A STUDY OF BACTERIA ISOLATES FROM GIN TRASH FOR COTTON FIBRE WAX REMOVAL DURING BIO SCOURING

BY

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DECLARATION

STUDENT'S DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

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DEDICATION

I hereby dedicate this report to my beloved family for the moral encouragement they offered to me throughout this research work.

ABSTRACT

Alkaline pectinase scoured cotton fibres show low absorbency levels. Thisis mainly due to the inability of the enzyme to remove cotton wax concurrently with the other impurities. The enzyme requires temperature of up to 50°C for activity while cotton fibre wax requires temperatures of 75-100^{o}C to melt, at which the pectinase would be denatured. This research therefore identified the need to usecotton fibre wax degrading microbial isolates at mutually favorable temperatures, pH and incubation timeto enhance the wax removal during bio scouring. The main objective was to investigate the role of the bacteria isolates from gin trash in cotton fibre wax biodegradation during the bio scouring process. Bacteria cultures were grown and isolated from cotton gin trashes collected from Kibos and Kitui ginneries as case studies. All the three pure isolates,yellow, white and blue obtained were found to have activity on paraffin wax at 37^{0}Cto 45^{0}C.Universal Rotatable Design (URD) was used to design the optimized process parameters during cotton wax removal by the isolates. Wax removal efficiency was then assessed by the percentage weight loss after solvent extraction of the treated fibres. Maximumwax of 0.765% out of 1.2% present in the fibres was removed at 45°C, pH 8 within 1 hour. Asecond-order polynomial regression model was fittedand found adequate with a determination coefficient, R^2 ,of 0.94032 ($p < 0.005$). Minitab 15, Monte Carlosand SPSS statistical tools were used for the parametersmodeling and sensitivity analysis.Temperature was found to be the most significant variable in thebacterial cotton wax removal. At 45 °C, pH 8, maximum 0.747%wax was removed by the bacteria with pectinase treatment within 1 hour. This amount was comparable to the 0.72% and 0.844% wax removed by caustic and pectinase with surfactants scour procedures. The isolates treated cotton fibres showed a slight3% increase in strength.

Key words: Gin trash; Bacteria isolates;pectinase enzyme; cotton wax removal;Optimization; Modeling parameters sensitivity.

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LIST OF ACRONYMS

VLIR-UOS	VlaamseInteruniversitaireRaad – UniversityOntwikkelingssamenwerking
ASTM	American Society for Testing and Materials

LIST OF ABBREVIATIONS

BOD	Bio-Chemical Oxygen Demand
COD	Chemical Oxygen Demand
TDS	Total Dissolved Solid
HPLC	High Performance Liquid Chromatography
GC–MS	Gas Chromatography- Mass Spectrometer
URD	Universal Rotatable Design
rpm	Rotation per Minute
ANOVA	Analysis of Variance
HVI	High Volume Instrument

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CHAPTER 1 INTRODUCTION

This chapter outlines a brief introduction on the role played by the conventional caustic scouring process in removing the natural impurities from cotton fibres and the eventual fibre absorbency. It also elaborates the process's shortcomings in terms of deteriorating some of the fibre properties, operational economies as well as the environmental repercussion resulting from the process's effluent discharge. The chapter further outlines the prospects and shortfalls of bio scouring as a viable alternative to the conventional scouring. The problem that hinders the commercial implementation of the bio scouring process is outlined. Objectives are also stated, projecting the plan on how the bio scouring gap can be filled. Scope of the research that demonstrates how the objectives are realised is hence given.

1.0COTTON FIBRE

Cotton is the most important of the raw materials for the textile industry. Cotton has a distinction among fibres due to numerous reasons. Particularly, its softness, absorbency, luster, strength and wearing comfort have contributed a lot to make it most liked and used fibres since ancient times. Even, today cotton has 50% apportion in total fibre consumption in the world (Anita, 2004). From the previous studiesdone byAgrawal (2005),Qiang*et al.* (2006) andBouwhuis(2011), cotton fibre is a single biological cell which is built up of four parts – lumen, secondary wall, primary wall and cuticle. Lumen is thenutrient transportation tube while the secondary wall is built up ofcellulose layers which contribute about 91.5% of fibre. The primary cell wallmainly consists of protein, pectic substances, cellulose, hemicelluloses, colouring matter and traces of metal. The cellulose in the primary wall has a lower degree of polymerization than the cellulose in thesecondary wall.The protective

cuticle is mainly made of wax, unsaponifiable high molecular alcohols and their esters, higher fatty acids of high molecular weight, which give the cuticle a strong hydrophobic character.However, majority of these non-cellulosic impurities are taken away during the scouring process, which makes the fibre absorbent.

