1.519	2.144	4.867	3.278	5.356	0.137
7	3.653	5.669	4.359	4.359	3.034	4.359	0.158
8	2.745	2.316	2.389	3.448	1.601	2.232	0.385
9	1.863	1.591	1.984	1.295	3.664	2.605	0.217
10	2.586	2.992	2.625	2.918	2.175	2.301	0.417

Table 7.26: OD_{550nm} values for the determination of effect of temperature on flocculating activity

28^0C	Isolates	10	8	6	4	2
	A22	0.513	0.459	0.435	0.333	0.216
		0.495	0.444	0.459	0.321	0.222
		0.507	0.471	0.447	0.327	0.225
	Average	**0.505**	**0.452**	**0.447**	**0.327**	**0.221**
	Standard deviation	0.007	0.011	0.010	0.005	0.004
	D1	0.558	0.471	0.417	0.381	0.294
		0.540	0.453	0.408	0.357	0.276
		0.549	0.462	0.411	0.375	0.285
	Average	**0.549**	**0.462**	**0.412**	**0.371**	**0.285**
	Standard deviation	0.007	0.007	0.004	0.010	0.007
	A17	0.501	0.480	0.429	0.405	0.273
		0.471	0.450	0.411	0.384	0.243
		0.492	0.471	0.420	0.393	0.258
	Average	**0.488**	**0.467**	**0.42**	**0.394**	**0.258**
	Standard deviation	0.013	0.012	0.007	0.009	0.012
	A14	0.417	0.375	0.363	0.333	0.327
		0.402	0.366	0.354	0.306	0.309
		0.411	0.369	0.357	0.321	0.321
	Average	**0.410**	**0.370**	**0.358**	**0.320**	**0.317**
	Standard deviation	0.006	0.004	0.004	0.011	0.007
	E1	0.345	0.339	0.285	0.273	0.255
		0.318	0.318	0.303	0.264	0.246
		0.336	0.330	0.294	0.267	0.249
	Average	**0.332**	**0.329**	**0.294**	**0.268**	**0.250**
	Standard deviation	0.011	0.009	0.007	0.004	0.004
	R 2	0.351	0.288	0.273	0.261	0.243
		0.339	0.294	0.261	0.252	0.228
		0.345	0.297	0.267	0.255	0.237
	Average	**0.345**	**0.293**	**0.267**	**0.256**	**0.236**
		0.005	0.004	0.005	0.007	0.006
	Control	2.874				
		2.883				
		2.868				
	Average	**2.875**				
	Standard deviation	0.006				

37^0C	Isolates	10	8	6	4	2
	A22	0.657	0.495	0.429	0.354	0.282
		0.636	0.471	0.405	0.348	0.264
		0.648	0.483	0.423	0.351	0.273
	Average	**0.647**	**0.483**	**0.419**	**0.351**	**0.273**
	Standard deviation	0.009	0.010	0.010	0.002	0.007
	D1	0.585	0.489	0.447	0.381	0.243
		0.555	0.462	0.432	0.363	0.234
		0.576	0.486	0.438	0.372	0.237
	Average	**0.572**	**0.479**	**0.439**	**0.372**	**0.238**
	Standard deviation	0.012	0.012	0.006	0.007	0.004
	A17	0.477	0.423	0.402	0.321	0.294
		0.456	0.408	0.402	0.294	0.279
		0.465	0.417	0.399	0.309	0.285
	Average	**0.466**	**0.416**	**0.401**	**0.308**	**0.286**
	Standard deviation	0.009	0.006	0.001	0.011	0.006
	A14	0.366	0.363	0.345	0.267	0.210
		0.381	0.357	0.321	0.249	0.210
		0.381	0.357	0.336	0.258	0.210
	Average	**0.376**	**0.359**	**0.334**	**0.258**	**0.021**
	Standard deviation	0.007	0.003	0.010	0.007	0
	E1	0.261	0.243	0.243	0.240	0.168
		0.249	0.249	0.240	0.228	0.144
		0.255	0.252	0.240	0.234	0.156
	Average	**0.255**	**0.248**	**0.241**	**0.734**	**0.156**
	Standard deviation	0.005	0.004	0.001	0.004	0.010
	R 2	0.225	0.273	0.261	0.213	0.189
		0.213	0.267	0.240	0.198	0.159
		0.219	0.270	0.249	0.207	0.174
	Average	**0.219**	**0.270**	**0.250**	**0.206**	**0.174**
	Standard deviation	0.005	0.002	0.009	0.006	0.012
	Control	2.874				
		2.883				
		2.868				
	Average	**2.875**				
	Standard deviation	0.006				

Table 7.27: Effect of temperature on the flocculating activity as derived from Table 7.26 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.9)

	A22	D1	A17	A14	E1	R2
37^oC	3.301	2.707	2.430	5.138	9.953	9.478
Standard deviation	0.183	0.178	0.168	0.315	0.163	0.155
28^oC	2.796	1.928	2.155	0.625	5.395	6.585
Standard deviation	0.217	0.300	0.041	0.091	0.382	0.110

Table 7.28: OD_{550nm} values for the determination of effect of cationic compounds on the flocculating activity

CTAB	Isolates	2	4	6	8	10
	A22	0.348	0.414	0.528	0.597	0.657
		0.348	0.387	0.498	0.582	0.648
		0.354	0.402	0.519	0.588	0.651
	Average	**0.350**	**0.401**	**0.515**	**0.234**	**0.652**
	Standard deviation	0.003	0.011	0.013	0.006	0.004
	D1	0.237	0.423	0.576	0.651	0.702
		0.240	0.402	0.573	0.639	0.699
		0.237	0.414	0.570	0.642	0.699
	Average	**0.238**	**0.413**	**0.571**	**0.644**	**0.7**
	Standard deviation	0.001	0.009	0.002	0.005	0.001
	A17	0.276	0.351	0.567	0.651	0.660
		0.264	0.351	0.552	0.636	0.669
		0.267	0.354	0.561	0.645	0.681
	Average	**0.269**	**0.352**	**0.560**	**0.644**	**0.670**
	Standard deviation	0.005	0.001	0.006	0.006	0.009
	A14	0.321	0.432	0.597	0.603	0.621
		0.297	0.402	0.594	0.630	0.642
		0.309	0.423	0.594	0.618	0.630
	Average	**0.309**	**0.419**	**0.595**	**0.617**	**0.631**
	Standard deviation	0.010	0.013	0.001	0.011	0.009
	E1	0.195	0.327	0.432	0.597	0.666
		0.174	0.321	0.438	0.588	0.645
		0.186	0.324	0.441	0.591	0.657
	Average	**0.182**	**0.324**	**0.437**	**0.592**	**0.656**
	Standard deviation	0.009	0.002	0.004	0.004	0.009
	R 2	0.213	0.387	0.477	0.687	0.735
		0.195	0.375	0.462	0.672	0.729
		0.204	0.381	0.468	0.678	0.732
	Average	**0.204**	**0.381**	**0.469**	**0.679**	**0.732**
	Standard deviation	0.007	0.005	0.006	0.006	0.002
	Control	2.718				
		2.688				
		2.709				
	Average	**2.705**				
	Standard deviation	0.013				

$MgSO_4$	Isolates	2	4	6	8	10
	A22	0.246	0.393	0.477	0.564	0.675
		0.216	0.381	0.468	0.546	0.669
		0.231	0.387	0.471	0.555	0.672
	Average	**0.231**	**0.387**	**0.471**	**0.555**	**0.672**
	Standard deviation	0.013	0.005	0.004	0.007	0.002
	D1	0.258	0.426	0.519	0.624	0.687
		0.240	0.423	0.495	0.606	0.678
		0.249	0.432	0.507	0.615	0.681
	Average	**0.248**	**0.432**	**0.507**	**0.615**	**0.682**
	Standard deviation	0.007	0.004	0.010	0.007	0.004
	A17	0.378	0.465	0.471	0.531	0.624
		0.351	0.435	0.450	0.516	0.615
		0.366	0.456	0.459	0.522	0.618
	Average	**0.365**	**0.452**	**0.46**	**0.523**	**0.619**
	Standard deviation	0.011	0.013	0.009	0.006	0.004
	A14	0.252	0.396	0.465	0.537	0.645
		0.264	0.363	0.456	0.519	0.624
		0.258	0.381	0.459	0.528	0.633
	Average	**0.258**	**0.380**	**0.460**	**0.528**	**0.634**
	Standard deviation	0.005	0.014	0.004	0.007	0.009
	E1	0.363	0.447	0.591	0.597	0.633
		0.384	0.411	0.585	0.588	0.618
		0.372	0.426	0.588	0.591	0.624
	Average	**0.373**	**0.431**	**0.588**	**0.592**	**0.625**
	Standard deviation	0.009	0.015	0.004	0.004	0.006
	R 2	0.315	0.477	0.516	0.693	0.708
		0.291	0.462	0.528	0.675	0.669
		0.303	0.468	0.528	0.684	0.699
	Average	**0.303**	**0.469**	**0.524**	**0.684**	**0.692**
	Standard deviation	0.010	0.006	0.006	0.007	0.017
	Control	2.742				
		2.733				
		2.751				
	Average	**2.742**				
	Standard deviation	0.007				

$MnCl_2$	Isolates	2	4	6	8	10
	A22	0.207	0.423	0.447	0.579	0.609
		0.201	0.405	0.435	0.579	0.594
		0.216	0.441	0.459	0.582	0.621
	Average	**0.208**	**0.423**	**0.447**	**0.58**	**0.608**
	Standard deviation	0.006	0.015	0.099	0.001	0.011
	D1	0.306	0.480	0.540	0.576	0.690
		0.297	0.468	0.528	0.561	0.681
		0.318	0.492	0.555	0.588	0.702
	Average	**0.307**	**0.48**	**0.541**	**0.575**	**0.691**
	Standard deviation	0.009	0.010	0.011	0.011	0.009
	A17	0.279	0.477	0.501	0.579	0.582
		0.267	0.471	0.495	0.573	0.576
		0.288	0.486	0.507	0.582	0.591
	Average	**0.278**	**0.478**	**0.501**	**0.578**	**0.583**
	Standard deviation	0.009	0.006	0.005	0.004	0.006
	A14	0.336	0.462	0.477	0.525	0.657
		0.330	0.465	0.471	0.519	0.642
		0.345	0.462	0.486	0.531	0.669
	Average	**0.337**	**0.463**	**0.478**	**0.525**	**0.656**
	Standard deviation	0.006	0.001	0.006	0.005	0.011
	E1	0.372	0.456	0.477	0.576	0.594
		0.363	0.441	0.477	0.567	0.588
		0.378	0.468	0.483	0.582	0.597
	Average	**0.371**	**0.455**	**0.479**	**0.575**	**0.593**
	Standard deviation	0.006	0.011	0.003	0.006	0.004
	R 2	0.300	0.417	0.465	0.468	0.486
		0.297	0.402	0.459	0.465	0.48
		0.306	0.432	0.471	0.474	0.495
	Average	**0.301**	**0.417**	**0.465**	**0.469**	**0.487**
	Standard deviation	0.004	0.012	0.005	0.004	0.006
	Control	2.853				
		2.838				
		2.868				
	Average	**2.853**				
	Standard deviation	0.012				

$CaCl_2$	Isolates	10	8	6	4	2
	A22	1.308	0.948	0.750	0.570	0.324
		1.302	0.939	0.720	0.480	0.315
		1.317	0.954	0.780	0.660	0.330
	Average	**1.309**	**0.947**	**0.750**	**0.510**	**0.323**
	Standard deviation	0.006	0.006	0.024	0.073	0.006
	D1	1.572	1.539	0.876	0.549	0.411
		1.569	1.527	0.867	0.543	0.402
		1.575	1.551	0.888	0.555	0.417
	Average	**1.572**	**1.539**	**0.877**	**0.549**	**0.410**
	Standard deviation	0.003	0.0010	0.009	0.005	0.006
	A17	1.617	0.948	0.747	0.534	0.312
		1.611	0.939	0.738	0.525	0.303
		1.626	0.954	0.756	0.546	0.318
	Average	**1.618**	**0.947**	**0.747**	**0.535**	**0.311**
	Standard deviation	0.006	0.006	0.007	0.009	0.006
	A14	1.317	1.197	0.594	0.465	0.297
		1.311	1.200	0.600	0.456	0.300
		1.329	1.194	0.588	0.474	0.300
	Average	**1.319**	**1.197**	**0.594**	**0.465**	**0.299**
	Standard deviation	0.007	0.002	0.005	0.007	0.001
	E1	1.995	1.563	0.630	0.405	0.210
		1.986	1.554	0.621	0.402	0.198
		2.001	1.572	0.642	0.408	0.225
	Average	**1.994**	**1.563**	**0.631**	**0.405**	**0.211**
	Standard deviation	0.006	0.007	0.009	0.002	0.011
	R 2	1.374	1.218	0.846	0.579	0.378
		1.365	1.206	0.837	0.561	0.369
		1.380	1.227	0.855	0.597	0.387
	Average	**1.373**	**1.217**	**0.846**	**0.579**	**0.378**
	Standard deviation	0.006	0.009	0.007	0.015	0.007
	Control	2.877				
		2.874				
		2.886				
	Average	**2.879**				
	Standard deviation	0.0051				

Table 7.29: Effect of cationic compounds on the flocculating activity as derived from Table 7.28 using an equation for flocculating activity in Section 3.2.1 (Fig. 3.10)

Isolate	CTAB	$MgSO_4$	$MnCl_2$	$CaCl_2$
A22	0.244	2.450	2.821	1.567
Standard deviation	0.111	0.003	0.004	0.084
D1	2.027	1.954	0.941	2.701
Standard deviation	0.125	0.139	0.013	0.027
A17	2.109	0.836	1.414	1.885
	0.125	0.171	0.398	0.300
A14	0.989	2.087	0.550	2.072
Standard deviation	0.133	0.217	0.147	0.168
E1	3.253	0.542	1.104	3.362
Standard deviation	0.099	0.155	0.119	0.161
R2	1.713	1.085	0.695	2.746
Standard deviation	0.0238	0.178	0.119	0.100

Table 7.30: OD_{550nm} values for the determination of flocculation inhibition assay at different concentration of potential inhibitors (Table 3.1)

E1	Conc (ppm)	K_2HPO_4	$NaNO_3$	CH_2COONa	Na_2CO_3	D-GLU
	10 000	0.747	0.010	0.919	1.025	0.015
	7 500	0.743	0.115	0.859	0.886	0.112
	5 000	0.663	0.221	0.737	0.740	0.135
	2 500	0.612	0.239	0.391	0.518	0.233
	1 000	0.471	0.257	0.242	0.495	0.241
	500	0.285	0.261	0.207	0.301	0.257
	100	0.253	0.283	0.142	0.218	0.262
	10	0.131	0.298	0.113	0.151	0.283

A14	Conc (ppm)	K_2HPO_4	$NaNO_3$	CH_2COONa	Na_2CO_3	D-GLU
	10 000	0.697	0.125	0.951	0.954	0.124
	7 500	0.449	0.175	0.787	0.842	0.151
	5 000	0.431	0.191	0.635	0.729	0.210
	2 500	0.385	0.217	0.299	0.677	0.254
	1 000	0.255	0.228	0.282	0.445	0.253
	500	0.227	0.256	0.251	0.215	0.291
	100	0.213	0.282	0.173	0.201	0.315
	10	0.157	0.291	0.139	0.123	0.337

DI	Conc (ppm)	K_2HPO_4	$NaNO_3$	CH_2COONa	Na_2CO_3	D-GLU
	10 000	0.953	0.117	0.640	1.570	0.110
	7 500	0.875	0.158	0.511	1.385	0.171
	5 000	0.724	0.172	0.382	0.612	0.218
	2 500	0.453	0.205	0.315	0.367	0.235
	1 000	0.320	0.213	0.297	0.359	0.257
	500	0.268	0.237	0.273	0.392	0.291
	100	0.241	0.281	0.162	0.261	0.297
	10	0.157	0.299	0.138	0.254	0.301

A17	Conc (ppm)	K_2HPO_4	$NaNO_3$	CH_2COONa	Na_2CO_3	D-GLU
	10 000	0.872	0.139	0.903	0.825	0.105
	7 500	0.777	0.202	0.824	0.633	0.127
	5 000	0.653	0.218	0.688	0.618	0.153
	2 500	0.417	0.257	0.308	0.542	0.192
	1 000	0.251	0.289	0.273	0.486	0.215
	500	0.232	0.260	0.277	0.320	0.238
	100	0.213	0.277	0.285	0.218	0.261
	10	0.135	0.286	0.193	0.207	0.266

R2	Conc (ppm)	K_2HPO_4	$NaNO_3$	CH_2COONa	Na_2CO_3	D-GLU
	10 000	1.411	0.119	0.937	0.993	0.151
	7 500	0.824	0.151	0.612	0.838	0.172
	5 000	0.740	0.212	0.315	0.542	0.198
	2 500	0.472	0.228	0.298	0.475	0.211
	1 000	0.257	0.237	0.263	0.396	0.223
	500	0.226	0.284	0.257	0.207	0.261
	100	0.212	0.289	0.209	0.172	0.281
	10	0.181	0.311	0.132	0.193	0.307

A22	Conc (ppm)	K_2HPO_4	$NaNO_3$	CH_2COONa	Na_2CO_3	D-GLU
	10 000	1.534	0.032	0.897	1.868	0.195
	7 500	0.985	0.105	0.738	1.835	0.172
	5 000	0.716	0.138	0.651	0.967	0.132
	2 500	0.567	0.213	0.325	0.857	0.258
	1 000	0.312	0.228	0.255	0.621	0.217
	500	0.267	0.257	0.281	0.426	0.231
	100	0.231	0.275	0.215	0.311	0.209
	10	0.216	0.288	0.173	0.230	0.317

Table 7.31: OD_{550nm} values for the determination of flocculating activity of whale dye using different concentrations of bioflocculants

Isolates	10	8	6	4	2
E1	0.165	0.174	0.369	0.501	0.561
	0.156	0.165	0.363	0.492	0.555
	0.174	0.180	0.378	0.510	0.567
Average	**0.165**	**0.173**	**0.370**	**0.501**	**0.561**
Standard deviation	0.007	0.006	0.006	0.007	0.005
D1	0.009	0.252	0.333	0.543	0.612
	0.003	0.252	0.321	0.528	0.591
	0.015	0.255	0.342	0.558	0.630
Average	**0.009**	**0.253**	**0.332**	**0.543**	**0.611**
Standard deviation	0.005	0.001	0.009	0.012	0.016
R 2	0.081	0.168	0.186	0.270	0.543
	0.072	0.159	0.177	0.255	0.543
	0.087	0.177	0.192	0.288	0.546
Average	**0.080**	**0.168**	**0.185**	**0.271**	**0.544**
Standard deviation	0.006	0.007	0.006	0.013	0.00141
A22	0.054	0.237	0.264	0.360	0.507
	0.039	0.234	0.261	0.348	0.495
	0.072	0.246	0.267	0.375	0.519
Average	**0.055**	**0.239**	**0.264**	**0.361**	**0.507**
Standard deviation	0.013	0.005	0.002	0.011	0.010
A17	0.057	0.096	0.138	0.255	0.456
	0.048	0.096	0.126	0.246	0.494
	0.066	0.099	0.150	0.261	0.465
Average	**0.057**	**0.097**	**0.138**	**0.254**	**0.427**
Standard deviation	0.007	0.001	0.010	0.006	0.016
A14	0.045	0.063	0.075	0.180	0.414
	0.030	0.051	0.063	0.165	0.423
	0.060	0.072	0.087	0.198	0.417
Average	**0.045**	**0.062**	**0.075**	**0.181**	**0.418**
Standard deviation	0.012	0.009	0.010	0.013	0.004
Control	1.521				
	1.512				
	1.527				
Average	**1.520**				
Standard deviation	0.006				

Table 7.32: Percentage removal (C_0–C/C_0) of whale dye using different concentrations of flocculants as derived from Table 7.31 (Fig. 4.1)

Conc (ppm)	E1	D1	R2	A22	A17	A14
10	89.145	99.408	94.737	96.382	96.118	97.039
8	88.618	83.355	88.947	84.276	93.618	95.921
6	75.658	78.158	87.829	82.632	90.921	95.066
4	67.039	64.276	82.171	76.250	83.289	88.092
2	63.092	59.803	70.066	66.645	69.934	72.500

Table 7.33: Flocculating activity of the whale dye as derived from Table 7.31 using an equation for flocculating activity in Section 4.2.3 (Fig. 4.2)

Isolate	Flocculating activity	Standard deviation
E1	9.397	0.132
D1	8.552	0.009
R2	7.688	0.156
A22	8.473	0.136
A17	6.904	0.022
A14	6.075	0.198

Table 7.34: OD_{550nm} values for the determination of effect of pH on the removal of whale dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.3)

pH6	Isolates	10	8	6	4	2
	E1	0.171	0.213	0.324	0.399	0.447
		0.162	0.210	0.324	0.390	0.441
		0.180	0.216	0.327	0.405	0.450
	Average	**0.171**	**0.213**	**0.325**	**0.398**	**0.446**
	Standard deviation	0.007	0.002	0.001	0.006	0.004
	D1	0.177	0.195	0.243	0.435	0.474
		0.174	0.186	0.237	0.426	0.465
		0.186	0.201	0.246	0.441	0.480
	Average	**0.179**	**0.194**	**0.242**	**0.434**	**0.473**
	Standard deviation	0.005	0.006	0.004	0.006	0.006
	R 2	0.165	0.285	0.330	0.387	0.468
		0.153	0.279	0.327	0.387	0.459
		0.177	0.291	0.333	0.390	0.474
	Average	**0.165**	**0.285**	**0.330**	**0.388**	**0.467**
	Standard deviation	0.010	0.005	0.002	0.001	0.006
	A22	0.267	0.306	0.402	0.471	0.492
		0.252	0.297	0.396	0.468	0.489
		0.279	0.312	0.405	0.477	0.495
	Average	**0.266**	**0.305**	**0.401**	**0.472**	**0.492**
	Standard deviation	0.011	0.006	0.004	0.004	0.00245
	A17	0.264	0.321	0.381	0.501	0.519
		0.249	0.318	0.381	0.492	0.519
		0.279	0.327	0.384	0.507	0.522
	Average	**0.264**	**0.322**	**0.382**	**0.5**	**0.52**
	Standard deviation	0.012	0.004	0.001	0.006	0.001
	A14	0.156	0.198	0.237	0.474	0.489
		0.141	0.189	0.231	0.489	0.504
		0.171	0.207	0.246	0.459	0.498
	Average	**0.156**	**0.195**	**0.238**	**0.474**	**0.497**
	Standard deviation	0.012	0.023	0.006	0.012	0.006
	Control	1.551				
		1.542				
		1.557				
	Average	**1.550**				
	Standard deviation	0.006				

pH 6	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	88.968	88.452	89.08	82.839	82.968	89.935
	8	86.258	87.284	81.138	80.323	79.226	87.226
	6	79.032	84.387	78.16	74.129	75.355	84.645
	4	74.323	72.000	74.322	69.548	67.742	69.419
	2	71.226	69.484	69.093	68.258	66.452	67.935

pH 7	Isolates	10	8	6	4	2
	E1	0.036	0.063	0.102	0.129	0.444
		0.030	0.054	0.099	0.120	0.441
		0.039	0.069	0.105	0.135	0.447
	Average	**0.035**	**0.062**	**0.102**	**0.128**	**0.444**
	Standard deviation	0.004	0.006	0.002	0.006	0.002
	D1	0.021	0.042	0.078	0.312	0.345
		0.018	0.039	0.075	0.309	0.336
		0.024	0.045	0.084	0.315	0.351
	Average	**0.021**	**0.042**	**0.079**	**0.312**	**0.344**
	Standard deviation	0.002	0.002	0.004	0.002	0.006
	R 2	0.093	0.156	0.237	0.504	0.528
		0.084	0.153	0.234	0.501	0.525
		0.099	0.159	0.243	0.507	0.534
	Average	**0.092**	**0.156**	**0.238**	**0.504**	**0.529**
	Standard deviation	0.006	0.002	0.004	0.002	0.004
	A22	0.069	0.276	0.315	0.420	0.528
		0.084	0.273	0.309	0.417	0.522
		0.072	0.282	0.318	0.426	0.534
	Average	**0.070**	**0.277**	**0.314**	**0.421**	**0.527**
	Standard deviation	0.006	0.004	0.004	0.004	0.005
	A17	0.105	0.339	0.378	0.390	0.483
		0.099	0.339	0.372	0.381	0.480
		0.111	0.342	0.381	0.396	0.486
	Average	**0.105**	**0.340**	**0.377**	**0.389**	**0.483**
	Standard deviation	0.005	0.001	0.004	0.006	0.002
	A14	0.078	0.123	0.324	0.438	0.483
		0.075	0.120	0.328	0.432	0.480
		0.084	0.126	0.327	0.441	0.486
	Average	**0.079**	**0.123**	**0.323**	**0.437**	**0.483**
	Standard deviation	0.004	0.002	0.002	0.004	0.002
	Control	1.512				
		1.506				
		1.515				
	Average	**1.511**				
	Standard deviation	0.004				

pH 7	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	97.684	98.610	94.065	90.012	93.051	94.772
	8	95.897	97.220	89.935	75.050	77.498	91.794
	6	93.249	94.772	84.645	74.249	75.314	78.623
	4	91.529	79.351	67.484	74.076	74.255	71.079
	2	70.612	77.234	65.871	70.439	68.034	68.034