1.1COTTON SCOURING

In the conventional textile wet processing, grey cotton fibres have to undergo a series of chemical treatments, including scouring which is done in order to remove non-cellulosic and foreign constituents either partially or completely. Mohamed (2007) and Bahrum(2012) emphasized that, the main target of scouring is to remove waxes, pectins, hemi-celluloses and minerals from the raw cotton fibres during the early stage of textile wet processing to make the fibres highly absorbent, which is necessary for the subsequent processes such as mercerizing, bleaching, dyeing, printing and finishing. For this purpose, Caustic soda (Sodium Hydroxide) treatment is used in conventional scouring, whereas, Enzymes (Cutinases, Pectinases, protease, cellulase.) treatment is applied in bio-scouring process (Petra,2011).

The scouring process is ultimately aimed at increasing the water adsorption ability as well as the whiteness of the fibres. The cotton fibres wet-ability can only be achieved by breaking the outermost cuticle layer which is a thin film of mostly fats and waxes. Conventional Caustic soda scouring process involves treatment of the cotton substrate with hot solutions of sodium hydroxide, chelating agents, and surface active agents. Such solutions attack the primary wallmatrix and result in a very high removal of thenon cellulosic components. However, unless the process is carefully controlled, the cotton fibremay be damaged during the scouring process by the formation ofoxycellulose (Emre

and Merih, 2004;Bouwhuis, 2011;Kiromojsov, 2012a). The usual practice is to neutralize the alkali in the substrate by adding acetic acid to the rinse water. Sodium acetate is formed by the neutralization reaction, and if this compound is not completely rinsed out, fabric yellowing can result during high-temperature drying or curing (Etters, 1999).

The scouring process is designed to break down or release natural waxes, oils, and other impurities and emulsify or suspend them in the scouring bath, as reported by Petra (2011). Typically, conventional scouring process is water and energy intensive and the wastes generated contribute high Bio-Chemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) loads to the effluent. This effluent once discharged compromises on the environmental integrity. The process also subjects the fibre to chemical damage as well as the resultant fibres exhibiting harsh hand due to removal of most of the lubricating wax from the fibre (Kiromojsov2012b).Qiang*et al*. (2006) and Sonia and Koushic (2011) reported that caustic scouring result into more fibre weight and strength loss owing to the chemical damage to the fibres. Due to these challenges, enzymatic scouring has been evaluated as an alternative to the caustic scouring method.Enzymes are high molecular weight proteins that are produced by living organisms. They are catalysts that accelerate the rate of biological processes and hence are referred to as biocatalysts. Generally, enzymes contain an “active site”, where the reaction is carried out and a “binding site”, which links to the substrate. When in the active site, the enzyme can catalyse the reaction (Petra, 2011;Vigneswaran*et al*., 2011; Sonia and Kouchic, 2011). Generally, enzymes work in mild conditions: atmospheric pressure, low temperatures and slight acidic, neutral or alkaline pH and therefore the assurance of optimal reaction conditions is needed throughout the whole process (Anita, 2004).

Use of enzymes during scouring allows the development of environmentally friendly technologies in fibre processing and strategies to improve final product quality. The consumption of energy and raw materials as well as increased awareness of environmental concerns related to the use and disposal of chemicals into landfills, water or release into the air during chemical processing of textiles are the principal reasons for the application of enzymes in processing of textile materials.

1.2ENZYMATIC SCOURING (BIO-SCOURING)

The enzymes used for bio-scouring do not have any effect on the cellulose backbone and consequently, fibre damage is very limited. Different enzymes like pectinases, proteases, cellulases and recently cutinaseshave been examined to degrade and subsequently remove the natural component present in the outer layer of cottonfibresas indicated byBuchert and Pere (2000).According to Kiromojsov (2012b), the cotton fabrics bio-scoured with pectinase show improved softness and wet ability but much less wax is removed than by the conventional alkaline process. The improved wetness of the bio-scoured cotton is however reverted to that of the original following a thermal process, indicating that the thermal process cause the wax that has remained inside the bio-scoured cotton to migrate to the surface (Sawada *et al*.,1998). Agrawal (2008a) reported that pre-rinsing in hot water ($\geq 90^0$C) with a surfactant or extraction with boiled n-hexane helps to reduce wax impurities and, subsequently results in better pectinase performance in destabilizing the primary cell wall. Introduction of such a high temperature treatments however, hinders the development of a low temperature process.Hartzell and Durrant(2000),Agrawal (2008a) andVigneswaran (2013) studied the effect of agitation in pectinase scouring, and concluded that agitation during scouring improves the fabric absorbency. Enzymatic scouring has, however, not yet

been implemented by textile industries, due to several problems such as longer incubation time, high enzyme doses, uneven dyeing, non-uniformity of enzyme treatment, overall slow process speed andthe most important identified aspect being the inability of wax removal during enzymatic cotton scouring (Agrawal, 2005;Vigneswaran, 2013;Vigneswaran, 2012b).A rational approach is therefore necessary to design a more efficient enzymatic scouring process. Several aspects such as the specificity of enzymes, the complexity of the substrate (cotton fibre) and mass transfer, need to be considered for a successful and efficient enzymatic scouring process, the main challenge being the removal of cotton waxes in an efficient, economic and environmentally friendly way.

1.3STATEMENT OF PROBLEM

The waxy layer of the cotton fibre contributes significantly to the fibre'shydrophobicity.Due to the inability of pectinase to remove the cotton fibre wax during bioscouring,the process hasbeen conservatively implemented by textile industries. Pre-rinsing in hot water ($\geq 90^0$C) with a surfactant or extraction with boiled n-hexane helps in removal of wax impurities and, subsequently results in better pectinase performance. Introduction of such a high temperature chemical treatmentshowever, hinders the development of a low temperature and environmentally friendly process. Therefore the main challenge is to remove the waxy materials in cotton fibre effectively at low temperatures via environmentally and economically friendly means.

1.4OBJECTIVES

Main Objective

To establish the feasibility of using bacteria isolates from cotton gin trash to enhance cotton fibre wax removal during pectinase bio scouring.

Specific Objectives

i. Culture, isolate andidentifybacteria from cotton gin trash collected from Kibos and Kitui ginneries as case studies.
ii. Determinethe isolates activity on hydrocarbons by using paraffin wax.
iii. Establish cotton fibre wax removal efficiencies by the caustic and pectinase, with and without a surfactant scour procedures.
iv. Determine optimal temperature, pH and incubation time for wax removal by the isolates
v. Investigate presence of an enzyme in the bacteria isolates toascertain the source of activity on the wax.

1.5 SCOPE OF THE RESEARCH

This research was aimed at developing a mechanism that would enable efficient performance of the pectinase enzyme during the cotton fibre bio-scouring. The need for eliminating the use of surfactants and replacing them with a waxdegradingmicrobes(bio surfactant) during the pectinase scouring was identified. This beingto minimize the chemical loadsand high cost aspectsassociated with the surfactants. The target was then to enhance efficiency of pectinase in cottonfibreswax removal to ensure proper fibre wet-ability for easier downstream processing of the fibres. Microbial isolates were cultured and isolated from cotton gin trashes, tested for activity on paraffin wax and subsequentlytested for action on the cotton fibre wax. The isolates were then incorporated into the pectinase scour bath to determine whether the enzyme’s efficiency on the cotton fibre wax removal

would be improved. The results were compared with those obtained from conventional caustic and pectinase, with surfactantsscour procedures.

CHAPTER 2 LITERATURE REVIEW

This chapter gives a preliminary study of the cotton fibre structure in order to reveal significance of removing cotton fibre wax in enhancing the fibre's absorbency. A detailed review on the previous studies done on the cotton scouring methods has been presented. The chapter has also given an account of the alternative solvent and bio scouring methods that have been evaluated for replacing the caustic scouring method in an attempt to overcome its associated drawbacks. Bio scouring as the most viable alternative to the caustic method have therefore been extensively analysed and a measure for enhancing its efficiency in cotton fibre wax removal investigated.

2.0COTTON FIBRE STRUCTURE

Cotton, the seed hair of the plants of genus *Gossypium*, is the purest form of cellulose readily available in nature. Despite the relative growth of the synthetic fibre, cottonstill has an important position due to its properties. The major comfort properties are thermalinsulation, moisture handling and next-to-skin comfort. These comfort aspects are due to relativefine and flexible fibres. Cotton can absorb water up to 20% of the fibre weight, without feeling wet. The wateruptake capacity is up to 60%of the fibre weight, without the fabric starting to drip. The next-to-skin comfort is highbecause the fibre is fine and soft. Cotton develops no electrostaticcharging under average use as it always contains water, resulting in discharging of the fabric. The combination ofthese properties and the relative low price give cotton this important position in the worldwideuse of fibres (Bouwhuis, 2011).

The fibre is composed of concentric layers. The cuticle layer on the fibre itself is separable from the fibre and consists of wax and pectin materials. The primary wall, the most

peripheral layer of the fibre, is composed of cellulosic crystalline fibrils, hemicelluloses, pectin and protein. The secondary wall of the fibre consists of three distinct layers. All three layers of the secondary wall include closely packed parallel fibrils with spiral winding of 25-35^{o} and represent the majority of cellulose within the fibre. The innermost part of cotton fibre, the lumen, is composed of the remains of the cell contents. Before boll opening, the lumen is filled with liquid containing the cell nucleus and protoplasm. The twists and convolutions of the dried fibre are due to the removal of this liquid. The cross section of the fibre is bean-shaped, swelling almost round when moisture absorption takes place(Kiromojsov, 2012a;Agrawal, 2005).Raw cotton contains impurities such as oil, waxes, pectins, proteins, organic acids, mineral matter and natural coloring-matter, in addition to cellulose.Cotton contains nearly 90% of cellulose and around 10% of the non-cellulosic substances, which are mainly located inthe cuticle and primary walls of the fibreas reported by Emre and Merih (2004) and Kiromojsov (2012a).