pH 8	Isolates	10	8	6	4	2
	E1	0.264	0.411	0.420	0.564	0.603
		0.258	0.405	0.423	0.558	0.600
		0.267	0.417	0.420	0.570	0.606
	Average	**0.263**	**0.411**	**0.421**	**0.564**	**0.603**
	Standard deviation	0.004	0.005	0.001	0.005	0.002
	D1	0.066	0.078	0.258	0.396	0.441
		0.063	0.078	0.252	0.396	0.438
		0.072	0.081	0.264	0.399	0.447
	Average	**0.067**	**0.079**	**0.258**	**0.397**	**0.442**
	Standard deviation	0.004	0.001	0.005	0.001	0.004
	R 2	0.180	0.237	0.306	0.339	0.576
		0.174	0.228	0.306	0.333	0.573
		0.186	0.243	0.309	0.345	0.579
	Average	**0.180**	**0.236**	**0.307**	**0.339**	**0.576**
	Standard deviation	0.005	0.006	0.001	0.005	0.002
	A22	0.174	0.432	0.447	0.447	0.513
		0.168	0.426	0.441	0.447	0.507
		0.177	0.438	0.450	0.453	0.516
	Average	**0.173**	**0.432**	**0.446**	**0.449**	**0.512**
	Standard deviation	0.004	0.005	0.004	0.003	0.004
	A17	0.249	0.297	0.387	0.627	0.636
		0.243	0.306	0.381	0.621	0.630
		0.252	0.288	0.390	0.633	0.642
	Average	**0.248**	**0.297**	**0.386**	**0.627**	**0.636**
	Standard deviation	0.004	0.007	0.004	0.005	0.005
	A14	0.147	0.342	0.519	0.588	0.591
		0.141	0.339	0.513	0.582	0.585
		0.15	0.348	0.522	0.594	0.594
	Average	**0.146**	**0.343**	**0.518**	**0.588**	**0.59**
	Standard deviation	0.004	0.004	0.004	0.005	0.004
	Control	1.563				
		1.557				
		1.566				
	Average	**1.562**				
		0.004				

pH 8	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	83.163	95.711	88.476	86.494	84.123	90.653
	8	73.688	94.942	84.891	74.068	80.986	78.041
	6	73.892	83.483	80.346	73.744	75.288	66.837
	4	63.892	74.584	78.297	71.583	59.859	62.356
	2	61.396	71.703	63.124	69.746	59.283	62.227

pH 9	E1	0.363	0.411	0.417	0.456	0.471
		0.357	0.405	0.411	0.447	0.471
		0.366	0.417	0.420	0.462	0.474
	Average	**0.362**	**0.411**	**0.416**	**0.455**	**0.472**
	Standard deviation	0.004	0.005	0.004	0.006	0.001
	D1	0.198	0.306	0.336	0.354	0.423
		0.195	0.297	0.330	0.345	0.423
		0.201	0.312	0.339	0.36	0.426
	Average	**0.198**	**0.305**	**0.335**	**0.353**	**0.424**
	Standard deviation	0.002	0.006	0.004	0.006	0.001
	R 2	0.118	0.141	0.405	0.525	0.555
		0.177	0.140	0.399	0.522	0.552
		0.189	0.143	0.411	0.531	0.558
	Average	**0.183**	**0.140**	**0.405**	**0.526**	**0.555**
	Standard deviation	0.031	0.001	0.005	0.004	0.002
	A22	0.249	0.480	0.486	0.525	0.567
		0.249	0.474	0.483	0.522	0.543
		0.252	0.486	0.489	0.531	0.570
	Average	**0.250**	**0.480**	**0.486**	**0.276**	**0.560**
	Standard deviation	0.001	0.005	0.002	0.004	0.012
	A17	0.252	0.411	0.438	0.492	0.507
		0.249	0.405	0.435	0.489	0.507
		0.258	0.414	0.444	0.495	0.513
	Average	**0.252**	**0.410**	**0.439**	**0.492**	**0.509**
	Standard deviation	0.004	0.004	0.004	0.002	0.003
	A14	0.384	0.522	0.531	0.546	0.570
		0.384	0.519	0.528	0.543	0.567
		0.387	0.528	0.537	0.549	0.573
	Average	**0.385**	**0.523**	**0.532**	**0.546**	**0.570**
	Standard deviation	0.0010	0.004	0.004	0.002	0.002
	Control	1.731				
		1.731				
		1.734				
	Average	**1.732**				
	Standard deviation	0.001				

pH 9	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	79.099	88.568	89.434	95.519	85.393	77.771
	8	76.270	82.390	78.002	82.266	76.328	69.804
	6	75.982	80.658	76.617	79.898	74.654	69.284
	4	73.730	79.619	69.630	73.047	71.594	68.822
	2	72.748	75.520	67.956	66.261	70.612	67.090

pH 10	Isolates	10	8	6	4	2
	E1	0.564	0.576	0.591	0.591	0.615
		0.561	0.570	0.588	0.588	0.612
		0.567	0.582	0.594	0.597	0.618
	Average	**0.564**	**0.576**	**0.591**	**0.592**	**0.615**
	Standard deviation	0.002	0.005	0.002	0.004	0.002
	D1	0.459	0.537	0.543	0.549	0.582
		0.453	0.528	0.540	0.546	0.582
		0.462	0.543	0.546	0.555	0.585
	Average	**0.458**	**0.536**	**0.543**	**0.55**	**0.583**
	Standard deviation	0.004	0.006	0.002	0.004	0.001
	R 2	0.522	0.537	0.537	0.558	0.561
		0.513	0.531	0.534	0.552	0.555
		0.528	0.540	0.543	0.561	0.567
	Average	**0.521**	**0.536**	**0.538**	**0.557**	**0.561**
	Standard deviation	0.006	0.004	0.004	0.004	0.005
	A22	0.255	0.393	0.399	0.42	0.477
		0.249	0.387	0.399	0.414	0.474
		0.258	0.399	0.402	0.426	0.480
	Average	**0.254**	**0.393**	**0.4**	**0.42**	**0.477**
	Standard deviation	0.004	0.005	0.001	0.005	0.002
	A17	0.495	0.510	0.519	0.522	0.558
		0.495	0.507	0.513	0.516	0.555
		0.498	0.516	0.522	0.528	0.561
	Average	**0.496**	**0.511**	**0.178**	**0.522**	**0.558**
	Standard deviation	0.001	0.004	0.004	0.005	0.002
	A14	0.501	0.519	0.564	0.579	0.636
		0.495	0.513	0.564	0.573	0.636
		0.507	0.525	0.567	0.585	0.639
	Average	**0.501**	**0.519**	**0.565**	**0.579**	**0.637**
	Standard deviation	0.005	0.005	0.001	0.005	0.001
	Control	1.851				
		1.845				
		1.857				
	Average	**1.851**				
	Standard deviation	0.005				

pH 10	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	69.530	75.257	71.853	83.190	73.204	72.934
	8	68.882	71.043	71.043	73.991	72.393	71.961
	6	68.071	70.665	70.935	73.527	72.015	69.476
	4	68.017	70.286	69.908	72.204	71.799	68.720
	2	66.775	68.504	69.692	68.432	69.854	65.586

Table 7.35: OD_{550nm} values for the determination of effect of temperature on the removal of whale dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.4)

28°C	Isolates	10	8	6	4	2
	E1	0.096	0.153	0.156	0.174	0.210
		0.096	0.147	0.159	0.171	0.210
		0.099	0.159	0.156	0.180	0.213
	Average	**0.097**	**0.153**	**0.157**	**0.175**	**0.211**
	Standard deviation	0.001	0.005	0.001	0.004	0.001
	D1	0.102	0.111	0.132	0.141	0.219
		0.105	0.105	0.129	0.138	0.213
		0.108	0.114	0.138	0.144	0.222
	Average	**0.105**	**0.110**	**0.133**	**0.141**	**0.218**
	Standard deviation	0.002	0.004	0.004	0.002	0.004
	R 2	0.141	0.156	0.219	0.249	0.300
		0.147	0.156	0.228	0.249	0.315
		0.132	0.159	0.213	0.252	0.285
	Average	**0.140**	**0.157**	**0.220**	**0.250**	**0.300**
	Standard deviation	0.006	0.001	0.006	0.001	0.012
	A22	0.051	0.129	0.243	0.282	0.297
		0.054	0.141	0.240	0.285	0.294
		0.045	0.135	0.249	0.288	0.297
	Average	**0.050**	**0.135**	**0.244**	**0.285**	**0.294**
	Standard deviation	0.004	0.005	0.004	0.002	0.001
	A17	0.090	0.102	0.225	0.246	0.291
		0.087	0.102	0.225	0.249	0.297
		0.096	0.105	0.222	0.240	0.312
	Average	**0.091**	**0.103**	**0.224**	**0.245**	**0.292**
	Standard deviation	0.004	0.001	0.001	0.004	0.009
	A14	0.051	0.063	0.186	0.186	0.294
		0.045	0.057	0.183	0.183	0.294
		0.057	0.066	0.189	0.189	0.297
	Average	**0.051**	**0.062**	**0.186**	**0.186**	**0.295**
	Standard deviation	0.005	0.004	0.002	0.002	0.001
	Control	0.48				
		0.477				
		0.486				
	Average	**0.481**				
	Standard deviation	0.004				

28°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	79.834	78.170	76.299	88.009	81.081	87.770
	8	68.191	77.131	67.360	67.626	78.586	85.132
	6	67.360	72.249	54.262	41.487	53.430	67.386
	4	63.617	70.686	46.362	31.655	49.064	55.396
	2	56.133	54.678	37.630	29.496	39.293	29.257

35°C	Isolates	10	8	6	4	2
	E1	0.009	0.033	0.117	0.123	0.174
		0.006	0.024	0.111	0.117	0.168
		0.015	0.039	0.120	0.129	0.180
	Average	**0.010**	**0.032**	**0.116**	**0.122**	**0.174**
	Standard deviation	0.004	0.006	0.004	0.005	0.005
	D1	0.006	0.201	0.207	0.228	0.255
		0.003	0.201	0.204	0.228	0.246
		0.006	0.204	0.213	0.231	0.261
	Average	**0.005**	**0.202**	**0.208**	**0.229**	**0.254**
	Standard deviation	0.001	0.001	0.004	0.001	0.006
	R 2	0.006	0.042	0.057	0.087	0.132
		0.006	0.039	0.057	0.087	0.129
		0.006	0.048	0.060	0.093	0.135
	Average	**0.006**	**0.043**	**0.058**	**0.089**	**0.132**
	Standard deviation	0	0.004	0.001	0.003	0.005
	A22	0.012	0.066	0.108	0.114	0.117
		0.006	0.075	0.105	0.114	0.108
		0.015	0.063	0.114	0.117	0.123
	Average	**0.011**	**0.068**	**0.109**	**0.115**	**0.116**
	Standard deviation	0.004	0.005	0.004	0.001	0.006
	A17	0.021	0.087	0.129	0.162	0.216
		0.015	0.081	0.123	0.153	0.210
		0.027	0.090	0.132	0.168	0.219
	Average	**0.021**	**0.086**	**0.128**	**0.161**	**0.215**
	Standard deviation	0.005	0.004	0.004	0.006	0.004
	A14	0.009	0.042	0.072	0.114	0.117
		0.012	0.039	0.069	0.105	0.111
		0.006	0.048	0.075	0.12	0.123
	Average	**0.009**	**0.043**	**0.072**	**0.113**	**0.117**
	Standard deviation	0.002	0.004	0.002	0.006	0.004
	Control	0.417				
		0.414				
		0.402				
	Average	**0.417**				
	Standard deviation	0.006				

35°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	97.602	98.993	98.561	97.713	94.964	98.129
	8	92.326	59.256	89.688	85.863	79.376	91.06
	6	72.182	58.149	86.091	77.339	69.305	85.031
	4	70.743	53.924	78.657	76.091	61.391	76.507
	2	58.273	48.893	68.345	75.884	48.441	75.676

40°C	Isolates	10	8	6	4	2
	E1	0.021	0.114	0.171	0.186	0.192
		0.015	0.108	0.168	0.186	0.183
		0.027	0.117	0.177	0.189	0.198
	Average	**0.021**	**0.113**	**0.172**	**0.187**	**0.191**
	Standard deviation	0.005	0.004	0.004	0.001	0.006
	D1	0.111	0.150	0.159	0.186	0.210
		0.105	0.150	0.153	0.186	0.210
		0.114	0.153	0.165	0.189	0.213
	Average	**0.110**	**0.151**	**0.159**	**0.187**	**0.211**
	Standard deviation	0.004	0.001	0.005	0.001	0.001
	R 2	0.084	0.12	0.168	0.243	0.297
		0.084	0.117	0.159	0.237	0.306
		0.087	0.126	0.174	0.246	0.288
	Average	**0.085**	**0.121**	**0.167**	**0.242**	**0.297**
	Standard deviation	0.001	0.004	0.006	0.004	0.007
	A22	0.063	0.174	0.183	0.240	0.279
		0.060	0.174	0.177	0.240	0.270
		0.066	0.177	0.189	0.243	0.285
	Average	**0.063**	**0.178**	**0.183**	**0.241**	**0.278**
	Standard deviation	0.002	0.001	0.005	0.001	0.006
	A17	0.081	0.087	0.102	0.129	0.150
		0.078	0.087	0.099	0.126	0.159
		0.087	0.09	0.108	0.135	0.159
	Average	**0.082**	**0.088**	**0.103**	**0.130**	**0.156**
	Standard deviation	0.004	0.001	0.004	0.004	0.004
	A14	0.162	0.195	0.249	0.276	0.300
		0.156	0.186	0.249	0.270	0.297
		0.165	0.201	0.252	0.279	0.306
	Average	**0.161**	**0.194**	**0.25**	**0.275**	**0.301**
	Standard deviation	0.004	0.006	0.001	0.004	0.004
	Control	0.498				
		0.489				
		0.495				
	Average	**0.497**				
	Standard deviation	0.004				

40°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	94.866	73.105	82.897	87.324	79.951	67.606
	8	72.372	63.081	75.654	64.789	78.484	60.966
	6	57.946	61.125	66.398	63.179	74.817	49.698
	4	54.279	54.279	51.308	51.509	68.215	44.668
	2	53.301	48.411	40.241	44.064	61.858	39.437

45°C	Isolates	10	8	6	4	2
	E1	0.207	0.237	0.273	0.300	0.333
		0.210	0.234	0.264	0.297	0.330
		0.210	0.240	0.279	0.306	0.336
	Average	**0.209**	**0.237**	**0.272**	**0.301**	**0.333**
	Standard deviation	0.001	0.002	0.006	0.004	0.002
	D1	0.129	0.234	0.282	0.303	0.339
		0.129	0.231	0.282	0.300	0.336
		0.132	0.237	0.285	0.306	0.345
	Average	**0.130**	**0.234**	**0.283**	**0.303**	**0.340**
	Standard deviation	0.001	0.002	0.001	0.002	0.004
	R 2	0.105	0.228	0.150	0.231	0.252
		0.102	0.102	0.147	0.228	0.246
		0.108	0.114	0.156	0.234	0.255
	Average	**0.105**	**0.108**	**0.151**	**0.231**	**0.251**
	Standard deviation	0.002	0.057	0.004	0.002	0.004
	A22	0.075	0.120	0.174	0.180	0.198
		0.072	0.114	0.168	0.180	0.195
		0.078	0.126	0.177	0.183	0.204
	Average	**0.075**	**0.120**	**0.173**	**0.181**	**0.199**
	Standard deviation	0.002	0.005	0.004	0.001	0.004
	A17	0.171	0.180	0.243	0.276	0.300
		0.165	0.174	0.237	0.270	0.297
		0.177	0.186	0.246	0.279	0.306
	A17	0.171	0.180	0.243	0.276	0.300
	Standard deviation	0.005	0.005	0.004	0.004	0.004
	A14	0.141	0.249	0.285	0.294	0.300
		0.141	0.246	0.285	0.297	0.297
		0.144	0.252	0.288	0.294	0.306
	Average	**0.142**	**0.249**	**0.286**	**0.295**	**0.301**
	Standard deviation	0.001	0.002	0.001	0.001	0.004
	Control	0.408				
		0.405				
		0.414				
	Average	**0.409**				
	Standard deviation	0.004				

45°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	57.948	68.825	74.328	81.663	65.594	65.281
	8	52.314	43.884	73.594	70.660	63.783	39.120
	6	45.272	32.134	63.081	57.702	51.308	31.540
	4	39.437	27.338	43.521	55.746	44.668	27.873
	2	32.998	18.465	38.631	51.345	39.437	26.406

Table 7.36: OD_{550nm} values for the determination of effect of cations on the removal of whale dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.5)

CTAB	Isolates	10	8	6	4	2
	E1	0.729	0.879	0.891	0.894	0.909
		0.729	0.873	0.888	0.894	0.903
		0.732	0.885	0.897	0.897	0.918
	Average	**0.730**	**0.879**	**0.892**	**0.895**	**0.910**
	Standard deviation	0.001	0.005	0.004	0.001	0.006
	D1	0.441	0.531	0.768	0.879	0.951
		0.438	0.525	0.762	0.864	0.933
		0.447	0.540	0.771	0.891	0.942
	Average	**0.442**	**0.532**	**0.767**	**0.878**	**0.942**
	Standard deviation	0.004	0.006	0.004	0.011	0.007
	R 2	0.825	0.846	0.873	0.888	0.966
		0.807	0.831	0.870	0.882	0.957
		0.840	0.828	0.876	0.891	0.978
	Average	**0.824**	**0.845**	**0.873**	**0.887**	**0.967**
	Standard deviation	0.013	0.008	0.002	0.004	0.009
	A22	0.816	0.834	0.861	0.897	0.915
		0.804	0.825	0.852	0.897	0.928
		0.825	0.840	0.870	0.900	0.927
	Average	**0.815**	**0.833**	**0.861**	**0.898**	**0.914**
	Standard deviation	0.009	0.006	0.007	0.001	0.006
	A17	0.717	0.834	0.864	0.891	0.921
		0.711	0.825	0.852	0.888	0.918
		0.723	0.84	0.879	0.894	0.927
	Average	**0.717**	**0.833**	**0.865**	**0.891**	**0.322**
	Standard deviation	0.005	0.006	0.011	0.002	0.004
	A14	0.702	0.726	0.813	0.903	0.924
		0.696	0.711	0.804	0.897	0.915
		0.711	0.738	0.822	0.912	0.936
	Average	**0.703**	**0.725**	**0.813**	**0.904**	**0.925**
	Standard deviation	0.006	0.011	0.007	0.006	0.007
	Control	1.152				
		1.146				
		1.155				
	Average	**1.151**				
	Standard deviation	0.004				

CTAB	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	36.577	61.599	28.410	29.192	37.706	38.923
	8	23.632	53.779	26.586	27.628	27.628	37.011
	6	22.502	33.362	24.153	25.195	24.848	29.366
	4	22.242	23.719	22.937	21.981	22.589	21.460
	2	20.938	18.158	15.986	20.591	19.896	19.635

$CaCl_2$	Isolates	10	8	6	4	2
	E1	0.087	0.087	0.09	0.09	0.144
		0.075	0.078	0.087	0.099	0.135
		0.099	0.102	0.096	0.096	0.15
	Average	**0.087**	**0.089**	**0.091**	**0.097**	**0.143**
	Standard deviation	0.008	0.010	0.004	0.004	0.006
	D1	0.066	0.084	0.087	0.099	0.102
		0.048	0.078	0.072	0.09	0.096
		0.081	0.090	0.102	0.108	0.108
	Average	**0.065**	**0.084**	**0.087**	**0.099**	**0.102**
	Standard deviation	0.013	0.005	0.012	0.007	0.005
	R 2	0.072	0.096	0.111	0.123	0.147
		0.060	0.084	0.105	0.108	0.138
		0.084	0.108	0.117	0.135	0.156
	Average	**0.072**	**0.096**	**0.111**	**0.122**	**0.147**
	Standard deviation	0.010	0.010	0.005	0.011	0.007
	A22	0.063	0.072	0.084	0.084	0.114
		0.057	0.063	0.078	0.078	0.108
		0.066	0.078	0.087	0.093	0.123
	Average	**0.062**	**0.071**	**0.083**	**0.085**	**0.115**
	Standard deviation	0.004	0.006	0.004	0.006	0.006
	A17	0.069	0.078	0.084	0.09	0.135
		0.066	0.069	0.066	0.078	0.132
		0.072	0.087	0.102	0.102	0.141
	Average	**0.069**	**0.078**	**0.084**	**0.09**	**0.136**
	Standard deviation	0.002	0.007	0.015	0.010	0.004
	A14	0.069	0.072	0.078	0.087	0.090
		0.060	0.066	0.069	0.069	0.075
		0.081	0.081	0.084	0.105	0.105
	Average	**0.070**	**0.073**	**0.077**	**0.087**	**0.090**
	Standard deviation	0.009	0.006	0.006	0.015	0.012
	Control	0.276				
		0.267				
		0.282				
	Average	**0.275**				
	Standard deviation	0.006				

$CaCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	68.364	75.364	73.818	77.455	74.909	74.545
	8	67.636	69.455	65.091	74.182	71.636	73.455
	6	66.909	68.364	60.000	69.818	69.455	72.000
	4	64.727	64.000	55.636	69.091	67.273	68.364
	2	48.000	62.909	46.546	58.182	50.545	67.273

$MnCl_2$	Isolates	10	8	6	4	2
	E1	0.003	0.006	0.009	0.012	0.015
		0	0.009	0.012	0.006	0.009
		0	0.006	0.006	0.015	0.018
	Average	**0.001**	**0.007**	**0.009**	**0.011**	**0.014**
	Standard deviation	0.001	0.001	0.002	0.004	0.004
	D1	0.003	0.009	0.012	0.018	0.024
		0.003	0.012	0.006	0.009	0.009
		0	0.006	0.021	0.027	0.036
	Average	**0.002**	**0.009**	**0.013**	**0.018**	**0.023**
	Standard deviation	0.001	0.002	0.006	0.007	0.011
	R 2	0.003	0.003	0.015	0.018	0.021
		0.003	0.006	0.006	0.006	0.018
		0	0.003	0.021	0.015	0.027
	Average	**0.002**	**0.004**	**0.014**	**0.017**	**0.022**
	Standard deviation	0.001	0.001	0.006	0.005	0.004
	A22	0.003	0.009	0.015	0.021	0.024
		0	0.012	0.006	0.018	0.018
		0	0.006	0.024	0.024	0.030
	Average	**0.001**	**0.009**	**0.015**	**0.021**	**0.024**
	Standard deviation	0.001	0.002	0.007	0.002	0.005
	A17	0.003	0.003	0.006	0.009	0.009
		0	0.006	0.003	0.012	0.012
		0	0.003	0.012	0.006	0.009
	Average	**0.001**	**0.004**	**0.007**	**0.009**	**0.010**
	Standard deviation	0.005	0.001	0.004	0.002	0.001
	A14	0.003	0.009	0.012	0.015	0.009
		0.003	0.012	0.006	0.012	0.018
		0.003	0.006	0.021	0.021	0.018
	Average	**0.003**	**0.009**	**0.013**	**0.016**	**0.018**
	Standard deviation	0	0.002	0.006	0.004	0.004
	Control	0.111				
		0.105				
		0.117				
	Average	**0.111**				
	Standard deviation	0.005				

$MnCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	99.099	98.198	98.198	99.099	99.099	97.209
	8	93.694	91.892	96.396	91.892	96.396	91.892
	6	91.892	88.288	87.387	86.486	93.694	88.288
	4	90.090	83.784	84.685	81.081	91.892	85.586
	2	87.387	79.279	80.180	78.378	90.991	83.784

$MgSO_4$	Isolates	10	8	6	4	2
	E1	0.003	0.003	0.006	0.009	0.012
		0	0.003	0.003	0.009	0.006
		0	0.003	0.006	0.006	0.015
	Average	**0.001**	**0.003**	**0.005**	**0.008**	**0.011**
	Standard deviation	0.001	0	0.001	0.001	0.004
	D1	0.012	0.015	0.018	0.048	0.051
		0.009	0.012	0.021	0.036	0.045
		0.015	0.018	0.018	0.057	0.057
	Average	**0.012**	**0.015**	**0.019**	**0.047**	**0.051**
	Standard deviation	0.002	0.002	0.001	0.009	0.005
	R 2	0.006	0.021	0.027	0.039	0.039
		0.003	0.018	0.03	0.033	0.03
		0.009	0.021	0.027	0.042	0.051
	Average	**0.006**	**0.020**	**0.028**	**0.038**	**0.04**
	Standard deviation	0.002	0.001	0.001	0.004	0.009
	A22	0.027	0.030	0.036	0.039	0.042
		0.021	0.027	0.03	0.036	0.030
		0.033	0.036	0.039	0.042	0.054
	Average	**0.027**	**0.131**	**0.035**	**0.039**	**0.042**
	Standard deviation	0.005	0.004	0.004	0.002	0.010
	A17	0.015	0.021	0.027	0.033	0.030
		0.012	0.012	0.027	0.033	0.030
		0.018	0.027	0.033	0.03	0.045
	Average	**0.015**	**0.02**	**0.029**	**0.032**	**0.035**
	Standard deviation	0.002	0.006	0.003	0.001	0.007
	A14	0.012	0.012	0.018	0.024	0.027
		0.006	0.006	0.012	0.018	0.021
		0.015	0.021	0.021	0.03	0.033
	Average	**0.011**	**0.013**	**0.017**	**0.024**	**0.027**
	Standard deviation	0.004	0.006	0.004	0.005	0.005
	Control	0.234				
		0.234				
		0.237				
	Average	**0.235**				
	Standard deviation	0.001				

$MgSO_4$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	99.574	94.894	97.447	88.511	93.617	95.319
	8	98.723	93.617	91.489	86.809	91.489	94.468
	6	97.872	91.915	88.085	85.106	87.660	92.766
	4	96.596	80.000	83.830	83.404	86.383	89.787
	2	95.319	78.298	82.979	82.128	85.106	88.511

Table 7.37: OD_{550nm} values for the determination of flocculating activity of medi-blue dye using different concentrations of bioflocculants