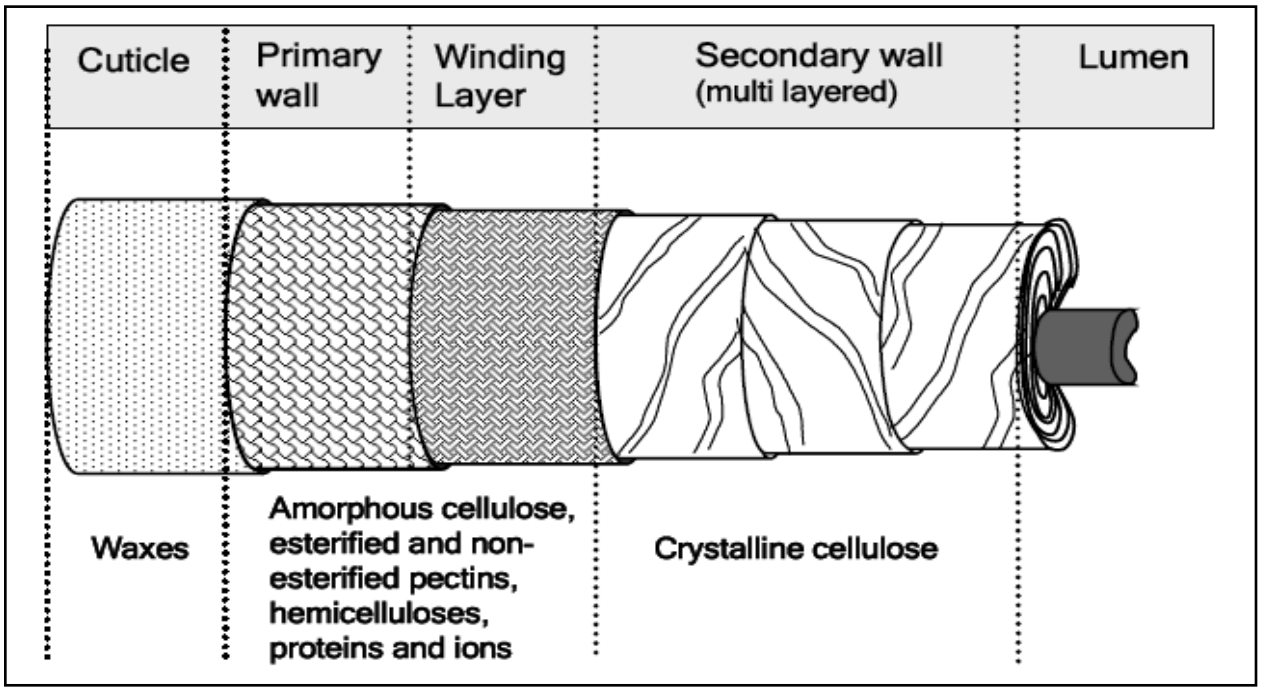

Figure2.1:Structure of a mature cotton fibre with its various layers as adapted from Agrawal (2005).

Figure 2.1 gives the schematic representation of cotton fibre structure.The outermost layer is the cuticle which is a thin film of mostly fats and waxes. The waxy layer forms a thin sheet over the primary wall that forms grooves on the cotton surface. The primary wall comprises of non-cellulosic materials and amorphous cellulose in which the fibrils are arranged in a criss-cross pattern (Agrawal,2005).Primary wall also contains insoluble calcium, magnesium, iron, and other salts of polygalacturonic acids (pectins) contribute to the hydrophobic nature of un-scoured cotton fibre. Pectin contributes to the firmness and structure of cotton fibre, both as a part of the primary wall and as a component of the winding layer (Vigneswaran*et al*., 2012a). These pectate salts also act as biological glue, binding the non-cellulosic components of the primary wall within the cellulosic matrix (Ridley and Malcom 2001).Cotton contains two types of pectin. The outer layer of the primary wallcontains esterified pectins. Esterified pectins allow the primary wall to extend

during growth, asthey are less stable to humidity and heat. Non-esterified pectin contains more charged groupsand is straighter than esterified pectin and forms a Ca^{2+} bridge. The Ca^{2+} -bridge cross-links withother non-esterified pectins.These cross-links function as anetwork to hold the cell wall components together(Bouwhuis, 2011).

According to studies from Anita (2004), Mohamed (2007) and Bahrum (2012),the percentage compositions of constituents of a mature cotton fibre are Cellulose (88-96), Protein (1.1-1.9), Wax (0.4-1.0), Ash (inorganic salts) (0.7-1.6), Pectins (0.7-1.2), Others (e.g seed coat fragments) (0.5-1.0). The exact percentage of each component of the primary wall is determined by the type of the cotton plant, its origin, the growth conditions, and the degree of maturity;there is an increase in the cellulosic percentage with the increase in immaturity level (Mohamed, 2007).

Raw cotton fibres have to go through several chemical processes to obtain properties suitable for use. With scouring, non-cellulosic substances (wax, pectin, protein, hemicelluloses) that surround the fibre cellulose core are removed, and as a result, fibres become hydrophilic and suitable for mercerizing, bleaching, dyeing and other processes as reported by Saravanan*et al*. (2010) and Mohamed (2007).The waxy layer contributes significantly to the hydrophobicity of the cotton fibre and scouring materials have to cross this first barrier in order to attack the primary wall components of the fibre(Sonia and Koushic, 2011).

2.1 COTTON FIBRE WAXES

Protective cuticle layer of cotton fibre is made of wax, mineral matters, pectins, fatty acids, highmolecular weight alcohols and their esters.Saravanan*et al*. (2008) and Perez *et al*. (2000) have reported that, structurally,Pectic substances situated on the primary layer act as

glue in binding the waxes on the cotton fibre.Vaughn and Turley (1999) had demonstrated that about 85% of carboxyl groups in the pectic substances of cotton are methylated and are in the form of insoluble calcium, magnesium and iron salts of polygalacturonic acid, which also constitutes to the non-absorbent characteristics of raw cotton.Varadarajan*et al.*(1990), Dusty (1996) and Willison and Malcolm (1977)hadalso established that the cuticle layer contains primary alcohols, higher fatty acids, hydrocarbons,aldehydes, glycerides, sterols, acyl components, resins, cutin and suberin, which arecalled waxes.It was then determined by Saravanan*et al*. (2008) that the cotton fibre waxy impurities are composed of 25% fatty acids, 52% alcohols, 10% sterols, 7% hydrocarbons, and 6% inert matter. The constituents of wax include 1-triacontanol, montanol, beta-sisterol and a mixture of high molecular weight esters.According to Agrawal (2005), the cotton waxes are solid substances with rather high and wide range ofmelting points (64^0C to 214^0C) and constitute around 0.4 to 1.3 % dry weight of rawcotton. The waxes contain Carbon, 80.38%, Hydrogen, 14.52%.The main function of wax is to protect the fibre from dehydration. Other functions areprotection against external physical and chemical attacks. In the spinning process the waxy layergives a soft hand but also improves spinning quality due to less friction.Levels of cotton fibre wax are determined by the fibre'stypeand maturity as well as environmental conditions and are usually measured by solvent extraction. Cuticular waxes are synthesized in response to environmental signals such as light intensity, photoperiod, humidity, chilling, and seasonal variation (Dusty, 1996).The amount of wax per unit surface is fairly constant, and finer cottons therefore contain more wax per unit weight than do coarser cottons, as reported by Anita (2004) and Bahrum (2012).

The Non-cellulosic cotton impurities, which are mainly located inthe cuticle and primary wall of the fibre, are removed during the scouring process. The treatment is traditionally performed at high temperature and involves the use of strong alkaline solutions, such as sodium hydroxide as reported byEmre and Merih (2004).Although alkaline scouring is effective and the cost of sodium hydroxide is low, the process is costly because it consumes large quantities of energy, water, and auxiliary agents. The potential for the environmental contamination and depletion of natural resources is also serious. The strict pH and temperature requirements for alkaline scouring are damaging tofibres (Bahrum, 2012).

2.2COTTON SCOURING METHODS AND THE FIBRE WAX REMOVAL

It is necessary to recognise the importance of the removal of the waxy layer in cotton scouring. This can be achieved by studying the effect of wax removal on hydrophilicity of the cotton fibre. It has been postulated that the removal of waxy layer will make the primary wall better accessible during scouring, resulting in an improved wet ability (Qiang*et al*., 2006). According to Nalankilli*et al*. 2008, Scouring materials such as sodium hydroxide, enzymes or solvents, have to cross this first barrier therefore in order to attack the primary wall components of the fibre. Sodium hydroxide is used in the textile industry to remove the outermost waxy layer during cotton scouring process. At high temperatures (90°C to100°C), it removes cotton waxes by saponification and emulsification. High temperature in the presence of sodium hydroxide melts some of the waxy material and converts some of it to a water-soluble form. It also convertsnon-cellulosic material (pectins, hemicelluloses and proteins) in the cuticle-primary wall to water soluble forms to effect removal. The caustic scouring process requires large quantities of chemicals, energy and water and is rather time consuming as argued by Etters (1999) and Petra (2011). Owing to

the high sodium hydroxide concentration and its corrosive nature, intensive rinsing is required that leads to high water consumption. According to Petra (2011), the use of high concentrations of sodium hydroxide also requires the neutralisation of wastewater, which requires addition of acetic acid.Kiromojsov (2012b) has indicated that Biological Oxygen Demand (BOD) andChemical Oxygen Demand (COD) of enzymatic scouring process are 20-45 % as compared toalkaline scouring (100 %). Total Dissolved Solid (TDS) of enzymatic scouring process is 20-50% ascompared to alkaline scouring (100%). Handle is very soft in enzymatic scouring compared to harshfeel in alkaline scouring process (Rita *et al*., 2008; Petra, 2011). Apart from the above wet processing problems, the biggest drawback of alkaline scouring is its non-specific degradation of cellulose that produces fibres of lower tensile strength and therefore lower quality. It is from the above discussed impediments associated with caustic scouring that the enzymatic and solvent scouring alternatives have been developed.