Isolates	10	8	6	4	2
E1	0.117	0.114	0.126	0.138	0.141
	0.084	0.117	0.117	0.114	0.126
	0.111	0.123	0.120	0.126	0.132
Average	**0.104**	**0.118**	**0.121**	**0.126**	**0.133**
Standard deviation	0.014	0.004	0.004	0.010	0.006
D1	0.111	0.129	0.147	0.156	0.147
	0.123	0.111	0.129	0.135	0.165
	0.117	0.120	0.138	0.147	0.156
Average	**0.117**	**0.12**	**0.138**	**0.046**	**0.156**
Standard deviation	0.005	0.007	0.007	0.009	0.007
R 2	0.111	0.114	0.123	0.111	0.135
	0.087	0.093	0.096	0.126	0.12
	0.099	0.102	0.108	0.117	0.129
Average	**0.099**	**0.103**	**0.109**	**0.118**	**0.128**
Standard deviation	0.010	0.009	0.011	0.006	0.006
A22	0.114	0.129	0.123	0.138	0.141
	0.102	0.102	0.108	0.12	0.117
	0.108	0.117	0.138	0.129	0.135
Average	**0.108**	**0.116**	**0.123**	**0.129**	**0.131**
Standard deviation	0.005	0.011	0.012	0.007	0.010
A17	0.111	0.126	0.114	0.126	0.129
	0.087	0.078	0.105	0.117	0.132
	0.099	0.108	0.108	0.12	0.12
Average	**0.099**	**0.104**	**0.109**	**0.121**	**0.127**
Standard deviation	0.010	0.020	0.004	0.004	0.005
A14	0.105	0.111	0.12	0.123	0.126
	0.087	0.087	0.086	0.105	0.114
	0.096	0.105	0.117	0.114	0.112
Average	**0.096**	**0.101**	**0.107**	**0.114**	**0.12**
Standard deviation	0.007	0.010	0.015	0.007	0.006
Control	0.513				
	0.477				
	0.495				
Average	**0.495**				
Standard deviation	0.015				

Table 7.38: Percentage removal (C_0–C/C_0 x 100) of medi-blue dye using different concentrations of flocculants as derived from Table 7.37 (Fig. 4.6)

Conc (ppm)	E1	D1	R2	A22	A17	A14
10	78.990	76.364	80.000	78.182	80.000	80.606
8	76.162	75.758	79.192	76.566	78.990	79.596
6	75.556	72.121	77.980	75.152	77.980	78.384
4	74.545	70.505	76.162	73.939	75.556	76.970
2	73.131	68.485	74.141	73.535	74.343	75.758

Table 7.39: Flocculating activity of the medi-blue dye as derived from Table 7.37 using an equation for flocculating activity in Section 4.2.3 (Fig. 4.7)

Isolate	Flocculating activity	Standard deviation
E1	26.117	0.177
D1	21.964	0.102
R2	22.456	0.063
A22	29.489	0.154
A17	22.651	0.218
A14	24.993	0.127

Table 7.40: OD_{550nm} values for the determination of effect of pH on the removal of medi-blue dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.8)

pH 6	Isolates	10	8	6	4	2
	E1	0.228	0.213	0.234	0.243	0.249
		0.219	0.237	0.219	0.222	0.228
		0.222	0.225	0.228	0.231	0.240
	Average	**0.223**	**0.225**	**0.227**	**0.232**	**0.239**
	Standard deviation	0.004	0.010	0.006	0.009	0.009
	D1	0.207	0.243	0.252	0.255	0.243
		0.231	0.213	0.228	0.246	0.225
		0.237	0.234	0.240	0.249	0.234
	Average	**0.225**	**0.230**	**0.240**	**0.250**	**0.234**
	Standard deviation	0.013	0.013	0.010	0.004	0.007
	R 2	0.240	0.222	0.234	0.249	0.276
		0.213	0.237	0.225	0.216	0.255
		0.228	0.228	0.231	0.237	0.264
	Average	**0.227**	**0.229**	**0.23**	**0.234**	**0.265**
	Standard deviation	0.011	0.006	0.004	0.014	0.009
	A22	0.225	0.231	0.225	0.225	0.240
		0.204	0.216	0.228	0.24	0.234
		0.216	0.222	0.228	0.231	0.237
	Average	**0.215**	**0.223**	**0.227**	**0.232**	**0.237**
	Standard deviation	0.009	0.006	0.001	0.006	0.002
	A17	0.216	0.219	0.225	0.234	0.246
		0.201	0.210	0.237	0.243	0.255
		0.213	0.213	0.231	0.237	0.252
	Average	**0.210**	**0.214**	**0.231**	**0.238**	**0.251**
	Standard deviation	0.006	0.004	0.005	0.004	0.004
	A14	0.222	0.225	0.222	0.24	0.252
		0.216	0.219	0.225	0.231	0.243
		0.219	0.222	0.231	0.237	0.249
	Average	**0.219**	**0.222**	**0.226**	**0.236**	**0.248**
	Standard deviation	0.002	0.002	0.004	0.004	0.004
	Control	0.426				
		0.390				
		0.414				
	Average	**0.410**				
	Standard deviation	0.015				

pH 6	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	45.610	45.122	52.439	47.561	48.780	46.585
	8	45.122	43.902	51.220	45.610	47.805	45.854
	6	44.634	41.463	46.829	44.634	43.659	44.878
	4	43.415	39.024	46.098	43.415	41.951	42.439
	2	41.707	38.293	40.976	42.195	38.780	39.512

pH 7	Isolates	10	8	6	4	2
	E1	0.234	0.249	0.249	0.252	0.27
		0.240	0.234	0.246	0.249	0.261
		0.231	0.243	0.246	0.249	0.264
	Average	**0.232**	**0.247**	**0.247**	**0.250**	**0.265**
	Standard deviation	0.004	0.006	0.001	0.001	0.004
	D1	0.234	0.234	0.24	0.243	0.258
		0.219	0.228	0.231	0.24	0.243
		0.228	0.231	0.234	0.24	0.246
	Average	**0.227**	**0.231**	**0.285**	**0.241**	**0.046**
	Standard deviation	0.006	0.002	0.004	0.001	0.006
	R 2	0.228	0.198	0.216	0.225	0.249
		0.228	0.201	0.216	0.216	0.234
		0.225	0.201	0.222	0.222	0.243
	Average	**0.195**	**0.200**	**0.218**	**0.221**	**0.242**
	Standard deviation	0.001	0.001	0.003	0.004	0.006
	A22	0.219	0.231	0.243	0.240	0.267
		0.234	0.228	0.228	0.240	0.252
		0.225	0.228	0.234	0.240	0.261
	Average	**0.226**	**0.229**	**0.235**	**0.240**	**0.260**
	Standard deviation	0.006	0.001	0.006	0	0.006
	A17	0.219	0.246	0.243	0.246	0.276
		0.216	0.225	0.234	0.249	0.291
		0.216	0.237	0.240	0.246	0.285
	Average	**0.217**	**0.236**	**0.239**	**0.247**	**0.284**
	Standard deviation	0.001	0.009	0.004	0.001	0.006
	A14	0.228	0.237	0.255	0.291	0.297
		0.222	0.225	0.252	0.279	0.297
		0.225	0.231	0.252	0.285	0.297
	Average	**0.225**	**0.231**	**0.253**	**0.284**	**0.297**
	Standard deviation	0.002	0.005	0.001	0.005	0
	Control	0.450				
		0.447				
		0.456				
	Average	**0.451**				
	Standard deviation	0.004				

pH 7	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	48.559	49.667	49.667	50.554	51.885	50.111
	8	46.341	48.780	49.224	49.667	47.672	48.780
	6	45.233	47.894	49.002	48.78	47.007	43.902
	4	44.568	46.563	48.115	48.337	45.233	37.029
	2	41.242	45.455	41.242	46.341	37.029	34.146

pH 8	Isolates	10	8	6	4	2
	E1	0.639	0.675	0.702	0.738	0.738
		0.621	0.630	0.684	0.711	0.720
		0.630	0.657	0.693	0.720	0.729
	Average	**0.210**	**0.218**	**0.231**	**0.241**	**0.243**
	Standard deviation	0.007	0.018	0.007	0.011	0.007
	D1	0.675	0.684	0.711	0.729	0.765
		0.657	0.639	0.702	0.702	0.729
		0.657	0.72	0.675	0.711	0.747
	Average	**0.221**	**0.227**	**0.232**	**0.238**	**0.249**
	Standard deviation	0.008	0.033	0.015	0.011	0.015
	R 2	0.666	0.738	0.765	0.801	0.774
		0.657	0.72	0.765	0.756	0.801
		0.657	0.729	0.783	0.774	0.792
	Average	**0.220**	**0.243**	**0.257**	**0.259**	**0.263**
	Standard deviation	0.004	0.007	0.008	0.018	0.011
	A22	0.666	0.666	0.711	0.702	0.801
		0.639	0.666	0.684	0.729	0.819
		0.657	0.657	0.693	0.72	0.792
	Average	**0.218**	**0.221**	**0.232**	**0.239**	**0.266**
	Standard deviation	0.011	0.004	0.011	0.011	0.011
	A17	0.729	0.747	0.720	0.783	0.801
		0.693	0.711	0.765	0.720	0.747
		0.711	0.729	0.747	0.756	0.774
	Average	**0.237**	**0.243**	**0.248**	**0.251**	**0.258**
	Standard deviation	0.015	0.015	0.019	0.026	0.022
	A14	0.228	0.231	0.246	0.249	0.252
		0.228	0.237	0.234	0.24	0.258
		0.225	0.234	0.240	0.246	0.255
	Average	**0.227**	**0.234**	**0.240**	**0.245**	**0.255**
	Standard deviation	0.001	0.002	0.005	0.004	0.002
	Control	0.426				
		0.42				
		0.435				
	Average	**0.427**				
	Standard deviation	0.006				

pH 8	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	50.820	48.244	48.478	47.076	44.496	46.838
	8	48.946	46.838	43.091	46.370	43.091	45.199
	6	45.902	45.667	39.813	44.965	41.920	43.794
	4	43.560	44.262	39.344	43.794	41.218	42.623
	2	43.091	41.686	38.407	39.110	39.578	40.281

pH 9	Isolates	10	8	6	4	2
	E1	0.198	0.204	0.231	0.225	0.228
		0.195	0.219	0.204	0.219	0.228
		0.195	0.21	0.216	0.219	0.225
	Average	**0.194**	**0.211**	**0.217**	**0.221**	**0.227**
	Standard deviation	0.001	0.006	0.011	0.003	0.001
	D1	0.213	0.219	0.222	0.219	0.231
		0.210	0.207	0.216	0.225	0.216
		0.207	0.213	0.216	0.219	0.225
	D1	0.213	0.219	0.222	0.219	0.231
	Standard deviation	0.003	0.005	0.003	0.003	0.006
	R 2	0.204	0.216	0.228	0.234	0.243
		0.210	0.219	0.225	0.228	0.228
		0.216	0.216	0.219	0.231	0.234
	Average	**0.210**	**0.217**	**0.224**	**0.231**	**0.235**
	Standard deviation	0.006	0.001	0.004	0.003	0.006
	A22	0.027	0.030	0.036	0.057	0.075
		0.030	0.039	0.042	0.045	0.060
		0.036	0.045	0.048	0.051	0.069
	Average	**0.031**	**0.038**	**0.042**	**0.051**	**0.068**
	Standard deviation	0.004	0.006	0.005	0.005	0.006
	A17	0.204	0.219	0.222	0.222	0.237
		0.213	0.210	0.216	0.219	0.231
		0.219	0.216	0.219	0.222	0.234
	Average	**0.212**	**0.215**	**0.219**	**0.221**	**0.234**
	Standard deviation	0.006	0.004	0.002	0.001	0.002
	A14	0.219	0.216	0.219	0.219	0.240
		0.201	0.222	0.222	0.240	0.225
		0.216	0.216	0.222	0.228	0.234
	Average	**0.212**	**0.218**	**0.222**	**0.229**	**0.233**
	Standard deviation	0.008	0.003	0.001	0.009	0.006
	Control	0.438				
		0.432				
		0.435				
	Average	**0.435**				
	Standard deviation	0.002				

pH 9	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	55.402	51.724	51.724	49.885	51.264	51.264
	8	51.494	51.034	50.115	49.195	50.575	49.885
	6	50.115	49.885	48.506	46.667	49.655	48.966
	4	49.195	49.195	46.897	45.057	49.195	47.356
	2	47.816	48.506	45.977	38.851	46.207	46.437

pH 10	Isolates	10	8	6	4	2
	E1	0.123	0.138	0.165	0.168	0.177
		0.144	0.138	0.150	0.153	0.171
		0.132	0.138	0.159	0.162	0.174
	Average	**0.133**	**0.139**	**0.158**	**0.161**	**0.174**
	Standard deviation	0.009	0	0.006	0.006	0.006
	D1	0.117	0.111	0.129	0.141	0.153
		0.105	0.114	0.144	0.138	0.147
		0.111	0.12	0.123	0.144	0.150
	Average	**0.111**	**0.115**	**0.122**	**0.140**	**0.150**
	Standard deviation	0.005	0.004	0.009	0.002	0.002
	R 2	0.030	0.075	0.123	0.129	0.153
		0.036	0.078	0.108	0.123	0.138
		0.030	0.081	0.117	0.126	0.144
	Average	**0.032**	**0.078**	**0.116**	**0.126**	**0.145**
	Standard deviation	0.003	0.002	0.006	0.004	0.006
	A17	0.039	0.048	0.048	0.069	0.069
		0.048	0.057	0.060	0.06	0.066
		0.054	0.063	0.069	0.063	0.066
	Average	**0.047**	**0.056**	**0.059**	**0.064**	**0.067**
	Standard deviation	0.006	0.006	0.009	0.004	0.001
	A14	0.039	0.066	0.057	0.072	0.093
		0.036	0.054	0.063	0.078	0.081
		0.036	0.042	0.066	0.087	0.087
	Average	**0.037**	**0.054**	**0.062**	**0.079**	**0.087**
	Standard deviation	0.001	0.010	0.004	0.006	0.005
	A22	0.225	0.231	0.225	0.237	0.249
		0.222	0.222	0.231	0.234	0.234
		0.222	0.228	0.237	0.228	0.243
	Average	**0.223**	**0.227**	**0.231**	**0.233**	**0.242**
	Standard deviation	0.001	0.004	0.005	0.004	0.006
	Control	0.468	Control			
		0.462				
		0.453				
	Average	**0.461**				
	Standard deviation	0.006				

pH 10	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	71.150	75.922	93.059	93.275	89.805	91.974
	8	69.848	75.054	83.080	91.757	87.852	88.286
	6	65.727	73.536	74.837	90.889	87.202	86.551
	4	65.076	69.631	72.668	88.937	86.117	82.863
	2	62.256	67.462	68.547	85.249	85.466	81.128

Table 7.41: OD_{550nm} values for the determination of effect of temperature on the removal of medi-blue dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.9)

28°C	Isolates	10	8	6	4	2
	E1	0.114	0.123	0.132	0.147	0.165
		0.126	0.132	0.141	0.147	0.153
		0.120	0.129	0.135	0.153	0.159
	Average	**0.120**	**0.128**	**0.136**	**0.149**	**0.159**
	Standard deviation	0.005	0.004	0.004	0.003	0.005
	D1	1.230	0.135	0.138	0.141	0.153
		1.320	0.138	0.141	0.150	0.159
		1.290	0.135	0.147	0.144	0.156
	Average	**0.128**	**0.136**	**0.142**	**0.145**	**0.156**
	Standard deviation	0.037	0.001	0.004	0.004	0.002
	R 2	0.114	0.117	0.120	0.126	0.135
		0.120	0.123	0.132	0.141	0.138
		0.117	0.120	0.126	0.135	0.135
	Average	**0.117**	**0.120**	**0.126**	**0.134**	**0.136**
	Standard deviation	0.002	0.002	0.005	0.006	0.001
	A22	0.123	0.126	0.135	0.144	0.129
		0.129	0.132	0.123	0.126	0.138
		0.126	0.129	0.135	0.132	0.141
	Average	**0.126**	**0.128**	**0.131**	**0.134**	**0.136**
	Standard deviation	0.002	0.002	0.006	0.007	0.005
	A17	0.120	0.120	0.129	0.135	0.150
		0.117	0.123	0.135	0.141	0.156
		0.114	0.117	0.132	0.138	0.153
	Average	**0.117**	**0.12**	**0.132**	**0.138**	**0.153**
	Standard deviation	0.002	0.002	0.002	0.002	0.002
	A14	0.108	0.120	0.114	0.120	0.132
		0.117	0.120	0.123	0.129	0.132
		0.114	0.111	0.123	0.129	0.129
	Average	**0.113**	**0.117**	**0.12**	**0.126**	**0.13**
	Standard deviation	0.004	0.004	0.004	0.004	0.001
	Control	0.252				
		0.261				
		0.258				
	Average	**0.257**				
	Standard deviation	0.004				

28°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	53.307	50.195	54.475	50.972	54.475	56.031
	8	50.195	47.082	53.307	50.195	53.307	54.475
	6	47.082	44.747	50.973	49.027	48.638	53.307
	4	42.023	40.078	47.860	47.860	46.304	50.973
	2	38.132	39.300	46.693	47.082	40.467	49.416

35°C	Isolates	10	8	6	4	2
	E1	0.156	0.159	0.165	0.168	0.150
		0.141	0.144	0.144	0.147	0.153
		0.150	0.153	0.126	0.159	0.159
	Average	**0.149**	**0.152**	**0.155**	**0.158**	**0.16**
	Standard deviation	0.006	0.006	0.016	0.009	0.004
	D1	0.129	0.135	0.147	0.144	0.150
		0.111	0.120	0.108	0.141	0.150
		0.120	0.126	0.141	0.141	0.147
	Average	**0.12**	**0.127**	**0.132**	**0.142**	**0.149**
	Standard deviation	0.007	0.006	0.017	0.001	0.001
	R 2	0.156	0.156	0.159	0.165	0.165
		0.126	0.138	0.141	0.150	0.159
		0.147	0.147	0.150	0.156	0.162
	Average	**0.143**	**0.147**	**0.15**	**0.157**	**0.162**
	Standard deviation	0.013	0.007	0.007	0.006	0.002
	A22	0.147	0.153	0.156	0.141	0.177
		0.126	0.129	0.135	0.162	0.156
		0.138	0.141	0.144	0.150	0.165
	Average	**0.137**	**0.141**	**0.145**	**0.151**	**0.166**
	Standard deviation	0.009	0.010	0.009	0.009	0.009
	A17	0.159	0.144	0.171	0.165	0.183
		0.138	0.159	0.141	0.150	0.150
		0.150	0.153	0.153	0.159	0.171
	Average	**0.149**	**0.152**	**0.155**	**0.158**	**0.171**
	Standard deviation	0.009	0.006	0.012	0.006	0.014
	A14	0.144	0.132	0.141	0.153	0.171
		0.129	0.144	0.144	0.150	0.162
		0.135	0.138	0.135	0.144	0.165
	Average	**0.136**	**0.138**	**0.14**	**0.149**	**0.166**
	Standard deviation	0.006	0.005	0.004	0.004	0.004
	Control	0.471				
		0.459				
		0.483				
	Average	**0.471**				
	Standard deviation	0.010				

35°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	68.365	74.522	69.639	70.913	68.365	71.125
	8	67.728	73.036	68.970	70.064	67.091	70.701
	6	67.091	71.975	68.153	69.214	67.091	70.276
	4	66.454	69.851	66.667	67.941	66.454	68.365
	2	66.030	68.365	65.605	64.756	63.694	64.756

40°C	Isolates	10	8	6	4	2
	E1	0.165	0.183	0.162	0.195	0.198
		0.165	0.153	0.183	0.18	0.189
		0.171	0.174	0.174	0.189	0.192
	Average	**0.167**	**0.170**	**0.173**	**0.188**	**0.193**
	Standard deviation	0.003	0.013	0.009	0.006	0.004
	D1	0.180	0.183	0.195	0.195	0.198
		0.156	0.162	0.177	0.186	0.198
		0.174	0.174	0.186	0.192	0.195
	Average	**0.170**	**0.173**	**0.186**	**0.191**	**0.197**
	Standard deviation	0.011	0.009	0.007	0.004	0.001
	R 2	0.162	0.159	0.183	0.186	0.195
		0.138	0.168	0.174	0.183	0.183
		0.156	0.162	0.177	0.183	0.189
	Average	**0.152**	**0.163**	**0.178**	**0.184**	**0.189**
	Standard deviation	0.010	0.004	0.004	0.001	0.005
	A22	0.186	0.195	0.198	0.189	0.207
		0.165	0.183	0.189	0.201	0.198
		0.177	0.189	0.192	0.195	0.189
	Average	**0.176**	**0.189**	**0.193**	**0.195**	**0.198**
	Standard deviation	0.009	0.005	0.004	0.005	0.007
	A17	0.165	0.168	0.174	0.192	0.201
		0.156	0.171	0.180	0.177	0.213
		0.159	0.168	0.177	0.186	0.207
	Average	**0.160**	**0.169**	**0.177**	**0.185**	**0.207**
	Standard deviation	0.004	0.001	0.002	0.006	0.005
	A14	0.171	0.180	0.219	0.210	0.249
		0.186	0.195	0.210	0.225	0.240
		0.180	0.189	0.213	0.219	0.243
	Average	**0.179**	**0.188**	**0.21**	**0.218**	**0.244**
	Standard deviation	0.006	0.006	0.004	0.006	0.004
	Control	0.417				
		0.420				
		0.417				
	Average	**0.418**				
	Standard deviation	0.001				

40°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	60.048	59.330	63.636	57.895	61.722	57.177
	8	59.330	58.612	61.005	54.785	59.569	55.024
	6	58.612	55.502	57.416	53.828	57.656	49.761
	4	55.024	54.306	55.981	53.349	55.742	47.847
	2	53.828	52.871	54.785	52.632	50.478	41.627

45°C	Isolates	10	8	6	4	2
	E1	0.213	0.216	0.225	0.231	0.246
		0.198	0.201	0.210	0.222	0.222
		0.204	0.207	0.219	0.225	0.234
	Average	**0.205**	**0.208**	**0.218**	**0.226**	**0.234**
	Standard deviation	0.0061	0.006	0.006	0.004	0.010
	D1	0.210	0.210	0.219	0.225	0.225
		0.195	0.204	0.204	0.204	0.213
		0.201	0.207	0.210	0.216	0.219
	Average	**0.202**	**0.207**	**0.211**	**0.215**	**0.219**
	Standard deviation	0.006	0.002	0.006	0.009	0.005
	R 2	0.180	0.201	0.201	0.213	0.225
		0.171	0.183	0.189	0.201	0.213
		0.174	0.189	0.195	0.207	0.219
	Average	**0.175**	**0.189**	**0.195**	**0.207**	**0.219**
	Standard deviation	0.004	0.008	0.005	0.005	0.005
	A22	0.189	0.189	0.189	0.231	0.267
		0.180	0.189	0.195	0.219	0.261
		0.186	0.189	0.192	0.225	0.264
	Average	**0.185**	**0.189**	**0.192**	**0.225**	**0.264**
	Standard deviation	0.004	0	0.005	0.005	0.002
	A17	0.201	0.207	0.213	0.216	0.225
		0.186	0.189	0.189	0.198	0.204
		0.195	0.198	0.201	0.207	0.213
	Average	**0.194**	**0.198**	**0.201**	**0.207**	**0.214**
	Standard deviation	0.006	0.007	0.010	0.007	0.009
	A14	0.207	0.219	0.216	0.231	0.234
		0.186	0.204	0.210	0.216	0.225
		0.198	0.210	0.213	0.225	0.231
	Average	**0.197**	**0.211**	**0.213**	**0.224**	**0.23**
	Standard deviation	0.009	0.006	0.002	0.006	0.004
	Control	0.348				
		0.339				
		0.342				
	Average	**0.343**				
	Standard deviation	0.004				

45°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	40.233	41.108	48.980	46.064	43.440	42.566
	8	39.359	39.650	44.898	44.898	42.274	38.484
	6	36.443	38.484	43.149	44.023	41.399	37.901
	4	34.111	37.318	39.650	34.402	39.650	34.694
	2	31.778	36.152	36.152	23.032	37.609	32.945

Table 7.42: OD_{550nm} values for the determination of effect of cations on the removal of medi-blue dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.10)

CTAB	Isolates	10	8	6	4	2
	E1	0.591	0.915	0.975	0.984	1.089
		0.573	0.900	0.957	0.969	1.080
		0.564	0.906	0.966	0.978	1.068
	Average	**0.572**	**0.907**	**0.966**	**0.977**	**1.079**
	Standard deviation	0.011	0.006	0.007	0.006	0.009
	D1	0.588	0.873	0.873	0.915	0.948
		0.576	0.864	0.858	0.903	0.933
		0.582	0.867	0.885	0.909	0.942
	Average	**0.582**	**0.868**	**0.885**	**0.909**	**0.941**
	Standard deviation	0.005	0.004	0.011	0.005	0.006
	R 2	0.501	0.768	0.813	0.834	1.104
		0.483	0.756	0.798	0.819	1.083
		0.492	0.762	0.807	0.828	1.095
	Average	**0.492**	**0.762**	**0.806**	**0.827**	**1.094**
	Standard deviation	0.007	0.005	0.006	0.006	0.009
	A22	0.711	1.026	1.047	1.071	1.125
		0.699	1.011	1.038	1.044	1.107
		0.705	1.017	1.044	1.059	1.116
	Average	**0.705**	**1.018**	**1.043**	**0.058**	**1.116**
	Standard deviation	0.005	0.006	0.011	0.011	0.007
	A17	0.351	0.693	0.705	0.831	1.080
		0.330	0.678	0.678	0.816	1.068
		0.342	0.684	0.690	0.822	1.074
	Average	**0.341**	**0.685**	**0.691**	**0.823**	**1.074**
	Standard deviation	0.0086	0.006	0.011	0.006	0.005
	A14	0.483	0.777	0.783	0.984	1.002
		0.486	0.765	0.768	0.963	1.023
		0.483	0.771	0.777	0.975	1.014
	Average	**0.484**	**0.771**	**0.776**	**0.974**	**1.013**
	Standard deviation	0.001	0.005	0.006	0.009	0.009
	Control	2.115				
		2.100				
		2.109				
	Average	**2.108**				
	Standard deviation	0.006				

CTAB	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	72.865	72.391	76.660	66.556	83.824	77.039
	8	56.973	58.824	63.852	51.708	67.505	63.425
	6	54.175	58.017	61.765	50.522	67.220	63.188
	4	53.653	56.879	60.769	49.81	61.103	53.795
	2	48.814	55.361	48.102	47.059	49.051	51.945