Solvent scouring appears to be an alternative to the aqueous caustic scouring. Solvent processing has been developed due to the reduced water pollution, reduced energy consumption and costs apart from an effective removal of the impurities. Solvent scouring gives good results in terms of uniformity, reproducibility and high absorbency. Nalankilli*et al*. (2008) established that fibres extracted using carbon tetrachloride followed by ethanol show good correlation with enzyme treated samples in the case of fineness, moisture content, strength and elongation expressed by higher correlations.Inthe case of fineness, highest correlation was observed between alkali treated samples and carbon tetrachloride-ethanol extracted samples with a correlation coefficient of 0.984. Strength was 0.932 while moisture content and elongation correlations were 0.945.

The most widely used solvents for textile processing are the chlorinatedhydrocarbons, e.g. tetrachloroethylene(perchloroethylene), trichloroethylene and1,1,1-trichloroethane.Usually stabilisers and booster solvents are added during the process to stabilise the solvent and to make the process more efficient. The use of a detergent is also reported for enhancing the detergency of the scouring process (Agrawal, 2005). The use of solvent scouring is limited because of the increasing governmental and environmental restrictions. Several drawbacks are associated with the solvent scouring. Keith and Lynn (1997) and Tzanko*et al*. (2001) have demonstrated that only waxes areremoved by the solvents and therefore some form of alkaline scouring is still required. Most of the scouring solvents are flammablein nature. Moreover, there is the need of the system to recover the solvent from the fabric after processing;the reasons why solvents have very limited applications for cotton scouring, hence need for bio-scouring alternatives evaluation as emphsized by Tzanko*et al.* (2001).

2.3 BIO-SCOURING

Bio-scouring is an environmentally benign biological process, which applies enzymes capable of specific targeting and release of non-cellulosic impurities (Rita, 2008; Petra, 2011). Generally, the enzymes used for bio-scouring do not have any effect on the cellulose backbone and consequently, fibre damage is very limited. The effectiveness of the bio-scouring is evaluated by means of wetness testing, weight-loss measurement of cotton substrate and analyses of the amounts of pectic substance and cotton wax remaining in the substrate after scouring, though the methods are laborious and time consuming (Qiang*et al*., 2006).Various enzymes have been proposed by various scholars to provide effective scouring responses. As reported by Emre and Merih (2004),Qiang*et al*. (2006)

andVigneswaran*et al*. (2012b), pectinase enzymes have been found to be the most effective and suitable for cotton bio scouring. The mechanism of pectinase scouring reportedly assumes degradation and elimination of pectins and makes the loosened waxes more easily accessible with the help of a mechanical agitation (Vigneswaran*et al*., 2013). Pectinase, as the name suggests, hydrolysis pectins present in cotton as a non-cellulosic impurity (Rajendran*et al.*, 2011). The best kinds of pectinases as determined by Etters*et al*. (1999) are those which can function under slightly alkaline conditions even in the presence of chelating agents. Such enzymes are called "alkaline pectinases". Pectinase enzyme breaks down pectin, a polysaccharide substrate that is found in the cell walls and in the middle lamella of plants, where pectic substances account for up to 4.0% of the fresh weight of plant. Pectinase, however, is not generally active at temperatures over 50°C. These properties limits its application to bio-scouring of textiles, since the textiles must be pre-boiled to attenuate the waxy cuticle overlaying the pectin layer as indicated by Agrawal *et al*. (2008a) and Petra(2011).According toLi and Hardin(1997), the cotton fabrics bio-scoured with pectinase show improved softness and wet-ability but much less wax is removed than by the conventional alkaline process.The improved wetness of the bio-scoured cotton is however reverted to that of the original following a thermal process, indicating that the thermal process cause the wax that has remained inside the bio-scoured cotton to migrate to the surface(Emre and Merih, 2004; Sawada, 1998). Addition of surfactants has a big influence on removing noncellulose impurities.However,caution is advised when adding surfactant. Anionic surfactants can form complexes withproteins and influence the structure. Cationic surfactants have a similar influence onproteins, however, with a lower affinity. Enzymes usually retain their catalytic activity in asolution with non-

ionic surfactants, unless the concentration of the surfactants in thesolution exceeds the critical micelle concentration. Non-ionic surfactantsare compatible with enzymes and do not break their three-dimensional structure(Li & Hardin, 1998;Sawada, 1998; Petra, 2011). Small amounts of non-ionic surfactants when added in the bio-scouring solution,therefore, greatly enhance the effectiveness of the removal of cotton wax without inhibiting the activity of pectinase enzyme.Tzanko*et al.*(2001) has emphasized the significant role played by the surfactant in ensuring efficient pectinase bioscouring. According the work, the surfactant reduces the surface tension of the fibres and allows easier penetration of the enzymes into fibres micro pores and cracks. The surfactant then maintains the enzyme in the liquid phase, making it available for further catalytic action. Thus, the surfactant is a necessary component in the enzymatic composition for scouring and it seems to have a high impact in the removal of waxes and fats.

The cuticle of the cotton fibre which is cross linked to the primary cell wall by esterified pectic substances hinders pectinase action on the back bone. Cutin forms three dimensional network structures in which other amorphous waxy materials are embedded (Agrawal,*et al.*, 2008a;Degani*et al.*, 2002). Pre-rinsing in hot water ($\geq 90^0$C) with a surfactant or extraction with boiled n-hexane helps to reduce wax impurities and, subsequently results in better destabilization of the primary cell wall, as reported byQiang*et al*. (2006), Agrawal *et al*. (2008a) andKiromojsov (2012b). There is a need in the art for bio-scouring method, therefore, that can be performed in a single step, at temperatures near or above the melting temperature of the waxy cuticle of cotton using enzymes that effectively remove pectin and thereby facilitating the removal of pectin and other non-cellulosic impurities. Vigneswaran*et al.*(2011) reported that factors that influence enzymatic scouring are the

nature of substrate, the kind of enzyme used, the enzyme activity, the use of surfactants, and mechanical impact. Agrawal *et al.*(2008a)also highlighted the need for process parameters optimization with an aid of a mechanical agitation during the low temperatures enzymatic scouring.Vigneswaran*et al*. (2013) indicated that a sonicator would improve the bioscouring performance by 8-12%.Tzanko(2001) and Degani*et al.*(2002) in their research works had observed that, Lipases and cutinase are all lipolytic enzymes that show a high activity towards the wax substrate in their aggregated form, hence they are suitable candidates for the cotton wax degradation.Degani*et al.* (2002) reported acutinase from a bacterial source *Pseudomonas mandocino*for wax degradation in cotton scouring. As a proof for waxdegradation, the authors confirmed the release of C16 and C18fatty acids in the bulk medium, by means of advance techniquessuch as reverse phase-HPLC and GC–MS. Besides, the same authors successfullydemonstrated that a combination of cutinase and pectinase withsurfactants gives a synergistic effect in cotton scouring. Theseresults were encouraging; although, enzyme incubation timeswere between 10 and 20 hours, which are far too long to allow industrial implementation.They also observed that,Cutinase has some advantages over lipases for cotton wax degradation in that, lipases, in general, require interfacial activation at the lipid water interface, whereas cutinase does not. The interfacial activation is an extra energy need to expose the active site of Lipase enzymes to the substrate. Another important characteristic of cutinase is that, it can hydrolyse waxes in the absence ofCa^{2+} ions. Lipases need Ca^{2+} ions for their hydrolytic action. Since it is known that the presence of Ca^{2+} ions can interfere negatively with the pectinaseperformance, it will be difficult to combine lipases with pectinases during enzymaticcotton scouring (Agrawal *et al*., 2008b).Agrawal (2005) and Agrawal *et al.* (2008a) however

demonstratedthat with fast wetting, mass transfer improvement and the influence of mechanical action, a faster and more efficient enzymatic cotton scouring process can be realised within fifteen minutes using cutinase from fungus *Fusariumsolanipisi*. Cellulases are also capable of improving absorbency, but they provoke higher losses in weight and strength (Jordanov and Mangovska, 2002). A new pectatelyase from *Bacillus pumilus*-BK2 was recently reported,with optimum activity at pH 8.5 and around70 ^{0}C, and assessed forbio-scouring of cotton fabric. Removal of up to 80%of pectin was demonstrated by ruthenium red dyeing, and the hydrophilicility of the fabric,evaluated by liquid porosimetry, was also dramatically enhanced (Rita *et al*., 2008). However such a high temperature treatment on the other hand would compromise the desirable low temperature treatments associated to enzyme scouring hence increase on the process costs. Alternatively, the possibility of integrating wax degrading microbial isolates with the pectinase scouring bath conditions can also be evaluated to alleviate high costs of the cutinase enzyme and need for mass transfer as well as the pectatelyase from *Bacillus pumilus*high temperature aspects, as highlighted by Agrawal *et al*. (2008a) and Rita *et al*. (2008).