$CaCl_2$	Isolates	10	8	6	4	2
	E1	0.381	0.447	0.45	0.462	0.507
		0.354	0.438	0.438	0.450	0.453
		0.369	0.441	0.444	0.456	0.501
	Average	**0.368**	**0.442**	**0.444**	**0.456**	**0.487**
	Standard deviation	0.011	0.004	0.005	0.005	0.024
	D1	0.378	0.405	0.411	0.420	0.435
		0.372	0.390	0.390	0.405	0.417
		0.375	0.399	0.402	0.414	0.426
	Average	**0.375**	**0.398**	**0.401**	**0.413**	**0.1426**
	Standard deviation	0.002	0.006	0.009	0.006	0.007
	R 2	0.348	0.381	0.426	0.453	0.459
		0.333	0.360	0.414	0.438	0.477
		0.342	0.369	0.417	0.447	0.468
	Average	**0.341**	**0.37**	**0.419**	**0.446**	**0.468**
	Standard deviation	0.006	0.009	0.005	0.006	0.007
	A22	0.381	0.432	0.438	0.483	0.495
		0.360	0.423	0.423	0.471	0.486
		0.372	0.429	0.432	0.477	0.501
	Average	**0.371**	**0.428**	**0.431**	**0.477**	**0.490**
	Standard deviation	0.009	0.004	0.006	0.005	0.006
	A17	0.357	0.435	0.441	0.477	0.501
		0.348	0.438	0.447	0.468	0.489
		0.351	0.438	0.444	0.474	0.495
	Average	**0.352**	**0.473**	**0.444**	**0.473**	**0.495**
	Standard deviation	0.004	0.001	0.002	0.003	0.005
	A14	0.333	0.417	0.423	0.450	0.489
		0.318	0.396	0.411	0.444	0.477
		0.324	0.405	0.417	0.447	0.483
	Average	**0.325**	**0.406**	**0.417**	**0.447**	**0.483**
	Standard deviation	0.006	0.009	0.005	0.002	0.005
	Control	0.747				
		0.735				
		0.741				
	Average	**0.741**				
	Standard deviation	0.005				

$CaCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	50.337	49.393	53.981	49.933	52.497	56.140
	8	40.351	46.289	50.067	42.240	41.026	45.209
	6	40.081	45.884	43.455	41.835	40.081	43.725
	4	38.462	44.265	39.811	35.628	36.167	39.676
	2	34.278	42.510	36.842	33.873	33.198	34.818

$MnCl_2$	Isolates	10	8	6	4	2
	E1	0.003	0.015	0.027	0.057	0.069
		0	0.006	0.012	0.045	0.06
		0	0.012	0.018	0.051	0.06
	Average	**0.001**	**0.011**	**0.019**	**0.051**	**0.065**
	Standard deviation	0.001	0.004	0.006	0.005	0.004
	D1	0.030	0.048	0.054	0.051	0.057
		0.045	0.036	0.045	0.057	0.066
		0.039	0.042	0.051	0.054	0.060
	Average	**0.038**	**0.042**	**0.05**	**0.054**	**0.061**
	Standard deviation	0.006	0.005	0.004	0.002	0.004
	R 2	0.003	0.018	0.036	0.039	0.051
		0.024	0.021	0.033	0.045	0.063
		0.015	0.021	0.033	0.039	0.057
	Average	**0.014**	**0.020**	**0.034**	**0.041**	**0.057**
	Standard deviation	0.009	0.001	0.001	0.003	0.005
	A22	0.021	0.030	0.060	0.06	0.084
		0.036	0.033	0.069	0.075	0.075
		0.030	0.030	0.063	0.069	0.078
	Average	**0.029**	**0.031**	**0.064**	**0.068**	**0.079**
	Standard deviation	0.006	0.001	0.004	0.006	0.004
	A17	0.018	0.051	0.069	0.069	0.105
		0.006	0.063	0.054	0.075	0.078
		0.012	0.060	0.06	0.072	0.093
	Average	**0.012**	**0.058**	**0.061**	**0.072**	**0.092**
	Standard deviation	0.005	0.005	0.006	0.002	0.011
	A14	0.006	0.042	0.045	0.039	0.057
		0.015	0.024	0.030	0.039	0.045
		0.009	0.033	0.039	0.042	0.051
	Average	**0.010**	**0.033**	**0.038**	**0.04**	**0.051**
	Standard deviation	0.004	0.004	0.006	0.001	0.005
	Control	0.246				
		0.231				
		0.237				
	Average	**0.238**				
	Standard deviation	0.006				

$MnCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	99.580	84.034	94.118	87.815	94.958	95.798
	8	95.378	82.353	91.597	86.975	75.630	86.134
	6	92.017	78.992	85.714	73.109	74.370	84.034
	4	78.571	77.311	82.778	71.429	69.748	83.193
	2	72.689	74.370	76.050	66.807	61.345	78.571

$MgSO_4$	Isolates	10	8	6	4	2
	E1	0.060	0.081	0.078	0.075	0.084
		0.060	0.063	0.072	0.084	0.075
		0.060	0.072	0.075	0.081	0.081
	Average	**0.060**	**0.072**	**0.075**	**0.08**	**0.080**
	Standard deviation	0	0.0070	0.0020	0.004	0.004
	D1	0.081	0.096	0.096	0.108	0.126
		0.078	0.075	0.090	0.111	0.132
		0.078	0.087	0.090	0.114	0.129
	Average	**0.079**	**0.086**	**0.092**	**0.110**	**0.129**
	Standard deviation	0.001	0.009	0.003	0.002	0.002
	R 2	0.045	0.069	0.075	0.081	0.111
		0.030	0.069	0.063	0.084	0.096
		0.039	0.066	0.069	0.090	0.105
	Average	**0.038**	**0.065**	**0.069**	**0.085**	**0.104**
	Standard deviation	0.006	0.004	0.005	0.004	0.006
	A22	0.060	0.075	0.069	0.093	0.093
		0.051	0.069	0.090	0.087	0.093
		0.054	0.072	0.081	0.090	0.099
	Average	**0.055**	**0.072**	**0.079**	**0.090**	**0.095**
	Standard deviation	0.004	0.002	0.009	0.002	0.003
	A17	0.051	0.078	0.072	0.087	0.096
		0.048	0.057	0.078	0.081	0.099
		0.048	0.069	0.075	0.075	0.096
	Average	**0.049**	**0.068**	**0.075**	**0.081**	**0.097**
	Standard deviation	0.001	0.009	0.005	0.0049	0.00141
	A14	0.027	0.036	0.087	0.093	0.105
		0.030	0.045	0.072	0.075	0.108
		0.030	0.042	0.081	0.0843	0.105
	Average	**0.029**	**0.041**	**0.08**	**0.084**	**0.106**
	Standard deviation	0.001	0.004	0.006	0.007	0.001
	Control	0.264				
		0.273				
		0.270				
	Average	**0.269**				
	Standard deviation	0.004				

$MgSO_4$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	77.695	70.632	85.874	79.554	81.784	89.219
	8	73.234	68.030	75.836	73.234	74.721	84.758
	6	72.119	65.799	74.349	70.632	72.119	70.260
	4	70.260	59.108	68.401	66.543	69.888	68.773
	2	67.286	52.045	61.338	64.684	63.941	60.595

Table 7.43: OD_{550nm} values for the determination of flocculating activity of fawn dye using different concentrations of bioflocculants

Isolates	10	8	6	4	2
E1	0.135	0.207	0.228	0.246	0.252
	0.132	0.210	0.222	0.246	0.246
	0.141	0.210	0.234	0.090	0.258
Average	**0.136**	**0.209**	**0.228**	**0.247**	**0.252**
Standard deviation	0.004	0.001	0.004	0.074	0.005
D1	0.042	0.045	0.063	0.087	0.105
	0.036	0.036	0.057	0.078	0.099
	0.045	0.051	0.066	0.093	0.108
Average	**0.041**	**0.044**	**0.062**	**0.086**	**0.104**
Standard deviation	0.004	0.006	0.004	0.006	0.004
R 2	0.378	0.552	0.636	0.642	0.645
	0.369	0.549	0.633	0.636	0.642
	0.384	0.555	0.642	0.645	0.651
Average	**0.377**	**0.552**	**0.637**	**0.641**	**0.646**
Standard deviation	0.006	0.002	0.004	0.004	0.004
A22	0.597	0.603	0.633	0.642	0.654
	0.600	0.597	0.630	0.636	0.651
	0.594	0.606	0.636	0.645	0.657
Average	**0.597**	**0.602**	**0.633**	**0.641**	**0.654**
	0.002	0.004	0.002	0.004	0.002
A17	0.630	0.639	0.645	0.651	0.663
	0.624	0.630	0.636	0.648	0.657
	0.636	0.645	0.651	0.654	0.669
Average	**0.63**	**0.638**	**0.644**	**0.651**	**0.663**
Standard deviation	0.005	0.006	0.006	0.002	0.005
A14	0.582	0.627	0.633	0.639	0.672
	0.573	0.594	0.63	0.633	0.669
	0.588	0.660	0.636	0.645	0.675
Average	**0.581**	**0.627**	**0.633**	**0.369**	**0.272**
Standard deviation	0.006	0.027	0.002	0.005	0.002
Control	0.807				
	0.81				
	0.81				
Average	**0.809**				
Standard deviation	0.001				

Table 7.44: Percentage removal (C_0–C/C_0 x 100) of fawn dye using different concentrations of flocculants as derived from Table 7.43 (Fig. 4.11)

Conc (ppm)	E1	D1	R2	A22	A17	A14
10	83.189	94.932	53.399	26.205	22.126	28.183
8	74.166	94.561	31.768	25.587	21.137	22.467
6	71.817	92.336	21.261	21.755	20.396	21.755
4	69.468	89.369	20.766	20.766	19.530	21.014
2	68.850	87.145	20.148	19.159	18.047	16.934

Table 7.45: Flocculating activity of fawn dye as derived from Table 7.43 using an equation for flocculating activity in Section 4.2.3 (Fig. 4.12)

Isolate	Flocculating activity	Standard deviation
E1	14.158	0.198
D1	4.468	0.152
R2	17.613	0.369
A22	73.368	0.215
A17	146.413	0.166
A14	56.011	0.200

Table 7.46: OD_{550nm} values for the determination of effect of pH on the removal of fawn dye and the calculations for the percentage removal (C_0–C/C_0 x 100) (Fig. 4.13)

pH 6	Isolates	10	8	6	4	2
	E1	0.618	0.627	0.630	0.654	0.696
		0.612	0.618	0.624	0.648	0.693
		0.621	0.633	0.636	0.657	0.702
	Average	**0.617**	**0.626**	**0.630**	**0.653**	**0.697**
	Standard deviation	0.004	0.006	0.005	0.004	0.004
	D1	0.585	0.591	0.600	0.609	0.609
		0.576	0.588	0.600	0.600	0.609
		0.591	0.594	0.603	0.615	0.612
	Average	**0.584**	**0.591**	**0.601**	**0.608**	**0.61**
		0.006	0.002	0.001	0.006	0.001
	R 2	0.558	0.573	0.576	0.579	0.591
		0.555	0.564	0.570	0.576	0.591
		0.561	0.579	0.579	0.585	0.594
	Average	**0.558**	**0.572**	**0.575**	**0.58**	**0.592**
	Standard deviation	0.002	0.006	0.004	0.004	0.001
	A22	0.591	0.600	0.603	0.603	0.609
		0.588	0.600	0.597	0.600	0.603
		0.597	0.600	0.606	0.609	0.612
	Average	**0.592**	**0.600**	**0.602**	**0.604**	**0.608**
	Standard deviation	0.004	0	0.004	0.004	0.004
	A17	0.576	0.582	0.588	0.597	0.597
		0.576	0.582	0.594	0.600	0.603
		0.579	0.585	0.582	0.591	0.597
	Average	**0.577**	**0.583**	**0.588**	**0.596**	**0.599**
	Standard deviation	0.001	0.001	0.005	0.005	0.003
	A14	0.567	0.579	0.585	0.591	0.666
		0.570	0.570	0.582	0.588	0.657
		0.570	0.585	0.588	0.594	0.672
	Average	**0.569**	**0.578**	**0.585**	**0.591**	**0.665**
	Standard deviation	0.001	0.006	0.002	0.002	0.006
	Control	0.714				
		0.711				
		0.720				
	Average	**0.715**				
	Standard deviation	0.004				

pH 6	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	22.970	18.322	21.958	17.203	19.301	20.420
	8	21.848	17.343	20.000	16.084	18.462	19.161
	6	21.348	15.944	19.580	15.804	17.762	18.182
	4	18.477	14.965	18.881	15.524	16.643	17.343
	2	14.921	14.685	17.203	14.965	16.224	6.993

pH 7	Isolates	10	8	6	4	2
	E1	0.579	0.591	0.600	0.609	0.618
		0.573	0.588	0.597	0.609	0.612
		0.582	0.597	0.606	0.612	0.624
	Average	**0.578**	**0.592**	**0.601**	**0.610**	**0.618**
	Standard deviation	0.004	0.004	0.004	0.001	0.005
	D1	0.621	0.63	0.636	0.642	0.648
		0.615	0.624	0.63	0.636	0.639
		0.624	0.636	0.639	0.645	0.654
	Average	**0.620**	**0.630**	**0.635**	**0.641**	**0.647**
	Standard deviation	0.004	0.005	0.004	0.004	0.006
	R 2	0.624	0.636	0.642	0.642	0.645
		0.621	0.636	0.636	0.639	0.642
		0.627	0.639	0.645	0.648	0.648
	Average	**0.624**	**0.637**	**0.641**	**0.643**	**0.645**
	Standard deviation	0.002	0.001	0.004	0.004	0.002
	A22	0.576	0.636	0.642	0.645	0.648
		0.567	0.636	0.636	0.645	0.645
		0.582	0.639	0.348	0.648	0.654
	Average	**0.575**	**0.637**	**0.642**	**0.646**	**0.649**
	Standard deviation	0.006	0.001	0.137	0.001	0.004
	A17	0.639	0.642	0.648	0.651	0.687
		0.636	0.639	0.639	0.645	0.684
		0.642	0.648	0.654	0.654	0.69
	Average	**0.639**	**0.643**	**0.647**	**0.650**	**0.687**
	Standard deviation	0.002	0.004	0.006	0.004	0.002
	A14	0.633	0.648	0.651	0.666	0.681
		0.630	0.645	0.648	0.666	0.681
		0.639	0.651	0.657	0.669	0.684
	Average	**0.234**	**0.648**	**0.652**	**0.667**	**0.682**
	Standard deviation	0.004	0.002	0.004	0.001	0.001
	Control	0.822				
		0.816				
		0.825				
	Average	**0.821**				
	Standard deviation	0.004				

pH 7	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	29.598	22.597	22.097	28.215	20.225	20.849
	8	27.893	21.348	20.474	20.474	19.725	19.101
	6	26.797	20.724	19.975	19.850	19.226	18.602
	4	25.700	19.975	19.725	19.351	18.851	16.729
	2	24.726	19.226	19.476	18.976	14.232	14.856

pH 8	Isolates	10	8	6	4	2
	E1	0.489	0.57	0.582	0.597	0.645
		0.483	0.567	0.582	0.600	0.642
		0.492	0.576	0.585	0.600	0.651
	Average	**0.488**	**0.571**	**0.583**	**0.597**	**0.646**
	Standard deviation	0.004	0.004	0.001	0.001	0.004
	D1	0.588	0.594	0.594	0.597	0.597
		0.585	0.588	0.594	0.600	0.600
		0.594	0.597	0.597	0.600	0.600
	Average	**0.589**	**0.593**	**0.595**	**0.597**	**0.599**
	Standard deviation	0.004	0.004	0.001	0.001	0.001
	R 2	0.591	0.594	0.600	0.609	0.615
		0.585	0.594	0.597	0.606	0.609
		0.594	0.597	0.606	0.612	0.621
	Average	**0.590**	**0.595**	**0.601**	**0.609**	**0.615**
	Standard deviation	0.004	0.001	0.004	0.002	0.005
	A22	0.585	0.591	0.597	0.600	0.603
		0.579	0.591	0.600	0.540	0.600
		0.591	0.594	0.600	0.660	0.609
	Average	**0.585**	**0.592**	**0.597**	**0.600**	**0.604**
	Standard deviation	0.005	0.001	0.001	0.049	0.002
	A17	0.594	0.609	0.612	0.618	0.630
		0.591	0.609	0.606	0.618	0.624
		0.597	0.612	0.618	0.621	0.816
	Average	**0.594**	**0.610**	**0.612**	**0.619**	**0.630**
	Standard deviation	0.002	0.001	0.005	0.001	0.089
	A14	0.585	0.603	0.612	0.612	0.621
		0.582	0.603	0.603	0.612	0.618
		0.591	0.606	0.618	0.615	0.627
	Average	**0.586**	**0.604**	**0.611**	**0.613**	**0.622**
	Standard deviation	0.004	0.001	0.006	0.001	0.004
	Control	0.801				
		0.807				
		0.795				
	Average	**0.801**				
	Standard deviation	0.005				

pH 8	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	31.748	28.258	28.136	28.745	27.649	28.624
	8	20.140	27.771	27.771	27.893	25.700	26.431
	6	18.462	27.527	27.797	27.283	25.457	25.579
	4	16.503	27.284	25.822	26.918	24.604	25.335
	2	9.650	27.040	25.091	26.431	23.264	24.239

pH 9	Isolates	10	8	6	4	2
	E1	0.345	0.348	0.354	0.366	0.387
		0.342	0.342	0.354	0.366	0.378
		0.351	0.354	0.357	0.372	0.393
	Average	**0.346**	**0.348**	**0.355**	**0.368**	**0.386**
	Standard deviation	0.004	0.005	0.001	0.003	0.006
	D1	0.348	0.354	0.357	0.360	0.366
		0.342	0.351	0.351	0.357	0.363
		0.354	0.357	0.36	0.366	0.372
	Average	**0.348**	**0.354**	**0.356**	**0.361**	**0.367**
	Standard deviation	0.005	0.002	0.004	0.004	0.004
	R 2	0.435	0.450	0.453	0.453	0.462
		0.432	0.444	0.444	0.453	0.456
		0.441	0.453	0.459	0.456	0.465
	Average	**0.436**	**0.449**	**0.452**	**0.454**	**0.461**
	Standard deviation	0.004	0.004	0.006	0.001	0.004
	A22	0.447	0.456	0.465	0.471	0.501
		0.444	0.453	0.459	0.465	0.498
		0.450	0.459	0.471	0.474	0.507
	Average	**0.447**	**0.456**	**0.465**	**0.470**	**0.502**
	Standard deviation	0.002	0.002	0.005	0.004	0.004
	A14	0.348	0.351	0.456	0.468	0.510
		0.348	0.348	0.450	0.468	0.504
		0.351	0.354	0.462	0.471	0.516
	Average	**0.349**	**0.351**	**0.456**	**0.469**	**0.51**
	Standard deviation	0.001	0.002	0.005	0.001	0.005
	A17	0.351	0.366	0.372	0.399	0.405
		0.348	0.363	0.366	0.396	0.405
		0.357	0.372	0.375	0.402	0.408
	Average	**0.352**	**0.367**	**0.371**	**0.399**	**0.406**
	Standard deviation	0.004	0.004	0.004	0.002	0.001
	Control	0.825				
		0.822				
		0.828				
	Average	**0.825**				
	Standard deviation	0.002				

pH 9	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	58.061	57.818	47.152	45.818	57.333	57.697
	8	57.818	57.091	45.576	44.727	55.515	57.455
	6	56.970	56.848	45.212	44.848	55.030	44.727
	4	55.394	56.242	44.970	43.030	51.636	43.152
	2	53.212	55.515	44.121	39.152	50.788	38.182

pH 10	Isolates	10	8	6	4	2
	E1	0.312	0.321	0.342	0.348	0.366
		0.309	0.312	0.339	0.348	0.357
		0.315	0.327	0.345	0.351	0.372
	Average	**0.312**	**0.320**	**0.342**	**0.349**	**0.365**
	Standard deviation	0.002	0.006	0.002	0.001	0.006
	D1	0.339	0.348	0.354	0.372	0.387
		0.330	0.348	0.348	0.366	0.384
		0.345	0.351	0.357	0.375	0.390
	Average	**0.338**	**0.349**	**0.353**	**0.371**	**0.387**
	Standard deviation	0.006	0.001	0.004	0.004	0.002
	R 2	0.318	0.342	0.348	0.351	0.357
		0.315	0.339	0.342	0.348	0.351
		0.321	0.345	0.351	0.357	0.36
	Average	**0.318**	**0.342**	**0.347**	**0.352**	**0.356**
	Standard deviation	0.002	0.002	0.004	0.004	0.004
	A22	0.339	0.363	0.366	0.366	0.372
		0.333	0.354	0.357	0.363	0.366
		0.345	0.369	0.372	0.372	0.375
	Average	**0.339**	**0.362**	**0.365**	**0.367**	**0.371**
	Standard deviation	0.005	0.006	0.006	0.004	0.004
	A17	0.354	0.360	0.363	0.369	0.384
		0.354	0.351	0.354	0.363	0.381
		0.357	0.366	0.369	0.372	0.390
	Average	**0.355**	**0.359**	**0.362**	**0.368**	**0.385**
	Standard deviation	0.001	0.006	0.006	0.004	0.004
	A14	0.354	0.357	0.360	0.366	0.369
		0.348	0.351	0.357	0.363	0.363
		0.357	0.363	0.363	0.369	0.375
	Average	**0.353**	**0.357**	**0.36**	**0.366**	**0.369**
	Standard deviation	0.004	0.005	0.002	0.002	0.005
	Control	0.831				
		0.825				
		0.837				
	Average	**0.831**				
	Standard deviation	0.005				

pH 10	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	62.455	59.326	61.733	59.206	57.280	57.521
	8	61.492	58.002	58.845	56.438	56.799	57.040
	6	58.845	56.799	58.243	56.077	56.438	56.979
	4	58.002	55.355	57.641	55.836	55.716	55.957
	2	56.077	53.430	57.160	55.355	53.670	55.596

Table 7.47: OD_{550nm} values for the determination of effect of temperature on the removal fawn dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.14)

28°C	Isolates	10	8	6	4	2
	E1	0.639	0.645	0.648	0.651	0.657
		0.633	0.642	0.642	0.645	0.654
		0.645	0.648	0.651	0.657	0.663
	Average	**0.639**	**0.645**	**0.647**	**0.651**	**0.658**
	Standard deviation	0.005	0.002	0.004	0.005	0.004
	D1	0.642	0.651	0.654	0.657	0.663
		0.642	0.648	0.654	0.687	0.657
		0.645	0.657	0.657	0.633	0.669
	Average	**0.643**	**0.652**	**0.655**	**0.659**	**0.663**
	Standard deviation	0.001	0.004	0.001	0.020	0.0045
	R 2	0.645	0.648	0.657	0.660	0.663
		0.639	0.648	0.654	0.657	0.654
		0.648	0.651	0.660	0.663	0.669
	Average	**0.644**	**0.649**	**0.657**	**0.66**	**0.662**
	Standard deviation	0.004	0.001	0.002	0.002	0.006
	A22	0.642	0.648	0.651	0.660	0.669
		0.636	0.642	0.648	0.657	0.672
		0.645	0.651	0.654	0.663	0.669
	Average	**0.641**	**0.647**	**0.651**	**0.660**	**0.670**
	Standard deviation	0.004	0.004	0.002	0.002	0.001
	A17	0.657	0.66	0.663	0.666	0.702
		0.651	0.657	0.657	0.660	0.699
		0.660	0.663	0.669	0.669	0.705
	Average	**0.656**	**0.660**	**0.663**	**0.665**	**0.702**
	Standard deviation	0.004	0.002	0.005	0.004	0.002
	A14	0.546	0.612	0.627	0.657	0.666
		0.543	0.606	0.627	0.654	0.660
		0.552	0.615	0.63	0.660	0.669
	Average	**0.547**	**0.611**	**0.629**	**0.628**	**0.665**
	Standard deviation	0.004	0.004	0.001	0.003	0.004
	Control	0.858				
		0.846				
		0.867				
	Average	**0.857**				
	Standard deviation	0.009				

28°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	25.438	24.971	24.854	25.204	23.454	36.173
	8	24.737	23.921	24.271	24.504	22.987	28.705
	6	24.504	23.571	23.337	24.037	22.637	26.721
	4	24.037	23.104	22.987	22.987	22.404	23.337
	2	23.221	22.637	22.754	21.820	18.086	22.404

35°C	Isolates	10	8	6	4	2
	E1	0.444	0.486	0.492	0.498	0.504
		0.438	0.480	0.486	0.489	0.498
		0.447	0.492	0.495	0.480	0.507
	Average	**0.443**	**0.486**	**0.491**	**0.497**	**0.503**
	Standard deviation	0.004	0.005	0.004	0.007	0.004
	D1	0.465	0.471	0.483	0.486	0.507
		0.465	0.462	0.477	0.483	0.510
		0.468	0.477	0.486	0.492	0.510
	Average	**0.466**	**0.470**	**0.482**	**0.487**	**0.509**
	Standard deviation	0.001	0.006	0.004	0.004	0.001
	R 2	0.477	0.495	0.498	0.504	0.564
		0.471	0.489	0.495	0.498	0.564
		0.480	0.501	0.501	0.510	0.567
	Average	**0.476**	**0.495**	**0.498**	**0.504**	**0.565**
	Standard deviation	0.004	0.005	0.002	0.005	0.001
	A22	0.441	0.468	0.471	0.477	0.486
		0.441	0.462	0.465	0.477	0.483
		0.444	0.474	0.474	0.480	0.492
	Average	**0.442**	**0.468**	**0.47**	**0.478**	**0.487**
	Standard deviation	0.001	0.005	0.004	0.001	0.004
	A17	0.477	0.495	0.495	0.501	0.507
		0.471	0.489	0.489	0.492	0.504
		0.480	0.498	0.498	0.507	0.504
	Average	**0.476**	**0.494**	**0.494**	**0.500**	**0.505**
	Standard deviation	0.004	0.004	0.004	0.006	0.001
	A14	0.471	0.477	0.483	0.495	0.501
		0.465	0.474	0.477	0.492	0.501
		0.474	0.480	0.489	0.501	0.504
	Average	**0.470**	**0.477**	**0.483**	**0.496**	**0.502**
	Standard deviation	0.004	0.002	0.005	0.004	0.001
	Control	0.837				
		0.831				
		0.843				
	Average	**0.837**				
	Standard deviation	0.005				

35°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	47.073	44.325	43.130	47.192	43.130	43.847
	8	41.935	43.847	40.860	44.086	40.980	43.010
	6	41.338	42.413	40.502	43.847	40.502	42.294
	4	40.621	41.816	39.785	42.891	40.263	40.741
	2	39.904	39.188	32.497	41.816	39.665	40.024

40°C	Isolates	10	8	6	4	2
	E1	0.315	0.324	0.429	0.429	0.444
		0.321	0.324	0.435	0.444	0.447
		0.318	0.324	0.432	0.438	0.444
	Average	**0.318**	**0.326**	**0.432**	**0.437**	**0.445**
	Standard deviation	0.002	0	0.002	0.006	0.001
	D1	0.420	0.432	0.435	0.462	0.492
		0.435	0.410	0.447	0.450	0.498
		0.426	0.438	0.441	0.453	0.492
	Average	**0.427**	**0.437**	**0.441**	**0.454**	**0.494**
	Standard deviation	0.006	0.0120	0.005	0.005	0.003
	R 2	0.435	0.438	0.450	0.450	0.492
		0.444	0.450	0.459	0.465	0.498
		0.441	0.444	0.453	0.459	0.495
	Average	**0.440**	**0.444**	**0.454**	**0.458**	**0.495**
	Standard deviation	0.004	0.005	0.004	0.006	0.002
	A22	0.306	0.315	0.321	0.444	0.444
		0.312	0.327	0.336	0.438	0.447
		0.309	0.321	0.330	0.441	0.444
	Average	**0.309**	**0.321**	**0.329**	**0.441**	**0.445**
	Standard deviation	0.002	0.005	0.006	0.002	0.001
	A17	0.432	0.432	0.540	0.555	0.573
		0.438	0.447	0.549	0.561	0.582
		0.435	0.441	0.546	0.558	0.576
	Average	**0.435**	**0.440**	**0.545**	**0.558**	**0.577**
	Standard deviation	0.002	0.006	0.004	0.002	0.004
	A14	0.420	0.441	0.441	0.47	0.483
		0.429	0.444	0.45	0.453	0.471
		0.423	0.441	0.447	0.45	0.477
	Average	**0.424**	**0.442**	**0.446**	**0.45**	**0.477**
	Standard deviation	0.004	0.001	0.004	0.008	0.005
	Control	0.825				
		0.828				
		0.825				
	Average	**0.826**				
	Standard deviation	0.001				

40°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	61.501	48.305	46.731	62.591	47.337	48.668
	8	50.000	47.094	46.247	61.138	46.731	46.489
	6	47.700	46.610	45.036	60.169	34.019	46.005
	4	47.094	45.036	44.552	46.610	32.446	45.521
	2	46.126	40.193	40.073	46.126	30.145	42.252

45°C	Isolates	10	8	6	4	2
	E1	0.405	0.417	0.438	0.444	0.462
		0.399	0.420	0.435	0.438	0.462
		0.411	0.420	0.444	0.447	0.465
	Average	**0.405**	**0.419**	**0.439**	**0.443**	**0.463**
	Standard deviation	0.005	0.001	0.004	0.004	0.001
	D1	0.405	0.426	0.429	0.435	0.444
		0.405	0.420	0.426	0.432	0.438
		0.408	0.432	0.435	0.441	0.447
	Average	**0.406**	**0.426**	**0.430**	**0.436**	**0.443**
	Standard deviation	0.001	0.005	0.004	0.004	0.004
	R 2	0.324	0.327	0.351	0.351	0.393
		0.324	0.324	0.336	0.345	0.387
		0.327	0.333	0.354	0.357	0.396
	Average	**0.325**	**0.328**	**0.347**	**0.351**	**0.392**
	Standard deviation	0.001	0.004	0.008	0.005	0.004
	A22	0.432	0.435	0.438	0.438	0.447
		0.426	0.429	0.441	0.450	0.444
		0.435	0.441	0.438	0.444	0.450
	Average	**0.431**	**0.435**	**0.439**	**0.444**	**0.447**
	Standard deviation	0.004	0.005	0.001	0.004	0.002
	A17	0.411	0.417	0.429	0.444	0.453
		0.408	0.420	0.423	0.438	0.447
		0.417	0.420	0.432	0.447	0.456
	Average	**0.412**	**0.419**	**0.428**	**0.443**	**0.452**
	Standard deviation	0.004	0.001	0.004	0.004	0.004
	A14	0.411	0.42	0.423	0.426	0.471
		0.408	0.414	0.42	0.429	0.468
		0.417	0.426	0.426	0.420	0.474
	Average	**0.412**	**0.42**	**0.423**	**0.425**	**0.471**
	Standard deviation	0.004	0.005	0.002	0.004	0.002
	Control	0.831				
		0.831				
		0.834				
	Average	**0.832**				
	Standard deviation	0.001				

45°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	51.322	51.202	60.938	48.197	50.481	50.481
	8	49.639	48.798	60.577	47.716	49.639	49.519
	6	47.236	48.317	58.293	47.236	48.558	49.159
	4	46.755	47.596	57.813	46.635	46.755	48.918
	2	44.351	46.755	52.885	46.274	45.673	43.389

Table 7.48: OD_{550nm} values for the determination of effect of cations on the removal of fawn dye and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.15)

CTAB	Isolates	10	8	6	4	2
	E1	0.012	0.015	0.015	0.072	0.108
		0.012	0.012	0.009	0.066	0.108
		0.015	0.021	0.018	0.078	0.111
	Average	**0.013**	**0.016**	**0.014**	**0.072**	**0.109**
	Standard deviation	0.001	0.004	0.004	0.004	0.001
	D1	0.006	0.027	0.039	0.048	0.12
		0.003	0.021	0.036	0.042	0.117
		0.012	0.033	0.042	0.051	0.126
	Average	**0.007**	**0.027**	**0.039**	**0.047**	**0.121**
	Standard deviation	0.004	0.005	0.002	0.004	0.004
	R 2	0.135	0.153	0.171	0.225	0.252
		0.108	0.144	0.171	0.216	0.234
		0.153	0.171	0.180	0.234	0.261
	Average	**0.044**	**0.052**	**0.059**	**0.075**	**0.083**
	Standard deviation	0.018	0.011	0.004	0.007	0.011
	A22	0.048	0.057	0.060	0.102	0.126
		0.042	0.057	0.057	0.096	0.123
		0.051	0.060	0.066	0.105	0.132
	Average	**0.047**	**0.058**	**0.061**	**0.101**	**0.127**
	Standard deviation	0.004	0.001	0.009	0.004	0.004
	A17	0.102	0.132	0.147	0.153	0.192
		0.099	0.132	0.141	0.153	0.186
		0.108	0.135	0.126	0.156	0.198
	Average	**0.103**	**0.133**	**0.148**	**0.154**	**0.192**
	Standard deviation	0.004	0.001	0.009	0.001	0.005
	A14	0.219	0.291	0.3	0.354	0.372
		0.213	0.285	0.294	0.354	0.366
		0.222	0.297	0.309	0.357	0.375
	Average	**0.218**	**0.291**	**0.301**	**0.355**	**0.371**
	Standard deviation	0.004	0.005	0.006	0.001	0.004
	Control	0.411				
		0.405				
		0.414				
	Average	**0.410**				
	Standard deviation	0.004				

CTAB	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	96.829	98.293	89.268	88.537	74.878	46.829
	8	96.098	93.415	87.317	85.854	67.561	29.024
	6	90.244	90.488	85.610	85.122	63.902	26.585
	4	82.195	88.537	81.707	75.366	92.439	13.415
	2	73.415	70.488	79.756	69.024	53.171	9.512

$CaCl_2$	Isolates	10	8	6	4	2
	E1	0.327	0.351	0.39	0.399	0.417
		0.327	0.351	0.384	0.393	0.417
		0.333	0.354	0.396	0.402	0.423
	Average	**0.329**	**0.352**	**0.390**	**0.398**	**0.419**
	Standard deviation	0.003	0.001	0.005	0.004	0.003
	D1	0.351	0.357	0.393	0.399	0.411
		0.351	0.348	0.393	0.396	0.405
		0.354	0.363	0.396	0.405	0.417
	Average	**0.352**	**0.356**	**0.394**	**0.4**	**0.411**
	Standard deviation	0.001	0.006	0.001	0.004	0.005
	R 2	0.303	0.333	0.348	0.357	0.375
		0.300	0.330	0.348	0.357	0.369
		0.309	0.339	0.351	0.360	0.381
	Average	**0.304**	**0.334**	**0.349**	**0.358**	**0.375**
	Standard deviation	0.004	0.004	0.001	0.001	0.005
	A22	0.330	0.333	0.339	0.345	0.369
		0.324	0.330	0.339	0.336	0.366
		0.336	0.339	0.342	0.351	0.372
	Average	**0.33**	**0.334**	**0.340**	**0.344**	**0.369**
	Standard deviation	0.005	0.004	0.001	0.006	0.002
	A17	0.006	0.027	0.039	0.168	0.174
		0.003	0.027	0.036	0.159	0.171
		0.012	0.030	0.042	0.174	0.177
	Average	**0.007**	**0.028**	**0.039**	**0.167**	**0.174**
	Standard deviation	0.004	0.001	0.002	0.006	0.002
	A14	0.003	0.009	0.015	0.036	0.342
		0.003	0.006	0.009	0.03	0.339
		0.006	0.015	0.018	0.039	0.348
	Average	**0.004**	**0.010**	**0.014**	**0.035**	**0.343**
	Standard deviation	0.001	0.004	0.004	0.004	0.004
	Control	0.708				
		0.702				
		0.711				
	Average	**0.707**				
	Standard deviation	0.004				

$CaCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	53.465	50.212	57.001	53.324	99.010	99.434
	8	50.212	49.646	52.646	52.758	96.040	98.586
	6	44.837	44.272	50.636	51.909	94.484	98.020
	4	43.706	43.423	49.364	51.344	76.379	95.050
	2	40.736	41.867	46.959	47.808	75.389	93.918

$MnCl_2$	Isolates	10	8	6	4	2
	E1	0.120	0.141	0.159	0.156	0.195
		0.114	0.135	0.153	0.147	0.189
		0.126	0.144	0.162	0.162	0.198
	Average	**0.120**	**0.140**	**0.158**	**0.155**	**0.194**
	Standard deviation	0.005	0.004	0.004	0.006	0.004
	D1	0.105	0.120	0.144	0.150	0.189
		0.102	0.117	0.138	0.141	0.189
		0.108	0.126	0.147	0.156	0.192
	Average	**0.105**	**0.121**	**0.143**	**0.149**	**0.190**
	Standard deviation	0.003	0.004	0.004	0.006	0.001
	R 2	0.129	0.141	0.153	0.621	0.168
		0.120	0.141	0.153	0.612	0.162
		0.135	0.144	0.156	0.627	0.171
	Average	**0.128**	**0.142**	**0.154**	**0.62**	**0.167**
	Standard deviation	0.006	0.001	0.001	0.006	0.004
	A22	0.138	0.153	0.162	0.168	0.261
		0.138	0.150	0.156	0.165	0.258
		0.141	0.159	0.165	0.174	0.264
	Average	**0.139**	**0.154**	**0.161**	**0.169**	**0.261**
	Standard deviation	0.001	0.004	0.004	0.006	0.002
	A17	0.123	0.156	0.177	0.198	0.207
		0.120	0.153	0.174	0.198	0.204
		0.129	0.162	0.18	0.201	0.210
	Average	**0.124**	**0.157**	**0.177**	**0.199**	**0.207**
	Standard deviation	0.004	0.004	0.002	0.001	0.002
	A14	0.087	0.126	0.141	0.144	0.159
		0.090	0.120	0.141	0.144	0.153
		0.090	0.129	0.147	0.147	0.165
	Average	**0.089**	**0.125**	**0.141**	**0.145**	**0.159**
	Standard deviation	0.001	0.004	0.003	0.001	0.005
	Control	0.531				
		0.540				
		0.522				
	Average	**0.531**				
	Standard deviation	0.007				

$MnCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	77.401	80.226	76.038	73.823	76.648	83.239
	8	73.635	77.213	73.258	70.998	70.433	76.460
	6	71.186	73.070	70.998	69.680	66.667	73.446
	4	70.810	70.056	69.492	68.173	62.524	72.693
	2	63.465	64.218	68.550	50.847	61.017	70.056

$MgSO_4$	Isolates	10	8	6	4	2
	E1	0.156	0.18	0.186	0.192	0.198
		0.153	0.177	0.180	0.189	0.189
		0.162	0.183	0.192	0.195	0.204
	Average	**0.157**	**0.18**	**0.186**	**0.192**	**0.197**
	Standard deviation	0.004	0.002	0.005	0.002	0.006
	D1	0.144	0.162	0.165	0.171	0.177
		0.138	0.156	0.159	0.168	0.174
		0.147	0.165	0.168	0.177	0.180
	Average	**0.143**	**0.161**	**0.164**	**0.172**	**0.177**
	Standard deviation	0.004	0.004	0.004	0.003	0.002
	R 2	0.141	0.147	0.171	0.192	0.201
		0.135	0.144	0.168	0.189	0.198
		0.144	0.153	0.177	0.195	0.204
	Average	**0.140**	**0.148**	**0.172**	**0.192**	**0.201**
	Standard deviation	0.004	0.004	0.004	0.002	0.002
	A22	0.141	0.429	0.168	0.186	0.210
		0.135	0.153	0.162	0.180	0.210
		0.144	0.165	0.171	0.192	0.213
	Average	**0.140**	**0.159**	**0.167**	**0.186**	**0.211**
	Standard deviation	0.004	0.127	0.004	0.005	0.001
	A17	0.162	0.171	0.177	0.183	0.234
		0.156	0.165	0.174	0.183	0.231
		0.165	0.177	0.183	0.186	0.24
	Average	**0.161**	**0.171**	**0.178**	**0.184**	**0.235**
	Standard deviation	0.004	0.005	0.004	0.001	0.004
	A14	0.174	0.183	0.192	0.192	0.195
		0.165	0.183	0.186	0.189	0.195
		0.180	0.186	0.195	0.198	0.198
	Average	**0.173**	**0.184**	**0.191**	**0.193**	**0.196**
	Standard deviation	0.006	0.001	0.004	0.004	0.001
	Control	0.543				
		0.537				
		0.546				
	Average	**0.542**				
	Standard deviation	0.00374				

$MgSO_4$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	71.033	73.616	74.170	74.170	70.295	68.081
	8	67.159	70.295	72.694	70.664	68.450	66.052
	6	65.683	69.742	68.266	69.188	67.159	64.760
	4	64.576	68.266	64.576	65.683	66.052	64.391
	2	63.653	67.343	62.915	61.070	56.642	63.838

Table 7.49: OD_{550nm} values for the determination of flocculating activity of mixed dyes using different concentrations of bioflocculants (Fig. 4.16)

Isolates	10	8	6	4	2
E1	0.057	0.057	0.051	0.048	0.072
	0.045	0.048	0.06	0.066	0.057
	0.051	0.051	0.054	0.063	0.063
Average	**0.050**	**0.050**	**0.055**	**0.059**	**0.064**
Standard deviation	0.005	0.004	0.004	0.008	0.006
D1	0.054	0.054	0.060	0.072	0.069
	0.048	0.060	0.063	0.066	0.078
	0.051	0.057	0.060	0.069	0.075
Average	**0.051**	**0.057**	**0.061**	**0.069**	**0.074**
Standard deviation	0.002	0.002	0.001	0.002	0.004
R 2	0.045	0.048	0.051	0.069	0.069
	0.048	0.051	0.057	0.072	0.078
	0.051	0.057	0.054	0.069	0.075
Average	**0.048**	**0.057**	**0.054**	**0.070**	**0.074**
Standard deviation	0.002	0.004	0.002	0.001	0.004
A22	0.045	0.048	0.054	0.057	0.066
	0.045	0.051	0.066	0.069	0.069
	0.051	0.051	0.060	0.063	0.066
Average	**0.047**	**0.050**	**0.060**	**0.063**	**0.067**
Standard deviation	0.003	0.001	0.005	0.005	0.001
A17	0.033	0.042	0.045	0.054	0.063
	0.042	0.051	0.057	0.063	0.066
	0.039	0.048	0.051	0.060	0.063
Average	**0.038**	**0.047**	**0.051**	**0.059**	**0.064**
Standard deviation	0.004	0.004	0.005	0.004	0.001
A14	0.045	0.048	0.063	0.072	0.072
	0.048	0.063	0.066	0.063	0.078
	0.045	0.057	0.063	0.063	0.075
Average	**0.046**	**0.056**	**0.064**	**0.067**	**0.075**
Standard deviation	0.001	0.006	0.001	0.004	0.002
Control	0.201				
	0.213				
	0.207				
Average	**0.207**				
Standard deviation	0.005				

Table 7.50: Percentage removal (C_0–C/C_0 x 100) of mixed dyes using different concentrations of flocculants as derived from Table 7.49 (Fig. 4.16)

Conc (ppm)	E1	D1	R2	A22	A17	A14
10	75.845	75.362	76.812	77.295	81.643	77.778
8	74.879	72.464	75.362	75.845	77.295	72.947
6	73.430	70.531	73.913	71.014	75.362	69.082
4	71.498	66.667	66.184	69.565	71.498	67.633
2	69.082	64.251	63.768	67.633	69.082	63.768

Table 7.51: Flocculating activity of mixed dyes as derived from Table 7.49 using an equation for flocculating activity in Section 4.2.3 (Fig. 4.17)

Isolate	Flocculating activity	Standard deviation
E1	25.611	0.315
D1	20.261	0.279
R2	15.898	0.127
A22	19.268	0.199
A17	15.597	0.249
A14	17.538	0.328

Table 7.52: OD_{550nm} values for the determination of effect of pH on the removal of mixed dyes and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.18)

pH 6	Isolates	10	8	6	4	2
	E1	0.183	0.192	0.195	0.204	0.213
		0.189	0.195	0.207	0.210	0.216
		0.186	0.192	0.201	0.207	0.213
	Average	**0.186**	**0.193**	**0.201**	**0.209**	**0.214**
	Standard deviation	0.002	0.001	0.005	0.002	0.001
	D1	0.186	0.183	0.189	0.192	0.207
		0.183	0.195	0.192	0.198	0.213
		0.186	0.189	0.189	0.195	0.210
	Average	**0.185**	**0.189**	**0.19**	**0.195**	**0.210**
	Standard deviation	0.001	0.005	0.001	0.002	0.002
	R 2	0.159	0.18	0.183	0.195	0.201
		0.165	0.195	0.198	0.195	0.210
		0.162	0.189	0.189	0.195	0.207
	Average	**0.162**	**0.188**	**0.190**	**0.195**	**0.206**
	Standard deviation	0.002	0.006	0.006	0	0.004
	A22	0.144	0.195	0.216	0.204	0.207
		0.150	0.201	0.189	0.207	0.210
		0.147	0.198	0.195	0.204	0.207
	Average	**0.147**	**0.198**	**0.200**	**0.205**	**0.208**
	Standard deviation	0.002	0.002	0.012	0.001	0.001
	A17	0.162	0.171	0.177	0.180	0.183
		0.174	0.168	0.180	0.186	0.192
		0.168	0.171	0.177	0.183	0.189
	Average	**0.168**	**0.170**	**0.176**	**0.183**	**0.183**
	Standard deviation	0.005	0.001	0.001	0.002	0.004
	A14	0.177	0.174	0.177	0.186	0.189
		0.165	0.18	0.183	0.195	0.198
		0.168	0.177	0.186	0.192	0.185
	Average	**0.170**	**0.177**	**0.182**	**0.191**	**0.194**
	Standard deviation	0.0051	0.002	0.004	0.004	0.005
	Control	0.300				
		0.300				
		0.297				
	Average	**0.299**				
	Standard deviation	0.001				

pH 6	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	37.793	38.127	45.820	50.836	43.813	43.144
	8	35.452	36.789	37.123	33.779	43.144	40.803
	6	32.776	36.455	36.455	33.110	41.137	39.130
	4	31.104	34.782	34.783	31.438	38.796	36.120
	2	28.428	29.766	31.104	30.435	37.124	35.117

pH 7	Isolates	10	8	6	4	2
	E1	0.141	0.183	0.162	0.171	0.174
		0.147	0.165	0.165	0.180	0.183
		0.144	0.159	0.162	0.177	0.180
	Average	**0.144**	**0.159**	**0.163**	**0.176**	**0.179**
	Standard deviation	0.002	0.010	0.001	0.004	0.004
	D1	0.144	0.156	0.156	0.156	0.180
		0.150	0.150	0.159	0.165	0.165
		0.147	0.153	0.156	0.162	0.162
	Average	**0.147**	**0.153**	**0.157**	**0.161**	**0.169**
	Standard deviation	0.002	0.002	0.001	0.004	0.008
	R 2	0.096	0.141	0.147	0.15	0.156
		0.099	0.147	0.156	0.159	0.159
		0.096	0.144	0.153	0.03	0.156
	Average	**0.097**	**0.144**	**0.152**	**0.154**	**0.157**
	Standard deviation	0.001	0.002	0.004	0.059	0.001
	A22	0.138	0.147	0.159	0.159	0.174
		0.144	0.150	0.153	0.159	0.177
		0.141	0.147	0.156	0.162	0.174
	Average	**0.141**	**0.149**	**0.156**	**0.160**	**0.175**
	Standard deviation	0.002	0.001	0.002	0.001	0.001
	A17	0.144	0.153	0.156	0.162	0.171
		0.153	0.156	0.168	0.168	0.174
		0.147	0.153	0.162	0.165	0.171
	Average	**0.148**	**0.154**	**0.162**	**0.165**	**0.172**
	Standard deviation	0.004	0.001	0.005	0.002	0.001
	A14	0.147	0.165	0.159	0.174	0.189
		0.162	0.159	0.174	0.171	0.195
		0.156	0.171	0.168	0.174	0.204
	Average	**0.155**	**0.165**	**0.167**	**0.173**	**0.196**
	Standard deviation	0.006	0.005	0.006	0.001	0.006
	Control	0.276				
		0.297				
		0.288				
	Average	**0.287**				
	Standard deviation	0.009				

pH 7	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	49.826	48.780	66.202	50.871	48.432	45.993
	8	44.599	46.690	49.826	48.084	46.341	42.509
	6	43.206	45.296	47.038	45.645	43.554	41.812
	4	38.676	43.902	46.341	44.251	42.509	39.721
	2	37.631	41.115	45.296	39.024	40.070	31.707

pH 8	Isolates	10	8	6	4	2
	E1	0.162	0.159	0.171	0.174	0.183
		0.165	0.171	0.180	0.183	0.183
		0.162	0.165	0.177	0.180	0.183
	Average	**0.163**	**0.165**	**0.176**	**0.179**	**0.182**
	Standard deviation	0.001	0.005	0.004	0.004	0
	D1	0.153	0.159	0.168	0.183	0.189
		0.159	0.168	0.174	0.192	0.195
		0.156	0.162	0.171	0.186	0.192
	Average	**0.156**	**0.163**	**0.171**	**0.187**	**0.192**
	Standard deviation	0.002	0.004	0.002	0.004	0.002
	R 2	0.144	0.15	0.15	0.159	0.192
		0.15	0.156	0.159	0.171	0.207
		0.159	0.153	0.156	0.165	0.198
	Average	**0.151**	**0.153**	**0.155**	**0.165**	**0.199**
	Standard deviation	0.006	0.002	0.004	0.005	0.006
	A22	0.153	0.150	0.153	0.162	0.162
		0.159	0.165	0.168	0.168	0.162
		0.156	0.159	0.162	0.165	0.162
		0.156	**0.158**	**0.161**	**0.165**	**0.169**
		0.002	0.006	0.006	0.002	0
	A17	0.153	0.159	0.165	0.171	0.186
		0.159	0.162	0.177	0.168	0.192
		0.156	0.159	0.171	0.180	0.189
	Average	**0.156**	**0.16**	**0.171**	**0.176**	**0.189**
	Standard deviation	0.002	0.001	0.005	0.005	0.002
	A14	0.138	0.138	0.147	0.162	0.177
		0.138	0.147	0.15	0.171	0.183
		0.138	0.141	0.147	0.165	0.180
	Average	**0.138**	**0.142**	**0.148**	**0.166**	**0.180**
	Standard deviation	0	0.004	0.001	0.004	0.002
	Control	0.204				
		0.213				
		0.210				
	Average	**0.209**				
	Standard deviation	0.004				

pH 8	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	22.010	25.359	27.751	25.359	25.359	33.971
	8	21.053	22.010	26.794	24.402	23.445	32.057
	6	15.789	18.182	25.837	22.967	18.182	29.187
	4	14.354	10.526	21.053	21.053	15.789	20.574
	2	12.919	8.134	4.785	19.139	9.569	13.876

pH 9	Isolates	10	8	6	4	2
	E1	0.18	0.168	0.165	0.174	0.177
		0.159	0.177	0.183	0.180	0.186
		0.168	0.171	0.174	0.177	0.183
	Average	**0.169**	**0.172**	**0.174**	**0.177**	**0.182**
	Standard deviation	0.009	0.004	0.007	0.002	0.004
	D1	0.147	0.165	0.168	0.174	0.183
		0.150	0.174	0.174	0.186	0.186
		0.147	0.168	0.171	0.18	0.183
	Average	**0.148**	**0.169**	**0.171**	**0.18**	**0.184**
	Standard deviation	0.001	0.004	0.002	0.005	0.001
	R 2	0.129	0.138	0.144	0.153	0.156
		0.132	0.141	0.147	0.162	0.162
		0.129	0.138	0.144	0.156	0.159
	Average	**0.13**	**0.139**	**0.145**	**0.157**	**0.159**
	Standard deviation	0.001	0.001	0.001	0.004	0.002
	A22	0.135	0.153	0.174	0.183	0.195
		0.153	0.168	0.177	0.195	0.195
		0.144	0.162	0.174	0.189	0.201
	Average	**0.144**	**0.161**	**0.175**	**0.188**	**0.197**
	Standard deviation	0.007	0.006	0.001	0.005	0.003
	A17	0.135	0.138	0.144	0.147	0.168
		0.135	0.144	0.15	0.156	0.165
		0.135	0.141	0.147	0.153	0.168
	Average	**0.136**	**0.141**	**0.147**	**0.152**	**0.167**
	Standard deviation	0	0.002	0.002	0.004	0.001
	A14	0.147	0.147	0.156	0.162	0.174
		0.147	0.156	0.162	0.168	0.186
		0.153	0.153	0.159	0.165	0.180
	Average	**0.149**	**0.152**	**0.159**	**0.165**	**0.180**
	Standard deviation	0.003	0.004	0.002	0.002	0.005
	Control	0.273				
		0.276				
		0.273				
	Average	**0.274**				
	Standard deviation	0.001				

pH 9	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	38.321	45.985	52.555	47.445	50.365	45.620
	8	37.226	38.321	49.270	41.241	48.540	44.526
	6	36.496	37.591	47.080	36.496	46.351	41.971
	4	35.401	34.307	42.701	31.387	44.526	39.781
	2	33.577	32.847	41.971	28.102	39.051	34.307

pH 10	Isolates	10	8	6	4	2
	E1	0.027	0.03	0.045	0.039	0.042
		0.021	0.027	0.039	0.042	0.051
		0.024	0.030	0.030	0.039	0.042
	Average	**0.024**	**0.029**	**0.038**	**0.040**	**0.046**
	Standard deviation	0.002	0.001	0.006	0.001	0.004
	D1	0.027	0.054	0.054	0.063	0.075
		0.036	0.063	0.072	0.072	0.081
		0.030	0.060	0.069	0.069	0.087
	Average	**0.031**	**0.059**	**0.065**	**0.068**	**0.081**
	Standard deviation	0.004	0.004	0.008	0.004	0.005
	R 2	0.003	0.021	0.024	0.048	0.051
		0	0.021	0.027	0.051	0.066
		0.003	0.015	0.021	0.048	0.060
	Average	**0.002**	**0.019**	**0.024**	**0.049**	**0.059**
	Standard deviation	0.001	0.003	0.002	0.001	0.006
	A22	0.012	0.012	0.024	0.048	0.054
		0.018	0.027	0.027	0.051	0.063
		0.015	0.018	0.021	0.048	0.060
	Average	**0.015**	**0.019**	**0.024**	**0.049**	**0.059**
	Standard deviation	0.002	0.006	0.002	0.001	0.004
	A17	0.012	0.024	0.036	0.051	0.075
		0.015	0.024	0.048	0.063	0.081
		0.012	0.021	0.042	0.066	0.078
	Average	**0.013**	**0.023**	**0.042**	**0.06**	**0.078**
	Standard deviation	0.001	0.001	0.005	0.006	0.002
	A14	0.006	0.021	0.030	0.036	0.045
		0.006	0.027	0.033	0.039	0.054
		0.009	0.024	0.030	0.042	0.051
	Average	**0.007**	**0.024**	**0.031**	**0.039**	**0.049**
	Standard deviation	0.001	0.002	0.001	0.002	0.004
	Control	0.246				
		0.257				
		0.258				
	Average	**0.258**				
	Standard deviation	0.005				

pH 10	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	90.698	87.984	99.225	94.186	94.961	97.287
	8	88.760	77.132	96.124	92.636	91.085	90.698
	6	85.271	74.806	95.349	90.698	83.721	87.984
	4	84.496	73.643	92.636	81.008	76.744	84.884
	2	82.171	68.605	89.922	77.132	69.767	82.558

Table 7.53: OD_{550nm} values for the determination of effect of temperature on the removal mixed dyes and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.19)

28°C	Isolates	10	8	6	4	2
	E1	0.009	0.036	0.036	0.048	0.054
		0.015	0.039	0.048	0.054	0.063
		0.012	0.036	0.042	0.051	0.069
	Average	**0.012**	**0.037**	**0.042**	**0.051**	**0.062**
	Standard deviation	0.002	0.001	0.005	0.002	0.006
	D1	0.054	0.066	0.072	0.084	0.960
		0.063	0.069	0.075	0.093	0.102
		0.060	0.066	0.072	0.087	0.096
	Average	**0.059**	**0.067**	**0.073**	**0.088**	**0.098**
	Standard deviation	0.004	0.001	0.001	0.004	0.406
	R 2	0.018	0.024	0.024	0.051	0.057
		0.012	0.018	0.027	0.045	0.048
		0.021	0.021	0.03	0.051	0.051
	Average	**0.017**	**0.021**	**0.027**	**0.049**	**0.052**
	Standard deviation	0.004	0.002	0.002	0.003	0.004
	A22	0.027	0.057	0.066	0.063	0.075
		0.036	0.045	0.057	0.066	0.078
		0.03	0.051	0.063	0.063	0.075
	Average	**0.031**	**0.051**	**0.062**	**0.064**	**0.076**
	Standard deviation	0.004	0.005	0.004	0.001	0.001
	A17	0.012	0.018	0.024	0.042	0.048
		0.018	0.024	0.027	0.045	0.048
		0.015	0.021	0.03	0.042	0.051
	Average	**0.015**	**0.021**	**0.027**	**0.043**	**0.049**
	Standard deviation	0.002	0.0024	0.002	0.001	0.001
	A14	0.024	0.027	0.024	0.042	0.039
		0.015	0.021	0.03	0.036	0.051
		0.018	0.027	0.033	0.039	0.045
	Average	**0.015**	**0.021**	**0.027**	**0.043**	**0.049**
	Standard deviation	0.004	0.003	0.004	0.002	0.005
	Control	0.255				
		0.249				
		0.243				
	Average	**0.249**				
	Standard deviation	0.005				

28°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	95.181	76.305	95.283	87.550	93.976	92.369
	8	85.141	73.092	88.208	79.518	91.566	89.960
	6	83.133	70.683	63.208	75.100	89.157	88.353
	4	79.518	64.659	57.075	74.297	82.731	84.337
	2	75.100	60.643	55.189	69.478	80.321	81.928

35°C	Isolates	10	8	6	4	2
	E1	0.003	0.039	0.045	0.051	0.066
		0.003	0.048	0.048	0.063	0.075
		0.003	0.042	0.048	0.060	0.072
	Average	**0.003**	**0.043**	**0.047**	**0.058**	**0.071**
	Standard deviation	0	0.004	0.001	0.005	0.004
	D1	0	0.033	0.036	0.039	0.066
		0.003	0.036	0.039	0.051	0.057
		0.006	0.033	0.045	0.045	0.06
	Average	**0.003**	**0.034**	**0.040**	**0.045**	**0.061**
		0.002	0.001	0.004	0.005	0.004
	R 2	0.009	0.024	0.075	0.096	0.090
		0.012	0.027	0.081	0.087	0.099
		0.009	0.024	0.078	0.090	0.096
	Average	**0.010**	**0.025**	**0.078**	**0.091**	**0.095**
		0.001	0.001	0.002	0.004	0.004
	A22	0.021	0.051	0.054	0.078	0.087
		0.030	0.060	0.066	0.075	0.075
		0.027	0.057	0.060	0.072	0.081
	Average	**0.026**	**0.056**	**0.06**	**0.075**	**0.081**
		0.004	0.004	0.005	0.002	0.005
	A17	0.009	0.006	0.021	0.015	0.018
		0.006	0.009	0.009	0.021	0.024
		0.009	0.015	0.015	0.018	0.027
	Average	**0.008**	**0.01**	**0.015**	**0.018**	**0.023**
	Standard deviation	0.001	0.004	0.005	0.002	0.004
	A14	0.003	0.009	0.006	0.021	0.030
		0.003	0.012	0.021	0.015	0.045
		0.003	0.015	0.015	0.006	0.039
	Average	**0.003**	**0.012**	**0.014**	**0.017**	**0.038**
	Standard deviation	0	0.002	0.006	0.006	0.006
	Control	0.195				
		0.207				
		0.201				
	Average	**0.201**				
	Standard deviation	0.005				

35°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	98.507	98.507	84.080	87.736	96.020	98.507
	8	78.607	83.085	80.597	73.585	95.025	94.030
	6	76.617	80.100	74.627	71.698	92.537	93.035
	4	71.144	77.612	69.652	64.623	91.045	91.542
	2	64.677	69.652	63.184	61.792	88.557	81.095

40°C	Isolates	10	8	6	4	2
	E1	0.003	0.012	0.015	0.018	0.036
		0.006	0.006	0.027	0.027	0.048
		0.003	0.009	0.018	0.024	0.042
	Average	**0.004**	**0.009**	**0.02**	**0.023**	**0.041**
	Standard deviation	0.001	0.002	0.005	0.004	0.005
	D1	0.006	0.009	0.009	0.018	0.030
		0.009	0.015	0.021	0.036	0.033
		0.006	0.012	0.015	0.027	0.030
	Average	**0.007**	**0.012**	**0.015**	**0.027**	**0.031**
	Standard deviation	0.001	0.002	0.005	0.007	0.001
	R 2	0	0.003	0.006	0.027	0.030
		0	0.012	0.006	0.018	0.045
		0.003	0.006	0.015	0.024	0.036
	Average	**0.001**	**0.007**	**0.009**	**0.023**	**0.037**
	Standard deviation	0.001	0.004	0.004	0.004	0.006
	A22	0.015	0.027	0.03	0.024	0.036
		0.024	0.018	0.021	0.039	0.039
		0.018	0.021	0.024	0.033	0.042
	Average	**0.019**	**0.022**	**0.025**	**0.032**	**0.039**
	Standard deviation	0.004	0.004	0.004	0.006	0.002
	A17	0	0.006	0.006	0.006	0.015
		0	0.003	0.009	0.012	0.015
		0.003	0.006	0.006	0.009	0.021
	Average	**0.001**	**0.005**	**0.007**	**0.009**	**0.017**
	Standard deviation	0.001	0.001	0.001	0.002	0.003
	A14	0.003	0.003	0.006	0.009	0.009
		0.003	0.006	0.009	0.012	0.015
		0.006	0.009	0.009	0.009	0.021
	Average	**0.004**	**0.006**	**0.008**	**0.01**	**0.015**
	Standard deviation	0.001	0.002	0.001	0.001	0.005
	Control	0.204				
		0.207				
		0.204				
	Average	**0.205**				
	Standard deviation	0.001				

40°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	98.049	96.585	99.512	90.732	99.512	98.049
	8	93.780	94.146	96.585	89.268	97.561	97.073
	6	90.244	92.683	95.610	87.805	96.585	96.098
	4	88.780	86.829	88.780	84.390	95.610	95.123
	2	80.000	84.878	81.951	80.976	91.707	92.683

45°C	Isolates	10	8	6	4	2
	E1	0.006	0.018	0.009	0.030	0.033
		0.012	0.009	0.018	0.021	0.024
		0.009	0.012	0.027	0.024	0.027
	Average	**0.090**	**0.013**	**0.018**	**0.025**	**0.028**
	Standard deviation	0.002	0.004	0.007	0.004	0.004
	D1	0.012	0.009	0.018	0.045	0.048
		0.009	0.015	0.024	0.033	0.063
		0.015	0.021	0.027	0.039	0.057
	Average	**0.012**	**0.015**	**0.023**	**0.039**	**0.056**
	Standard deviation	0.002	0.005	0.004	0.005	0.006
	R 2	0.027	0.033	0.045	0.057	0.066
		0.036	0.045	0.057	0.066	0.081
		0.033	0.039	0.051	0.06	0.075
	Average	**0.032**	**0.039**	**0.051**	**0.061**	**0.074**
	Standard deviation	0.004	0.005	0.005	0.004	0.006
	A22	0.051	0.048	0.051	0.054	0.075
		0.054	0.054	0.063	0.069	0.078
		0.051	0.060	0.057	0.063	0.075
	Average	**0.052**	**0.054**	**0.056**	**0.062**	**0.076**
	Standard deviation	0.001	0.005	0.005	0.006	0.001
	A17	0.048	0.072	0.078	0.081	0.084
		0.072	0.087	0.090	0.096	0.099
		0.066	0.081	0.084	0.090	0.093
	Average	**0.062**	**0.080**	**0.084**	**0.089**	**0.092**
	Standard deviation	0.010	0.006	0.005	0.006	0.006
	A14	0.018	0.033	0.042	0.042	0.039
		0.021	0.045	0.051	0.054	0.057
		0.018	0.039	0.045	0.048	0.054
	Average	**0.019**	**0.039**	**0.046**	**0.048**	**0.05**
	Standard deviation	0.001	0.005	0.004	0.005	0.008
	Control	0.201				
		0.219				
		0.216				
	Average	**0.212**				
	Standard deviation	0.008				

45°C	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	95.755	94.340	93.173	74.129	70.755	91.038
	8	93.868	92.925	91.566	73.134	62.264	81.604
	6	91.509	89.151	89.157	72.139	60.377	78.302
	4	88.208	81.604	80.321	69.154	58.019	77.358
	2	86.792	73.585	79.116	62.189	56.604	76.415

Table 7.54: OD_{550nm} values for the determination of effect of cations on the removal of mixed dyes and the calculations for the percentage removal (C_0-C/C_0 x 100) (Fig. 4.20)

CTAB	Isolates	10	8	6	4	2
	E1	0.156	0.210	0.213	0.219	0.258
		0.165	0.216	0.225	0.228	0.273
		0.150	0.219	0.219	0.222	0.267
	Average	**0.157**	**0.213**	**0.219**	**0.223**	**0.266**
	Standard deviation	0.006	0.004	0.005	0.004	0.006
	D1	0.228	0.231	0.249	0.273	0.246
		0.234	0.237	0.228	0.279	0.255
		0.231	0.234	0.240	0.276	0.252
	Average	**0.231**	**0.234**	**0.239**	**0.276**	**0.251**
	Standard deviation	0.002	0.002	0.009	0.002	0.004
	R 2	0.237	0.237	0.285	0.309	0.354
		0.228	0.246	0.285	0.315	0.351
		0.234	0.240	0.291	0.312	0.354
	Average	**0.233**	**0.241**	**0.287**	**0.312**	**0.353**
	Standard deviation	0.004	0.004	0.003	0.002	0.001
	A22	0.213	0.24	0.267	0.228	0.285
		0.228	0.231	0.276	0.282	0.297
		0.222	0.234	0.270	0.276	0.291
	Average	**0.221**	**0.235**	**0.271**	**0.275**	**0.291**
	Standard deviation	0.006	0.004	0.004	0.024	0.005
	A17	0.306	0.303	0.321	0.333	0.372
		0.312	0.318	0.327	0.342	0.387
		0.309	0.312	0.324	0.336	0.381
	Average	**0.309**	**0.311**	**0.324**	**0.337**	**0.38**
	Standard deviation	0.002	0.006	0.002	0.004	0.006
	A14	0.246	0.324	0.336	0.369	0.366
		0.258	0.333	0.345	0.375	0.384
		0.252	0.327	0.339	0.372	0.381
	Average	**0.252**	**0.328**	**0.34**	**0.372**	**0.377**
	Standard deviation	0.005	0.004	0.004	0.002	0.008
	Control	0.393				
		0.390				
		0.390				
	Average	**0.391**				
	Standard deviation	0.001				

CTAB	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	59.847	40.921	40.409	43.478	20.972	35.294
	8	45.424	40.153	38.636	39.898	20.460	16.113
	6	43.990	38.875	26.598	30.691	17.136	13.043
	4	42.967	29.412	20.205	29.668	13.811	4.859
	2	31.969	10.230	9.719	25.575	2.831	3.581

$CaCl_2$	Isolates	10	8	6	4	2
	E1	0.039	0.042	0.045	0.051	0.063
		0.051	0.057	0.06	0.069	0.066
		0.045	0.048	0.054	0.060	0.063
	Average	**0.045**	**0.490**	**0.053**	**0.060**	**0.064**
	Standard deviation	0.005	0.006	0.006	0.007	0.001
	D1	0.072	0.078	0.087	0.090	0.093
		0.075	0.078	0.093	0.096	0.105
		0.072	0.084	0.090	0.093	0.099
	Average	**0.073**	**0.08**	**0.09**	**0.093**	**0.099**
	Standard deviation	0.001	0.003	0.002	0.002	0.005
	R 2	0.048	0.051	0.045	0.069	0.060
		0.054	0.060	0.075	0.06	0.075
		0.051	0.057	0.066	0.063	0.066
	Average	**0.051**	**0.056**	**0.062**	**0.064**	**0.067**
	Standard deviation	0.002	0.004	0.013	0.004	0.006
	A22	0.075	0.075	0.075	0.081	0.102
		0.078	0.069	0.087	0.087	0.099
		0.075	0.078	0.081	0.084	0.096
	Average	**0.075**	**0.079**	**0.081**	**0.084**	**0.097**
	Standard deviation	0.001	0.004	0.005	0.002	0.002
	A17	0.042	0.054	0.072	0.069	0.075
		0.048	0.066	0.06	0.075	0.081
		0.045	0.060	0.066	0.072	0.078
	Average	**0.045**	**0.06**	**0.066**	**0.072**	**0.078**
	Standard deviation	0.002	0.005	0.006	0.002	0.002
	A14	0.027	0.039	0.042	0.054	0.066
		0.039	0.045	0.051	0.057	0.075
		0.033	0.042	0.045	0.054	0.072
	Average	**0.033**	**0.042**	**0.046**	**0.055**	**0.071**
	Standard deviation	0.005	0.002	0.004	0.001	0.004
	Control	0.198				
		0.219				
		0.210				
	Average	**0.209**				
	Standard deviation	0.009				

$CaCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	78.469	65.072	75.598	64.115	78.469	84.211
	8	76.555	61.722	73.206	62.201	71.292	79.904
	6	74.641	56.938	70.335	61.244	68.421	77.990
	4	71.292	55.938	69.378	59.809	65.550	73.684
	2	69.378	52.632	67.943	53.589	62.679	66.029

$MnCl_2$	Isolates	10	8	6	4	2
	E1	0.027	0.045	0.063	0.069	0.084
		0.039	0.051	0.051	0.075	0.093
		0.033	0.054	0.057	0.072	0.084
	Average	**0.033**	**0.050**	**0.057**	**0.072**	**0.087**
	Standard deviation	0.005	0.004	0.005	0.002	0.004
	D1	0.039	0.06	0.048	0.045	0.069
		0.051	0.039	0.063	0.075	0.078
		0.045	0.048	0.057	0.066	0.075
	Average	**0.045**	**0.049**	**0.056**	**0.062**	**0.074**
	Standard deviation	0.005	0.009	0.006	0.013	0.004
	R 2	0.003	0.012	0.024	0.048	0.072
		0.003	0.024	0.039	0.051	0.087
		0.003	0.018	0.033	0.048	0.081
	Average	**0.003**	**0.019**	**0.032**	**0.049**	**0.080**
	Standard deviation	0	0.005	0.006	0.001	0.006
	A22	0.015	0.024	0.021	0.042	0.036
		0.006	0.027	0.039	0.033	0.051
		0.009	0.024	0.036	0.036	0.045
	Average	**0.010**	**0.025**	**0.032**	**0.037**	**0.044**
	Standard deviation	0.004	0.001	0.008	0.004	0.00616
	A17	0.003	0.006	0.006	0.018	0.018
		0	0.006	0.015	0.018	0.027
		0.003	0.012	0.012	0.018	0.021
	Average	**0.002**	**0.008**	**0.011**	**0.017**	**0.022**
	Standard deviation	0.001	0.003	0.00374	0	0.004
	A14	0.003	0.009	0.009	0.012	0.018
		0	0.006	0.009	0.009	0.021
		0.003	0.009	0.009	0.012	0.018
	Average	**0.002**	**0.008**	**0.009**	**0.011**	**0.019**
	Standard deviation	0.001	0.001	0	0.001	0.001
	Control	0.192				
		0.210				
		0.207				
	Average	**0.203**				
	Standard deviation	0.008				

$MnCl_2$	Conc (ppm)	E1	D1	R2	A22	A17	A14	Control
	10	0.033	0.045	0.003	0.010	0.002	0.002	0.203
	8	0.050	0.049	0.019	0.025	0.008	0.008	
	6	0.057	0.056	0.032	0.032	0.011	0.009	
	4	0.072	0.062	0.049	0.037	0.017	0.011	
	2	0.087	0.074	0.080	0.044	0.022	0.019	

$MgSO_4$	Isolates	10	8	6	4	2
	E1	0.078	0.075	0.084	0.096	0.198
		0.084	0.099	0.099	0.117	0.201
		0.081	0.093	0.096	0.108	0.198
	Average	**0.081**	**0.089**	**0.095**	**0.107**	**0.199**
	Standard deviation	0.002	0.010	0.006	0.009	0.001
	D1	0.090	0.090	0.105	0.108	0.131
		0.099	0.102	0.111	0.117	0.123
		0.093	0.096	0.108	0.114	0.117
	Average	**0.094**	**0.096**	**0.018**	**0.113**	**0.117**
	Standard deviation	0.004	0.005	0.002	0.004	0.006
	R 2	0.060	0.075	0.087	0.09	0.105
		0.075	0.081	0.102	0.108	0.105
		0.069	0.078	0.096	0.099	0.102
	Average	**0.068**	**0.078**	**0.095**	**0.099**	**0.103**
	Standard deviation	0.006	0.002	0.006	0.007	0.001
	A22	0.075	0.084	0.090	0.093	0.105
		0.090	0.090	0.093	0.102	0.111
		0.084	0.087	0.090	0.099	0.108
	Average	**0.083**	**0.087**	**0.091**	**0.098**	**0.108**
	Standard deviation	0.006	0.002	0.001	0.004	0.002
	A17	0.084	0.078	0.087	0.093	0.111
		0.090	0.099	0.096	0.108	0.111
		0.087	0.090	0.093	0.102	0.108
	Average	**0.087**	**0.089**	**0.092**	**0.101**	**0.107**
	Standard deviation	0.002	0.009	0.004	0.006	0.001
	A14	0.099	0.102	0.105	0.105	0.114
		0.105	0.108	0.114	0.117	0.108
		0.102	0.111	0.108	0.111	0.117
	Average	**0.102**	**0.107**	**0.109**	**0.111**	**0.113**
	Standard deviation	0.002	0.004	0.004	0.005	0.004
	Control	0.204				
		0.225				
		0.216				
	Average	**0.215**				
	Standard deviation	0.009				

$MgSO_4$	Conc (ppm)	E1	D1	R2	A22	A17	A14
	10	62.326	56.279	68.372	61.395	59.535	52.558
	8	58.605	55.349	63.721	59.535	58.605	50.233
	6	58.537	49.767	55.814	57.674	57.209	49.302
	4	50.233	47.442	53.953	54.419	53.023	48.372
	2	44.651	45.581	52.093	49.767	50.233	47.442

Table 7.55: Turbidity (ntu) of river water with bioflocculants and *Escherichia coli* at 30 min

30 min	Isolates	10	20	30	50
		17.400	16.290	15.210	13.410
		16.800	16.290	15.180	13.410
		18	16.290	15.210	13.410
	Average	17.400	**16.300**	**15.20**	**13.40**
	Standard deviation	0.489	0	0.014	0
	E1	17.190	14.490	13.110	12.510
		17.160	14.490	13.080	12.510
		17.220	14.490	13.110	12.510
	Average	**17.20**	**14.490**	**13.100**	**12.500**
	Standard deviation	0.024	0	0.014	0
	A17	14.910	13.500	12.210	11.700
		14.910	12.900	12.210	11.400
		14.910	14.100	12.210	12
	Average	**14.900**	**13.500**	**12.200**	**11.700**
	Standard deviation	0	0.490	0	0.245
	A14	17.790	16.290	15.810	14.490
		17.790	16.290	15.780	14.490
		17.820	16.290	15.810	14.490
	Average	**17.800**	**16.300**	**15.800**	**14.500**
	Standard deviation	0.014	0	0.014	0
	R 2	15.990	15.900	14.790	14.400
		15.990	15.600	14.790	13.800
		16.020	16.200	14.790	15
	Average	**16**	**15.900**	**14.800**	**14.400**
	Standard deviation	0.014	0.245	0	0.490
	A22	17.790	16.200	14.490	10.890
		17.790	15.900	14.490	10.890
		17.820	16.500	14.490	10.890
	Average	**17.800**	**16.200**	**14.500**	**10.900**
	Standard deviation	0.014	0.245	0	0
	Alum	21	17.910	16.290	14.490
		18	17.910	15.960	14.490
		15	17.910	16.320	14.490
	Average	**18**	**17.900**	**16.300**	**14.500**
	Standard deviation	2.449	0	0.1631	0

Table 7.56: Turbidity (ntu) of river water with bioflocculants and *Escherichia coli* at 60 min

60 min	Isolates	10	20	30	50
	D1	17.100	16.200	14.910	12.210
		16.800	15.600	14.910	12.210
		17.400	16.800	14.910	12.210
	Average	**17.100**	**16.200**	**14.900**	**12.200**
	Standard deviation	0.245	0.490	0	0
	E1	17.100	14.010	12.900	12.210
		16.800	13.980	12.600	12.210
		17.400	14.010	13.200	12.210
	Average	**17.100**	**14**	**12.900**	**12.200**
	Standard deviation	0.245	0.014	0.245	0
	A17	14.490	13.110	11.700	10.200
		14.490	13.080	11.400	9.600
		14.490	13.110	12	10.800
	Average	**14.500**	**13.100**	**11.700**	**10.200**
	Standard deviation	0	0.014	0.245	0.490
	A14	17.310	15.810	12.210	13.200
		17.310	15.780	12.180	12.600
		17.310	15.810	12.210	13.800
	Average	**17.300**	**15.800**	**15.200**	**13.200**
	Standard deviation	0	0.750	0.014	0.490
	R 2	15.510	15.210	14.100	13.890
		15.510	16.800	13.500	13.890
		15.510	15.210	14.700	13.920
	Average	**15.500**	**15.200**	**14.100**	**13.900**
	Standard deviation	0	0.750	0.4899	0.014
	A22	17.490	15.990	13.890	9.510
		17.490	15.990	13.890	9.510
		17.520	15.990	13.920	9.510
	Average	**17.500**	**16**	**13.900**	**9.500**
	Standard deviation	0.014	0	0.014	0
	Alum	17.700	16.290	15.390	13.800
		17.400	16.290	15.390	13.500
		18	16.290	15.420	14.100
	Average	**17.700**	**16.300**	**15.400**	**13.800**
	Standard deviation	0.245	0	0.014	0.250

Table 7.57: Turbidity (ntu) of river water with bioflocculants and *Escherichia coli* at 120 min

120 min	Isolates	10	20	30	50
	D1	16.710	15.510	14.190	11.010
		16.710	15.510	14.190	10.980
		16.710	15.510	14.190	11.010
	Average	**16.700**	**15.500**	**14.200**	**11**
	Standard deviation	0	0	0	0.014
	E1	16.290	13.800	12.210	11.490
		16.290	13.500	12.180	11.490
		16.320	14.100	12.210	11.490
	Average	**16.300**	**13.800**	**12.200**	**11.500**
	Standard deviation	0.014	0.245	0.014	0
	A17	13.710	12.300	11.100	9.600
		13.710	11.700	10.800	8.700
		13.710	12.900	11.400	10.500
	Average	**13.700**	**12.300**	**11.100**	**9.600**
	Standard deviation	0	0.490	0.245	0.735
	A14	16.500	14.190	13.710	12.390
		15.900	14.190	13.680	12.390
		17.100	14.190	13.710	12.390
	Average	**16.500**	**14.200**	**13.700**	**12.400**
	Standard deviation	0.490	0	0.014	0
	R 2	14.700	13.200	12.510	11.190
		14.400	12.600	12.480	11.190
		15	13.800	12.510	11.190
	Average	**14.700**	**13.200**	**12.500**	**11.200**
	Standard deviation	0.245	0.490	0.014	0
	A22	16.200	13.500	11.400	8.700
		15.600	13.200	10.800	8.400
		16.800	13.800	12	9
	Average	**16.200**	**13.500**	**11.400**	**8.700**
	Standard deviation	0.490	0.245	0.490	0.245
	Alum	16.410	15.210	13.200	11.100
		16.410	15.180	12.600	10.800
		16.410	15.210	13.800	11.400
	Average	**16.400**	**15.200**	**13.200**	**11.100**
	Standard deviation	0	0.014	0.490	0.245

Table 7.58: Percentage reduction (B−A/B x 100) of river water turbidity at different time intervals compared to alum at 10 ppm as derived from Table 7.55−7.57 (Fig. 5.1)

Bacterial bioflocculants & Alum	30 min	60 min	120 min
D1	60.364	61.047	61.959
E1	60.820	61.047	62.870
A17	66.059	66.973	68.793
A14	59.453	60.592	62.415
R2	63.554	64.692	66.515
A22	59.453	60.137	63.098
Alum	58.998	59.680	62.642

Table 7.59: Percentage reduction (B−A/B x 100) of river water turbidity at different time intervals compared to alum at 20 ppm as derived from Table 7.55−7.57

Bacterial bioflocculants & Alum	30 min	60 min	120 min
D1	62.870	63.098	64.692
E1	66.970	68.109	68.565
A17	69.248	70.159	71.982
A14	62.870	64.009	67.654
R2	63.781	65.376	69.932
A22	63.979	63.554	69.248
Alum	59.226	62.870	65.376

Table 7.60: Percentage reduction (B−A/B x 100) of river water turbidity at different time intervals compared to alum at 30 ppm as derived from Table 7.55−7.57

Bacterial bioflocculants & Alum	30 min	60 min	120 min
D1	65.376	66.059	67.654
E1	70.159	70.615	72.209
A17	72.209	73.349	74.715
A14	64.009	65.376	68.793
R2	66.287	67.882	71.526
A22	66.970	68.337	74.032
Alum	62.870	64.920	69.932

Table 7.61: Percentage reduction (B−A/B x 100) of river water turbidity at different time intervals compared to alum at 50 ppm as derived from Table 7.55−7.57 (Fig. 5.2)

Bacterial bioflocculants & Alum	30 min	60 min	120 min
D1	69.476	72.209	74.943
E1	71.526	72.209	73.804
A17	73.349	76.765	78.132
A14	66.970	69.205	71.754
R2	67.198	68.337	74.487
A22	75.171	78.359	80.182
Alum	66.970	68.565	74.715

Table 7.62: OD_{550nm} values for the determination of flocculating activity of both alum and bioflocculants in 30 min

Isolates	10	20	30	50
D1	0.099	0.081	0.051	0.036
	0.093	0.078	0.051	0.027
	0.105	0.870	0.054	0.042
Average	**0.099**	**0.082**	**0.052**	**0.035**
Standard deviation	0.005	0.373	0.001	0.006
E1	0.090	0.087	0.081	0.081
	0.087	0.084	0.078	0.075
	0.093	0.093	0.084	0.084
Average	**0.09**	**0.088**	**0.081**	**0.080**
Standard deviation	0.002	0.004	0.002	0.004
A17	0.087	0.072	0.069	0.054
	0.090	0.072	0.069	0.054
	0.090	0.075	0.072	0.057
Average	**0.089**	**0.073**	**0.070**	**0.055**
Standard deviation	0.001	0.001	0.001	0.001
A14	0.105	0.099	0.093	0.087
	0.111	0.096	0.090	0.090
	0.318	0.105	0.099	0.090
Average	**0.035**	**0.100**	**0.094**	**0.089**
Standard deviation	0.099	0.004	0.004	0.001
R 2	0.099	0.093	0.087	0.084
	0.108	0.084	0.084	0.081
	0.312	0.099	0.093	0.087
Average	**0.032**	**0.092**	**0.088**	**0.084**
Standard deviation	0	0.004	0.006	0.005
A22	0.012	0.081	0.078	0.033
	0.012	0.078	0.069	0.027
	0.012	0.087	0.084	0.039
Average	**0.036**	**0.082**	**0.077**	**0.033**
Standard deviation	0	0.004	0.006	0.005
Alum	0.105	0.090	0.084	0.078
	0.114	0.087	0.081	0.075
	0.108	0.093	0.087	0.081
Average	**0.109**	**0.090**	**0.084**	**0.078**
	0.004	0.002	0.002	0.002

Table 7.63: OD_{550nm} values for the determination of flocculating activity of both alum and bioflocculants in 60 min

Isolates	10	20	30	50
D1	0.096	0.081	0.051	0.033
	0.090	0.075	0.045	0.027
	0.102	0.084	0.057	0.039
Average	**0.096**	**0.08**	**0.051**	**0.033**
Standard deviation	0.005	0.004	0.005	0.005
E1	0.087	0.084	0.078	0.072
	0.084	0.084	0.069	0.066
	0.093	0.087	0.084	0.078
Average	**0.088**	**0.085**	**0.077**	**0.072**
Standard deviation	0.004	0.001	0.006	0.005
A17	0.078	0.069	0.066	0.051
	0.081	0.069	0.063	0.054
	0.087	0.072	0.072	0.051
Average	**0.082**	**0.070**	**0.067**	**0.052**
Standard deviation	0.004	0.001	0.004	0.001
A14	0.102	0.099	0.090	0.084
	0.099	0.093	0.090	0.084
	0.108	0.102	0.093	0.087
Average	**0.103**	**0.098**	**0.091**	**0.085**
Standard deviation	0.004	0.004	0.001	0.001
R 2	0.102	0.090	0.084	0.078
	0.096	0.060	0.078	0.075
	0.105	0.120	0.087	0.084
Average	**0.101**	**0.09**	**0.083**	**0.079**
Standard deviation	0.004	0.024	0.004	0.004
A22	0.093	0.081	0.072	0.030
	0.084	0.075	0.072	0.027
	0.099	0.084	0.075	0.036
Average	**0.092**	**0.08**	**0.073**	**0.031**
Standard deviation	0.006	0.004	0.001	0.004
Alum	0.105	0.087	0.081	0.075
	0.099	0.084	0.078	0.069
	0.108	0.090	0.084	0.081
Average	**0.104**	**0.087**	**0.081**	**0.075**
Standard deviation	0.004	0.002	0.002	0.005

Table 7.64: OD_{550nm} values for the determination of flocculating activity of both alum and bioflocculants in 120 min

Isolates	10	20	30	50
D1	0.093	0.078	0.048	0.027
	0.084	0.069	0.048	0.021
	0.099	0.084	0.051	0.033
Average	**0.092**	**0.077**	**0.049**	**0.027**
Standard deviation	0.006	0.006	0.001	0.005
E1	0.084	0.081	0.072	0.069
	0.078	0.078	0.069	0.063
	0.087	0.084	0.078	0.075
Average	**0.083**	**0.081**	**0.073**	**0.069**
Standard deviation	0.004	0.002	0.004	0.005
A17	0.081	0.069	0.063	0.048
	0.075	0.063	0.057	0.048
	0.084	0.072	0.069	0.051
Average	**0.08**	**0.068**	**0.063**	**0.049**
Standard deviation	0.004	0.004	0.005	0.001
A14	0.099	0.084	0.081	0.078
	0.093	0.084	0.078	0.072
	0.105	0.087	0.084	0.084
Average	**0.099**	**0.085**	**0.081**	**0.078**
Standard deviation	0.005	0.001	0.002	0.005
R 2	0.096	0.087	0.081	0.066
	0.093	0.078	0.084	0.063
	0.102	0.093	0.078	0.072
Average	**0.097**	**0.086**	**0.081**	**0.067**
Standard deviation	0.004	0.006	0.002	0.004
A22	0.087	0.078	0.072	0.024
	0.084	0.084	0.066	0.024
	0.09	0.069	0.078	0.027
Average	**0.087**	**0.077**	**0.072**	**0.025**
Standard deviation	0.002	0.006	0.005	0.001
Alum	0.099	0.084	0.075	0.069
	0.093	0.084	0.069	0.069
	0.105	0.087	0.081	0.072
Average	**0.099**	**0.085**	**0.075**	**0.070**
Standard deviation	0.005	0.001	0.005	0.001

Table 7.65: Turbidity (ntu) and the OD_{550nm} for the determination of flocculating activity of the controls

	Turbidity	OD_{550nm}
River water + *Escherichia coli*	43.890	0.150
	43.890	0.180
	43.920	0.120
Average	**43.9**	**0.150**
River water + distilled water	0.014	0.024
	11.700	0.102
	11.700	0.108
	11.730	0.096
	11.710	**0.102**
Average	0.014	0.005

Table 7.66: Flocculating activity of the bacterial bioflocculants at different time intervals compared to alum as derived from Table 7.62–7.65 using an equation for flocculating activity in Section 5.2.2 (Fig. 5.3)

Bacterial bioflocculants & Alum	30 min	Standard deviation	60 min	Standard deviation	120 min	Standard deviation
D1	57.268	0.154	56.802	0.163	54.284	0.185
E1	244.444	0.095	180.077	0.090	175.994	0.100
A17	99.397	0.351	94.936	0.327	93.293	0.300
A14	165.005	0.214	147.721	0.239	119.707	0.137
R2	213.229	0.416	196.183	0.216	140.287	0.057
A22	62.532	0.218	61.538	0.233	57.461	0.019
Alum	116.659	0.300	110.752	0.276	113.432	0.277

Table 7.67: The pH of river water before and after the addition of bioflocculants and alum (Fig. 5.4)

Bioflocculants/alum	Before	After
A22	7.380	6.800
	7.380	6.800
	7.380	6.800
Average	**7.380**	**6.800**
	0	0
A17	7.380	6.600
	7.380	6.60
	7.380	6.600
Average	**7.380**	**6.600**
	0	0
A14	7.380	6.830
	7.380	6.830
	7.380	6.830
Average	**7.38**	**6.830**
	0	0
R 2	7.380	6.550
	7.380	6.550
	7.380	6.550
Average	**7.38**	**6.550**
	0	0
E1	7.380	6.650
	7.380	6.650
	7.380	6.650
Average	**7.380**	**6.650**
	0	0
D1	7.380	6.920
	7.380	6.920
	7.380	6.920
Average	**7.380**	**6.920**
	0	0
Alum	7.380	4.140
	7.380	4.140
	7.380	4.140
Average	**7.380**	**4.140**
	0	0

Table 7.68: The turbidity (ntu) of river water with different concentrations of bioflocculants and alum

Isolates	10	20	30	50
D1	0.870	0.810	0.570	0.510
	0.840	0.750	0.570	0.510
	0.900	0.870	0.600	0.570
Average	**0.870**	**0.810**	**0.580**	**0.540**
Standard deviation	0.024	0.049	0.014	0.028
E1	1.290	3.210	4.500	5.250
	1.290	3.210	3.900	5.250
	1.290	3.240	5.100	5.250
Average	**1.280**	**3.220**	**4.500**	**5.260**
Standard deviation	0	0.014	0.490	0
A17	1.230	3.180	4.530	4.980
	1.230	3.180	4.530	4.950
	1.260	3.180	4.530	4.980
Average	**1.240**	**3.170**	**4.520**	**4.970**
Standard deviation	0.014	0	0	0.014
A14	2.010	2.370	3.570	4.320
	2.010	2.370	3.510	4.320
	2.010	2.370	3.600	4.320
Average	**2**	**2.380**	**3.560**	**4.320**
Standard deviation	0	0	0.037	0
A22	0.870	0.930	0.960	0.990
	0.870	0.930	0.990	1.020
	0.870	0.930	0.960	0.960
Average	**0.880**	**0.920**	**0.970**	**0.990**
Standard deviation	0	0	0.014	0.024
R 2	2.160	4.530	7.170	10.890
	2.130	4.470	7.140	10.920
	2.160	4.560	7.170	10.890
Average	**2.150**	**4.520**	**7.160**	**10.900**
Standard deviation	0.014	0.037	0.014	0.014
Alum	1.260	2.520	4.620	4.860
	1.170	2.550	4.560	4.860
	1.320	2.520	4.680	4.890
Average	**1.250**	**2.530**	**4.620**	**4.870**
Standard deviation	0.061	0.014	0.049	0.014
Control	13.500			
	13.500			
	13.500			
Average	**13.500**			
Standard deviation	0			

Table 7.69: Percentage reduction (B−A/B x 100) of river water turbidity by the bacterial bioflocculants at different concentrations compared to alum as derived from Table 7.68 (Fig. 5.5)

Bacterial bioflocculants & Alum	10 ppm	20 ppm	30 ppm	50 ppm
D1	93.556	94.000	95.704	96.000
E1	61.037	66.667	76.148	90.519
A17	63.185	66.519	76.519	90.815
A14	68.000	73.629	82.370	85.185
A22	92.667	92.815	93.185	93.481
R 2	19.259	46.963	66.519	84.074
Alum	63.926	65.778	81.259	90.741

Table 7.70: OD_{550nm} values for the determination of flocculating activity of both the bioflocculants and alum

Isolates	10	20	30	50
D1	0.072	0.051	0.027	0.015
Standard deviation	0.063	0.045	0.027	0.012
	0.078	0.054	0.030	0.018
Average	**0.071**	**0.05**	**0.028**	**0.015**
Standard deviation	0.006	0.004	0.001	0.002
E1	0.090	0.072	0.057	0.039
	0.090	0.069	0.057	0.039
	0.093	0.078	0.063	0.042
Average	**0.091**	**0.073**	**0.059**	**0.040**
Standard deviation	0.001	0.004	0.003	0.001
A17	0.078	0.051	0.045	0.039
	0.075	0.048	0.042	0.033
	0.081	0.057	0.051	0.045
Average	**0.078**	**0.052**	**0.046**	**0.039**
Standard deviation	0.002	0.004	0.004	0.005
A14	0.087	0.066	0.057	0.069
	0.090	0.057	0.054	0.066
	0.090	0.072	0.063	0.072
Average	**0.089**	**0.065**	**0.058**	**0.069**
Standard deviation	0.001	0.006	0.004	0.002
A22	0.075	0.057	0.030	0.018
	0.069	0.060	0.027	0.015
	0.081	0.060	0.036	0.021
Average	**0.075**	**0.059**	**0.031**	**0.018**
Standard deviation	0.005	0.001	0.004	0.002
R 2	0.099	0.072	0.051	0.063
	0.093	0.069	0.045	0.054
	0.105	0.078	0.054	0.069
Average	**0.099**	**0.078**	**0.005**	**0.062**
Standard deviation	0.005	0.004	0.004	0.006
Alum	0.084	0.075	0.051	0.045
	0.078	0.066	0.051	0.036
	0.087	0.081	0.054	0.051
Average	**0.083**	**0.074**	**0.052**	**0.044**
	0.004	0.006	0.001	0.006
Control	0.177			
	0.183			
	0.174			
Average	**0.178**			
	0.004			

Table 7.71: Flocculating activity of the bacterial bioflocculants compared to alum as derived from Table 7.70 using an equation for flocculating activity in Section 5.2.2 (Fig. 5.6)

Isolate	Flocculating activity	Standard deviation
D1	45.524	0.517
E1	68.076	0.273
A17	62.564	0.500
A14	64.459	0.215
A22	47.639	0.107
R2	60.685	0.418

Table 7.72: Total plate count per 0.1 ml

Isolates	10	20	30	50
D1	300	255	188	156
	303	252	188	150
	300	261	188	162
Average	**301**	**256**	**188**	**156**
Standard deviation	1.414	3.741	0	4.899
E1	393	354	237	195
	387	354	249	192
	396	357	243	198
Average	**392**	**355**	**243**	**195**
Standard deviation	3.742	1.414	4.899	2.449
A17	387	324	107	186
	384	318	107	180
	390	327	107	189
Average	**387**	**323**	**107**	**185**
Standard deviation	2.449	3.742	0	3.742
A14	390	360	321	243
	408	372	321	240
	405	372	321	249
Average	**401**	**368**	**321**	**244**
Standard deviation	7.874	5.657	0	3.741
A22	300	276	192	168
	318	267	201	168
	318	282	198	171
Average	**312**	**275**	**197**	**169**
Standard deviation	8.485	6.164	3.741	1.414
R 2	438	399	342	273
	433	393	330	264
	440	402	324	279
Average	**437**	**398**	**342**	**272**
Standard deviation	2.944	3.742	7.483	6.164
Alum	357	321	243	180
	360	315	234	177
	367	327	249	186
Average	**358**	**321**	**242**	**181**
Standard deviation	4.189	4.899	6.164	3.742
Control	1980			
	1974			
	1986			
Average	**1980**			
Standard deviation	4.898			

Table 7.73: Percentage reduction (B−A/B x 100) of bacterial load by bioflocculants at different concentrations compared to alum as derived from Table 7.72 (Fig. 5.7)

Bacterial bioflocculants & Alum	10 ppm	20 ppm	30 ppm	50 ppm
D1	84.798	87.071	90.505	92.121
E1	80.202	82.071	87.727	90.152
A17	80.455	83.687	89.343	90.657
A14	79.748	81.414	83.733	87.677
A22	84.242	86.111	90.051	91.465
R2	77.929	79.899	82.727	86.263
Alum	81.919	83.788	87.778	90.859

Table 7.74: Turbidity (ntu) of river water with bioflocculants and alum at pH 9

Isolates	10	20	30	50
D1	0.840	0.690	0.510	0.480
	0.840	0.690	0.450	0.450
	0.840	0.690	0.570	0.510
Average	**0.830**	**0.700**	**0.510**	**0.480**
Standard deviation	0	0	0.049	0.024
E1	0.720	0.690	0.690	0.660
	0.690	0.630	0.690	0.600
	0.720	0.750	0.690	0.720
Average	**0.710**	**0.690**	**0.680**	**0.660**
Standard deviation	0.014	0.049	0	0.049
A17	0.600	0.570	0.540	0.540
	0.600	0.570	0.540	0.480
	0.630	0.570	0.570	0.600
Average	**0.610**	**0.580**	**0.550**	**0.540**
Standard deviation	0.014	0	0.014	0.049
A14	0.690	0.660	0.660	0.560
	0.690	0.660	0.630	0.540
	0.690	0.690	0.660	0.570
Average	**0.700**	**0.670**	**0.650**	**0.550**
Standard deviation	0	0.014	0.014	0.012
A22	0.660	0.630	0.510	0.390
	0.630	0.570	0.510	0.390
	0.660	0.690	0.540	0.420
Average	**0.650**	**0.630**	**0.520**	**0.40**
Standard deviation	0.014	0.049	0.014	0.014
R 2	0.750	0.720	0.600	0.42
	0.750	0.690	0.570	0.42
	0.750	0.720	0.600	0.42
Average	**0.740**	**0.71**	**0.590**	**0.43**
Standard deviation	0	0.014	0.014	0
Alum	0.750	0.630	0.570	0.450
	0.750	0.570	0.510	0.450
	0.750	0.690	0.630	0.420
Average	**0.740**	**0.630**	**0.570**	**0.440**
Standard deviation	0	0.04899	0.049	0.014
Control	10.500			
	10.500			
	10.500			
Average	**10.500**			
Standard deviation	0			

Table 7.75: Percentage reduction (B−A/B x 100) of river water turbidity by the bacterial bioflocculants at different concentration compared to alum at pH 9 as derived from Table 7.74 (Fig. 5.8)

Bacterial bioflocculants & Alum	10 ppm	20 ppm	30 ppm	50 ppm
D1	92.095	93.333	95.143	95.429
E1	93.238	93.429	93.524	93.714
A17	94.190	94.476	94.762	94.857
A14	93.333	93.619	93.809	94.762
A22	93.809	94.000	95.048	96.190
R2	92.952	93.238	94.381	95.905
Alum	92.952	94.000	94.571	95.809

Table 7.76: OD_{550nm} values for the determination of flocculating activity of both alum and bioflocculants at pH 9

Isolates	10	20	30	50
D1	0.021	0.006	0.003	0.001
	0.015	0.003	0.003	0.001
	0.024	0.006	0.003	0.001
Average	**0.020**	**0.005**	**0.003**	**0.001**
Standard deviation	0.004	0.001	0	0
E1	0.012	0.009	0.006	0.003
	0.009	0.006	0.003	0.003
	0.015	0.012	0.012	0.006
Average	**0.012**	**0.009**	**0.007**	**0.004**
Standard deviation	0.002	0.002	0.004	0.001
A17	0.015	0.009	0.006	0.003
	0.012	0.003	0.006	0.003
	0.021	0.015	0.006	0
Average	**0.016**	**0.009**	**0.005**	**0.002**
Standard deviation	0.004	0.005	0	0.001
A14	0.009	0.009	0.006	0.003
	0.006	0.003	0	0.003
	0.015	0.012	0.009	0.003
Average	**0.010**	**0.008**	**0.005**	**0.003**
Standard deviation	0.004	0.004	0.004	0
A22	0.009	0.006	0.003	0
	0.003	0.006	0.003	0.003
	0.015	0.009	0.006	0
Average	**0.009**	**0.007**	**0.004**	**0.001**
Standard deviation	0.005	0.001	0.001	0.001
R 2	0.018	0.012	0.006	0.003
	0.015	0.009	0.003	0.003
	0.024	0.015	0.012	0.006
Average	**0.019**	**0.012**	**0.007**	**0.004**
Standard deviation	0.004	0.002	0.003	0.001
Alum	0.009	0.006	0.003	0.003
	0.003	0.003	0.003	0
	0.012	0.009	0.003	0
Average	**0.008**	**0.006**	**0.003**	**0.001**
Standard deviation	0.003	0.002	0	0.001
Control	0.174			
	0.174			
	0.177			
	0.175			
	0.001			

Table 7.77: Flocculating activity of both alum and bioflocculants at pH 9 as derived from Table 7.76 using an equation for flocculating activity in Section 5.2.2 (Fig. 5.9)

Isolate	Flocculating activity	Standard deviation
D1	20.739	0.111
E1	10.000	0.641
A17	18.272	0.216
A14	0.776	0.105
A22	2.687	0.129
R2	25.058	0.166
Alum	9.913	0.317

Table 7.78: Total plate count per 0.1 ml

Isolates	10	20	30	50
D1	222	171	120	99
	240	162	117	93
	237	177	126	102
Average	**233**	**170**	**121**	**98**
Standard deviation	7.874	6.164	3.742	3.742
E1	276	270	171	123
	267	261	162	120
	282	276	177	129
Average	**275**	**269**	**170**	**124**
Standard deviation	6.164	6.164	6.164	3.741
A17	201	132	123	114
	198	129	117	114
	204	138	129	117
Average	**201**	**133**	**123**	**115**
Standard deviation	2.449	3.742	4.899	1.414
A14	201	168	135	96
	198	162	132	90
	204	171	138	102
Average	**199**	**167**	**135**	**96**
Standard deviation	2.449	3.742	2.449	4.898
A22	180	177	141	117
	174	171	132	108
	192	183	147	123
Average	**182**	**177**	**140**	**116**
Standard deviation	7.483	4.898	6.164	6.164
R 2	84	153	150	120
	78	147	144	114
	90	159	153	123
Average	**84**	**153**	**149**	**119**
Standard deviation	4.899	4.898	3.742	3.742
Alum	87	72	48	36
	72	69	45	33
	105	78	51	42
Average	**88**	**73**	**48**	**37**
Standard deviation	13.491	3.742	2.450	3.742
Control	1965			
	1982			
	1972			
Average	**1978**			
Standard deviation	6.976			

Table 7.79: Percentage reduction (B−A/B x 100) of bacterial load by at different concentrations of bioflocculants compared to alum at pH 9 as derived from Table 7.78 (Fig. 5.10)

Bacterial bioflocculants & Alum	10 ppm	20 ppm	30 ppm	50 ppm
D1	88.179	91.750	93.861	95.028
E1	86.048	86.352	91.375	93.709
A17	89.802	93.252	93.759	94.165
A14	89.904	91.527	93.151	95.129
A22	90.766	91.019	92.897	94.115
R 2	92.237	92.440	93.962	95.738
Alum	95.535	96.296	97.565	98.123

Table 7.80: Data used for the calculation of flocculating activity (Section 5.2.2) (Table 7.81) percentage reduction in turbidity (Table 7.82) and total plate count (B−A/B x 100) (Table 7.83). Figure 5.11 and 5.12 show the reduction of river water turbidity spiked with two Gram-positive and Gram-negative bacteria by the bacterial bioflocculants compared to alum at 10 and 50 ppm respectively

		OD_{550nm}			
S. feacalis	Isolates	10	20	30	50
	A22	0.051	0.048	0.048	0.039
		0.060	0.066	0.054	0.042
		0.042	0.033	0.039	0.033
	Average	**0.051**	**0.049**	**0.047**	**0.038**
	Standard deviation	0.007	0.013	0.006	0.004
	A17	0.048	0.039	0.030	0.030
		0.045	0.042	0.030	0.033
		0.03	0.033	0.036	0.024
	Average	**0.047**	**0.038**	**0.031**	**0.029**
	Standard deviation	0.008	0.004	0.003	0.004
	D1	0.027	0.027	0.006	0.003
		0.027	0.03	0.006	0
		0.003	0.021	0.009	0.003
	Average	**0.028**	**0.026**	**0.007**	**0.002**
	Standard deviation	0.011	0.004	0.001	0.001
	R 2	0.033	0.027	0.012	0.006
		0.042	0.03	0.015	0.003
		0.024	0.03	0.006	0.009
	Average	**0.033**	**0.029**	**0.011**	**0.006**
	Standard deviation	0.007	0.001	0.004	0.002
	A14	0.054	0.051	0.048	0.021
		0.060	0.054	0.051	0.027
		0.051	0.045	0.042	0.018
	Average	**0.055**	**0.050**	**0.047**	**0.022**
	Standard deviation	0.004	0.004	0.004	0.004
	E1	0.033	0.018	0.069	0.051
		0.039	0.015	0.075	0.057
		0.027	0.021	0.066	0.042
	Average	**0.033**	**0.018**	**0.07**	**0.050**
	Standard deviation	0.005	0.002	0.003	0.006
	Control	1.110			
		1.230			
		0.990			
	Average	**1.110**			
	Standard deviation	0.098			

Turbidity (NTU)					
S. feacalis	Isolates	10	20	30	50
	A22	3.030	2.970	2.880	2.610
		3.090	2.970	2.910	2.640
		3	2.970	2.850	2.550
	Average	**3.040**	**2.970**	**2.880**	**2.600**
	Standard deviation	0.037	0	0.024	0.037
	A17	2.730	2.610	2.520	2.220
		2.790	2.670	2.55	2.250
		2.640	2.550	2.460	2.220
	Average	**2.720**	**2.610**	**2.510**	**2.230**
	Standard deviation	0.061	0.049	0.037	0.014
	D1	3.120	2.580	2.520	2.100
		3.150	2.640	2.490	2.100
		3.090	2.550	2.460	2.130
	Average	**3.120**	**2.590**	**2.490**	**2.110**
	Standard deviation	0.024	0.037	0.024	0.014
	R 2	2.820	2.370	2.250	2.160
		2.820	2.310	2.280	2.190
		2.760	2.250	2.190	2.160
	Average	**2.810**	**2.310**	**2.240**	**2.170**
	Standard deviation	0.028	0.048	0.037	0.014
	A14	2.940	2.940	2.610	2.220
		2.970	2.880	2.640	2.250
		2.910	2.970	2.610	2.190
	Average	**2.940**	**2.930**	**2.620**	**2.220**
	Standard deviation	0.024	0.037	0.014	0.024
	E1	2.400	2.310	2.310	2.130
		2.430	2.370	2.340	2.190
		2.370	2.280	2.250	2.100
	Average	**2.400**	**2.320**	**2.300**	**2.140**
	Standard deviation	0.024	0.037	0.037	0.037
	Control	32.490			
		32.550			
		32.460			
	Average	**32.500**			
	Standard deviation	0.037			

Plate count per 0.1 ml					
S. feacalis	Isolates	10	20	30	50
	A22	6	3	1	0
		3	2	1	0
		0	1	1	0
	Average	**3**	**2**	**1**	**0**
	Standard deviation	2.449	0.816	0	0
	A17	6	9	9	5
		9	6	3	3
		6	3	3	3
	Average	**7**	**6**	**5**	**4**
	Standard deviation	1.414	2.449	2.828	0.942
	D1	138	114	105	81
		135	117	99	87
		138	114	95	78
	Average	**137**	**115**	**98**	**82**
	Standard deviation	1.414	1.414	4.109	3.742
	R 2	69	55	9	3
		70	50	3	3
		71	51	9	0
	Average	**70**	**52**	**7**	**2**
	Standard deviation	0.816	2.160	2.828	1.414
	A14	126	1	1	0
		130	1	1	0
		122	1	1	0
	Average	**126**	**1**	**1**	**0**
	Standard deviation	3.265	0	0	0
	E1	18	3	3	1
		19	3	3	1
		20	3	0	1
	Average	**19**	**3**	**2**	**1**
	Standard deviation	0.816	0	1.414	0
	Control	180			
		183			
		183			
	Average	**182**			
	Standard deviation	1.414			

OD_{550nm}					
S. aureas	Isolates	10	20	30	50
	A22	0.045	0.042	0.039	0.027
		0.048	0.045	0.045	0.030
		0.039	0.036	0.030	0.036
	Average	**0.044**	**0.041**	**0.038**	**0.031**
	Standard deviation	0.004	0.004	0.006	0.004
	A17	0.045	0.039	0.027	0.012
		0.054	0.045	0.021	0.018
		0.039	0.033	0.015	0.021
	Average	**0.047**	**0.038**	**0.021**	**0.017**
	Standard deviation	0.006	0.005	0.005	0.004
	D1	0.060	0.024	0.009	0.003
		0.030	0.021	0.015	0.003
		0	0.021	0.006	0.006
	Average	**0.03**	**0.022**	**0.01**	**0.004**
	Standard deviation	0.024	0.001	0.004	0.001
	R 2	0.033	0.027	0.015	0.009
		0.036	0.027	0.015	0.012
		0.033	0.030	0.015	0.006
	Average	**0.034**	**0.028**	**0.015**	**0.009**
	Standard deviation	0.001	0.001	0	0.002
	A14	0.036	0.033	0.027	0.018
		0.042	0.036	0.024	0.021
		0.036	0.027	0.030	0.018
	Average	**0.041**	**0.032**	**0.027**	**0.019**
	Standard deviation	0.003	0.004	0.002	0.001
	E1	0.039	0.027	0.012	0.006
		0.042	0.021	0.015	0.003
		0.036	0.030	0.012	0.003
	Average	**0.039**	**0.026**	**0.013**	**0.004**
	Standard deviation	0.002	0.004	0.001	0.001
	Control	1.44			
		1.44			
		1.44			
	Average	**1.427**			
	Standard deviation	0			

Turbidity (NTU)					
S. aureas	Isolates	10	20	30	50
	A22	2.550	2.340	2.340	2.310
		2.520	2.370	2.370	2.340
		2.490	2.340	2.100	2.250
	Average	**2.540**	**2.350**	**2.330**	**0.300**
	Standard deviation	0.024	0.014	0.1201	0.037
	A17	2.610	2.340	2.280	2.010
		2.640	2.370	2.310	2.040
		2.610	2.310	2.250	2.010
	Average	**2.620**	**2.340**	**2.280**	**2.020**
	Standard deviation	0.014	0.024	0.024	0.014
	D1	2.640	2.460	2.400	2.310
		2.610	2.490	2.430	2.310
		2.580	2.430	2.340	2.310
	Average	**2.630**	**2.460**	**2.390**	**2.310**
	Standard deviation	0.024	0.024	0.037	0
	R 2	2.520	2.490	2.070	2.040
		2.550	2.490	2.100	2.070
		2.490	2.490	2.130	2.010
	Average	**2.520**	**2.490**	**2.080**	**2.040**
	Standard deviation	0.024	0	0.024	0.024
	A14	3.210	2.400	2.280	2.250
		3.240	2.400	2.310	2.310
		3.240	2.400	2.280	2.190
	Average	**3.230**	**2.390**	**2.290**	**2.250**
	Standard deviation	0.014	0	0.014	0.049
	E1	2.760	2.670	2.370	2.310
		2.520	2.670	2.400	2.310
		2.700	2.670	2.310	2.310
	Average	**2.760**	**2.680**	**2.360**	**2.320**
	Standard deviation	0.102	0	0.037	0
	Control	37.800			
		37.890			
		37.710			
	Average	**37.800**			
	Standard deviation	0.073			

Plate count per 0.1 ml					
S.aureas	Isolates	10	20	30	50
	A22	6	3	3	0
		3	3	3	0
		3	3	0	0
	Average	**4**	**3**	**2**	**0**
	Standard deviation	1.414	0	1.414	0
	A17	6	6	3	0
		3	3	3	0
		3	3	3	0
	Average	**4**	**4**	**3**	**0**
	Standard deviation	1.414	1.414	0	0
	D1	6	9	6	3
		9	6	3	3
		3	3	3	3
	Average	**6**	**6**	**4**	**3**
	Standard deviation	2.449	2.449	1.414	0
	R 2	0	0	0	0
		0	0	0	0
		0	0	0	0
	Average	**0**	**0**	**0**	**0**
	Standard deviation	0	0	0	0
	A14	6	3	3	0
		3	3	3	0
		6	3	3	0
	Average	**5**	**3**	**3**	**0**
	Standard deviation	1.414	0	0	0
	E1	0	0	0	0
		0	0	0	0
		0	0	0	0
	Average	**0**	**0**	**0**	**0**
	Standard deviation	0	0	0	0
	Control	258			
		260			
		253			
	Average	**257**			
	Standard deviation	2.944			

OD_{550nm}					
K. oxytoca	Isolates	10	20	30	50
	A22	0.030	0.027	0.015	0.009
		0.036	0.027	0.018	0.009
		0.027	0.027	0.012	0.006
	Average	**0.013**	**0.027**	**0.015**	**0.008**
	Standard deviation	0.004	0	0.002	0.001
	A17	0.033	0.027	0.021	0.018
		0.033	0.030	0.021	0.015
		0.036	0.030	0.021	0.018
	Average	**0.034**	**0.029**	**0.021**	**0.017**
	Standard deviation	0.001	0.001	0	0.001
	D1	0.018	0.012	0.009	0.003
		0.021	0.009	0.009	0.003
		0.018	0.018	0.009	0
	Average	**0.019**	**0.013**	**0.009**	**0.002**
	Standard deviation	0.001	0.004	0	0.001
	R 2	0.009	0.006	0.006	0.003
		0.012	0.012	0.003	0
		0.009	0.006	0.003	0
	Average	**0.010**	**0.009**	**0.004**	**0.001**
	Standard deviation	0.001	0.003	0.001	0.001
	A14	0.018	0.012	0.009	0.003
		0.018	0.009	0.003	0
		0.018	0.012	0.003	0
	Average	**0.018**	**0.011**	**0.005**	**0.001**
	Standard deviation	0	0.00141	0.00283	0.0015
	E1	0.024	0.018	0.009	0.006
		0.027	0.018	0.006	0.003
		0.018	0.018	0.006	0.003
	Average	**0.023**	**0.018**	**0.007**	**0.004**
	Standard deviation	0.003	0	0.001	0.001
	Control	1.272			
		1.272			
		1.272			
	Average	**1.271**			
	Standard deviation	0			

Turbidity (NTU)					
K. oxytoca	Isolates	10	20	30	50
	A22	0.720	0.690	0.540	0.510
		0.720	0.720	0.570	0.480
		0.720	0.630	0.510	0.570
	Average	**0.720**	**0.680**	**0.540**	**0.520**
	Standard deviation	0	0.037	0.024	0.037
	A17	1.620	1.020	0.600	0.510
		1.620	1.050	0.660	0.540
		1.620	0.990	0.510	0.600
	Average	**1.610**	**1.140**	**0.590**	**0.550**
	Standard deviation	0	0.024	0.061	0.037
	D1	2.370	1.140	0.450	0.420
		2.400	1.170	0.450	0.450
		2.370	1.110	0.450	0.360
	Average	**2.380**	**1.140**	**0.450**	**0.410**
	Standard deviation	0.014	0.025	0	0.037
	R 2	0.540	0.510	0.420	0.390
		0.570	0.510	0.450	0.390
		0.510	0.510	0.420	0.390
	Average	**0.540**	**0.510**	**0.430**	**0.380**
	Standard deviation	0.024	0	0.014	0
	A14	0.810	0.480	0.450	0.390
		0.870	0.510	0.450	0.420
		0.780	0.480	0.450	0.330
	Average	**0.820**	**0.490**	**0.450**	**0.380**
	Standard deviation	0.037	0.014	0	0.037
	E1	0.600	0.570	0.420	0.420
		0.660	0.570	0.450	0.450
		0.570	0.600	0.420	0.360
	Average	**0.61**	**0.580**	**0.430**	**0.410**
	Standard deviation	0.037	0.014	0.014	0.037
	Control	34.320			
		34.320			
		34.320			
	Average	**34.310**			
	Standard deviation	0			

Plate count per 0.1 ml					
K. oxytoca	Isolates	10	20	30	50
	A22	12	9	3	3
		6	7	2	0
		6	5	1	0
	Average	**8**	**5**	**2**	**1**
	Standard deviation	2.828	1.633	0.817	1.414
	A17	0	0	0	0
		0	0	0	0
		0	0	0	0
	Average	**0**	**0**	**0**	**0**
	Standard deviation	0	0	0	0
	D1	15	12	12	6
		20	13	15	9
		13	14	9	6
	Average	**15**	**13**	**12**	**7**
	Standard deviation	2.944	0.817	2.449	1.414
	R 2	12	9	6	3
		12	6	5	3
		14	6	4	0
	Average	**13**	**7**	**5**	**2**
	Standard deviation	0.942	1.414	0.817	1.414
	A14	6	3	1	0
		3	3	1	0
		3	3	1	0
	Average	**4**	**3**	**1**	**0**
	Standard deviation	1.414	0	0	0
	E1	0	0	0	0
		0	0	0	0
		0	0	0	0
	Average	**0**	**0**	**0**	**0**
	Standard deviation	0	0	0	0
	Control	210			
		215			
		208			
	Average	**211**			
	Standard deviation	2.944			

OD_{550nm}					
E. coli	Isolates	10	20	30	50
	A22	0.045	0.030	0.027	0.021
		0.510	0.030	0.024	0.018
		0.360	0.030	0.027	0.018
	Average	**0.044**	**0.031**	**0.026**	**0.019**
	Standard deviation	0.194	0	0.001	0.001
	A17	0.039	0.033	0.021	0.012
		0.042	0.039	0.021	0.015
		0.036	0.030	0.024	0.006
	Average	**0.039**	**0.034**	**0.022**	**0.011**
	Standard deviation	0.002	0.004	0.001	0.004
	D1	0.039	0.048	0.048	0.036
		0.039	0.051	0.048	0.039
		0.039	0.048	0.048	0.036
	Average	**0.053**	**0.049**	**0.048**	**0.037**
	Standard deviation	0	0.001	0	0.001
	R 2	0.027	0.021	0.009	0.009
		0.033	0.021	0.0165	0.012
		0.027	0.021	0.006	0.003
	Average	**0.029**	**0.021**	**0.01**	**0.008**
	Standard deviation	0.003	0	0.004	0.004
	A14	0.039	0.039	0.033	0.027
		0.045	0.042	0.036	0.027
		0.036	0.033	0.030	0.027
	Average	**0.040**	**0.038**	**0.033**	**0.027**
	Standard deviation	0.004	0.004	0.002	0
	E1	0.003	0.018	0.009	0.003
		0.003	0.021	0.009	0.003
		0	0.018	0.009	0.003
	Average	**0.002**	**0.019**	**0.009**	**0.003**
	Standard deviation	0.001	0.001	0	0
	Control	2.874			
		2.880			
		2.865			
	Average	**2.873**			
	Standard deviation	0.006			

Turbidity (NTU)					
E.coli	Isolates	10	20	30	50
	A22	3.060	2.910	2.670	2.430
		3.060	2.910	2.700	2.490
		3.060	2.940	2.670	2.370
	Average	**3.060**	**2.920**	**2.680**	**2.430**
	Standard deviation	0	0.014	0.014	0.049
	A17	0.840	2.640	2.580	2.400
		0.890	2.640	2.580	2.700
		0.780	2.640	2.580	2.100
	Average	**0.840**	**2.650**	**2.580**	**2.400**
	Standard deviation	0.045	0	0	0.245
	D1	2.880	2.760	2.730	2.460
		2.880	2.790	2.760	2.460
		2.880	2.730	2.670	2.460
	Average	**2.890**	**2.760**	**2.720**	**2.470**
	Standard deviation	0	0.0245	0.037	0
	R 2	2.520	2.370	1.020	0.480
		2.550	2.400	1.050	0.510
		2.520	2.370	1.020	0.450
	Average	**2.530**	**2.380**	**1.030**	**0.480**
	Standard deviation	0.014	0.014	0.014	0.025
	A14	2.940	2.820	2.700	2.460
		2.970	2.850	2.730	2.490
		2.910	2.790	2.700	2.400
	Average	**2.940**	**2.820**	**2.710**	**2.450**
	Standard deviation	0.024	0.025	0.014	0.037
	E1	0.840	0.720	0.540	0.450
		0.870	0.750	0.570	0.450
		0.840	0.720	0.510	0.480
	Average	**0.850**	**0.730**	**0.540**	**0.460**
	Standard deviation	0.014	0.014	0.024	0.014
	Control	41.100			
		41.400			
		40.800			
	Average	**41.100**			
	Standard deviation	0.245			

Plate count per 0.1 ml					
E. coli	Isolates	10	20	30	50
	A22	10	3	0	0
		11	3	0	0
		9	0	0	0
	Average	**10**	**2**	**0**	**0**
	Standard deviation	0.816	1.414	0	0
	A17	3	3	1	1
		3	3	1	1
		3	0	1	1
	Average	**3**	**2**	**1**	**1**
	Standard deviation	0	1.414	0	0
	D1	6	11	12	18
		9	6	16	21
		6	13	11	18
	Average	**7**	**10**	**13**	**19**
	Standard deviation	1.414	2.944	2.160	1.414
	R 2	6	12	18	24
		3	21	24	30
		3	6	15	21
	Average	**4**	**13**	**19**	**25**
	Standard deviation	0	1.414	0	1.414
	A14	1	3	3	6
		1	3	3	3
		1	0	3	3
	Average	**1**	**2**	**3**	**4**
	Standard deviation	0.024	0.024	0.014	0.037
	E1	0	0	0	0
		0	0	0	0
		0	0	0	0
	Average	**0**	**0**	**0**	**0**
	Standard deviation	0	0	0	0
	Control	1257			
		1259			
		1255			
	Average	**1257**			
	Standard deviation	0.245			

OD_{550nm}				
Alum	Isolates	10	20	30
	S. feacalis	0.255	0.210	0.147
		0.258	0.210	0.150
		0.252	0.210	0.147
	Average	**0.255**	**0.211**	**0.148**
	Standard deviation	0.002	0	0.001
	S. aureas	0.240	0.231	0.129
		0.249	0.237	0.132
		0.228	0.228	0.126
	Average	**0.239**	**0.232**	**0.129**
	Standard deviation	0.009	0.004	0.002
	K. oxytoca	0.219	0.207	0.153
		0.222	0.210	0.159
		0.216	0.207	0.147
	Average	**0.219**	**0.208**	**0.153**
	Standard deviation	0.002	0.001	0.005
	E. coli	0.333	0.222	0.213
		0.342	0.225	0.219
		0.327	0.219	2.040
	Average	**0.334**	**0.222**	**0.212**
	Standard deviation	0.006	0.002	0.860

Turbidity (NTU)				
Alum	Isolates	10	20	30
	S. feacalis	20.010	12.600	6.810
		20.040	12.900	6.900
		19.950	12.300	6.690
	Average	**20**	**12.600**	**6.800**
	Standard deviation	0.037	0.245	0.086
	S.aureas	27.690	15	10.410
		27.720	15	10.410
		27.690	15	10.410
	Average	**27.700**	**15**	**10.400**
	Standard deviation	0.014	0	0
	K.oxytoca	21.990	19.710	19.200
		22.020	19.770	19.200
		21.990	19.620	19.200
	Average	**22**	**19.700**	**19.200**
	Standard deviation	0.014	0.061	0
	E.coli	31.110	26.790	19.110
		31.110	26.850	19.050
		31.110	26.760	19.140
	Average	**31.100**	**26.800**	**19.100**
	Standard deviation	0	0.037	0.037

Plate count per 0.1 ml				
Alum	Isolates	10	20	30
	S. feacalis	21	9	4
		20	9	3
		25	6	2
	Average	**22**	**8**	**3**
	STDEVPA	2.160	1.414	0.817
	S. aureas	3	0	0
		3	0	0
		0	0	0
	Average	**2**	**0**	**0**
	STDEVPA	1.414	0	0
	K. oxytoca	9	3	0
		6	0	0
		3	0	0
	Average	**5**	**1**	**0**
	STDEVPA	2.449	1.414	0
	E. coli	0	0	0
		0	0	0
		0	0	0
	Average	**0**	**0**	**0**
	STDEVPA	0	0	0

Table 7.81: Flocculating activity of the bacterial bioflocculants compared to alum, as calculated from Table 7.80 for the removal of test organisms using an equation for flocculating activity in Section 5.2.2 (Fig. 5.13)

Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
A22	161.015	291.824	138.887	365.371
A17	114.481	97.815	190.031	322.624
D1	86.363	149.053	248.623	557.856
R2	82.074	144.990	443.446	401.249
A14	47.904	177.561	232.905	710.876
E1	56.923	104.109	175.245	106.109
Alum	34.813	36.603	30.961	16.185
Standard deviation				
A22	1.235	0.521	0.355	0.127
A17	0.188	0.374	0.222	0.174
D1	0.098	0.177	0.208	0.311
R 2	0.900	0.537	0.418	0.159
A14	0.139	0.217	0.400	0.960
E1	0.023	0.369	0.493	0.300
Alum	0.090	0	0	0.179

Table 7.82: Percentage reduction of river water turbidity (B−A/B x 100) at different concentrations of bacterial bioflocculants compared to alum at pH 9 as calculated from Table 7.80 (Fig. 5.14)

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
10 ppm	A22	90.646	93.280	97.901	92.555
	A17	91.631	93.069	95.307	97.956
	D1	90.400	93.042	93.063	92.968
	R2	91.354	93.333	98.426	93.844
	A14	90.954	91.455	97.610	92.487
	E1	92.615	92.698	98.222	97.932
	Alum	38.462	26.719	35.879	4.331

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
20 ppm	A22	90.862	93.783	98.018	92.895
	A17	91.969	93.890	97.027	93.552
	D1	92.030	93.492	96.677	93.285
	R2	92.892	93.413	98.514	94.209
	A14	90.985	93.677	98.572	93.139
	E1	92.862	92.910	98.309	98.224
	Alum	61.231	60.317	24.220	34.893

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
30 ppm	A22	91.138	93.836	93.426	93.479
	A17	92.277	93.968	98.280	93.723
	D1	92.338	93.677	98.688	93.382
	R2	93.108	94.497	98.747	97.494
	A14	91.938	93.942	98.688	93.406
	E1	92.923	93.757	98.747	98.686
	Alum	79.077	72.487	44.040	53.528

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
50 ppm	A22	92.000	93.915	98.484	94.088
	A17	93.138	94.656	98.397	94.161
	D1	93.508	93.889	98.805	93.990
	R2	93.323	94.603	98.892	98.832
	A14	93.169	94.048	98.892	94.039
	E1	93.415	93.864	98.805	98.881
	Alum	90.185	75.952	79.102	57.178

Table 7.83: Percentage reduction in total bacterial counts (B−A/B x 100) at different concentrations of bacterial bioflocculants compared to alum at pH 9 as calculated from Table 7.80. Figure 5.14 and 5.15 shows the reduction of the microbial load by the bioflocculants at different concentrations compared to alum at 10 ppm and 50 ppm respectively

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
10 ppm	A22	98.352	98.444	96.209	99.204
	A17	96.153	98.444	100.000	99.761
	D1	24.725	97.665	92.891	99.443
	R2	61.538	100.000	93.839	99.682
	A14	30.769	98.054	98.104	99.920
	E1	89.560	100.000	100.000	100.000
	Alum	87.912	99.222	97.630	100.000

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
20 ppm	A22	98.901	98.833	97.630	99.814
	A17	96.703	98.444	100.000	99.814
	D1	36.813	97.665	93.839	99.204
	R2	71.428	100.000	96.682	98.966
	A14	99.451	98.833	98.578	99.814
	E1	98.351	100.000	100.000	100.000
	Alum	95.604	100.000	99.526	100.000

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
30 ppm	A22	99.451	99.222	99.052	100.000
	A17	97.530	98.833	100.000	99.920
	D1	46.154	98.444	94.313	98.966
	R2	96.154	100.000	97.630	97.098
	A14	99.451	98.833	99.526	99.761
	E1	98.901	100.000	100.000	100.000
	Alum	98.352	100.000	100.000	100.000

Conc (ppm)	Bacterial bioflocculants & Alum	*Streptococcus faecalis*	*Staphylococcus aureus*	*Klebsiella oxytoca*	*Escherichia coli*
50 ppm	A22	100.000	100.000	99.526	100
	A17	97.802	100.000	100.000	99.920
	D1	54.945	98.833	96.682	98.488
	R2	98.901	100.000	99.052	97.098
	A14	100.000	100.000	100.000	99.682
	E1	99.451	100.000	100.000	100.000
	Alum	100.000	100.000	100.000	100.000