2.4 INCOORPORATION OF WAX DEGRADING MICROBES WITH PECTINASE

Since cotton fibres bio-scoured using the pectinase enzymes yield softer, less-toxic and high wet strengthfibresaccording to Kiromojsov (2012b) and Li and Hardin(1997),research can therefore be conducted to enhance the cotton fibre wax removal during the pectinase bio scouring. This can bepursuedby incorporating wax degrading bacterial isolates from cotton gin trash, into the pectinase scouring bath. The isolates, being microbes, would counteract the enzyme's inability to attenuate the cotton fibre wax layer by degrading the

wax at temperature that would not denature the enzyme. The hydrocarbons degrading bacteria isolates can be cultured and isolated from cotton gin trash accordingZoBell (2005).He has reported that, if a small sample of the dry gin trash (12 to 15% moisture content) be placed in water and the water examined microscopically every few hours one will find active bacterial microorganisms cruising about after 16 to 18 hours even though the gin trash has been in dry storage for over three years. Within 24 to 48 hours a very large population of microorganisms will be active in the water. These bacteria come from cotton field soil and feed on the 16 percent cottonseed protein, along with the many carbohydrates present in the cotton gin trash plant debris.Bacillus, a Gram-positive, rod-shaped, non-pathogenic and endospore-forming aerobic bacterium found in soil and rotting plant material has been reported to have hydrocarbons emulsification capacity. Emulsifiers or surfactants produced by a variety of microorganisms such as yeast, bacteria and filamentous fungi are referred to as bio surfactants.Biosurfactants, hence, are amphiphilic compounds produced extracellularly by microorganisms on cell surfaces, or excreted extracellularly. They contain hydrophilic and hydrophobic moieties that reduce surface and interfacial tension between molecules at the surface and interface respectively (Jaysree*et al*., 2011).

CHAPTER 3MATERIALS AND METHODS

This chapter gives the chronological steps followed in executing bacteria culture, isolation and characterization.The isolates'induction for activity on a hydrocarbon source, caustic and pectinase pre scours, isolates' cottonwaxremoval optimization and investigation of the bacteria source of activity procedures are also demonstrated.The chapter also outlines the respective materials used in each procedure. The chapter consecutively describes each procedure as adapted and/or modified from previous related works.

3.0BACTERIA CULTURES, ISOLATION AND IDENTIFICATION

Gin trash that was used for the bacteria culturing was collected from Kibos and Kituiginneries as case studies. Each sample was macerated and about 10g put into a beaker containing 200mL of water. The beakers were then covered and placed in the incubator at 30^0C for 24 h to allow microbial growth.

Bacteria cultures were grown using pour plate dilution technique that involves diluting the microorganisms' concentrations for easier isolation. According to Ian and Charles (2009), in pour plate dilution, the original sample is diluted several times to reduce the microbial population sufficiently in order to obtain separate colonies when plating. Each of the samples, in pour plate dilution method, was diluted five times to reduce the microbial concentration depending on the dilution factors and the procedure outlined in the General microbiology laboratory manual was followed in executing the bacteria culturing techniques.Accordingly, small volumes (about 0.1to 1 mL) of several diluted samples were mixed with about 15 mLliquid agar that had been cooled to about 45^oC in sterile culture dishes which were then incubated at 37^0C for 24 h.The numbers of bacteria colonies that

grew on each plate were then studied.Nutrient agar was used to provide a balanced mixture of the required nutrients that would permit good growth.

The different colonies observed after the bacteria growth from the technique were then isolated further by using the streak and spread methods to achieve pure colonies. In streak method, small amount of colour and morphologically distinct colony was placed on the tip of an inoculation loop and streaked across the surface of the agar medium. According to Sanders (2012), each streak represents a dilution process and eventually single cells are obtained along the streak. The successive streaks "thin out" the inoculum sufficiently and the microorganisms are separated from each other. Each of these streaks, on incubation, grows up into a separate colony, which can be used as pure culture. In spread plate method on the other hand, a small amount of distinct colony was picked, suspended in distilled water then mixed with about 15mL of sterile nutrient agar. All the streaked and spread plates were then incubated at 37^0C for 24 h to allow for pure culture growth.

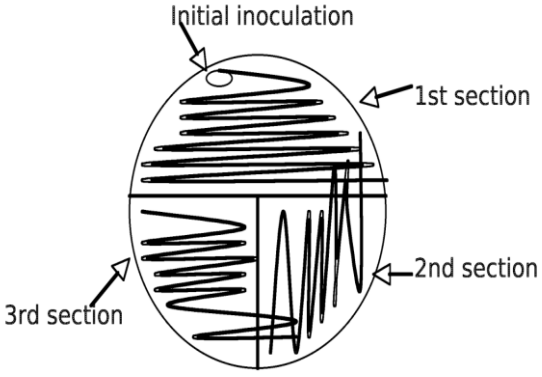

Figure 3.1:Representation of a streak pattern(adapted fromSanders (2012)).

Fig. 3.1 shows the thinning pattern streaks that were adopted during the streak procedure to avoid over lapping. A pure culture, according to Sanders (2012) is one in which the cells are all of one kind, i.e., demonstrate likeness. Hence proof of purity of the cultures was recognisedon the basis of the microorganisms demonstration of likenessas outlined

inSanders (2012) work.Accordingly, aculture proof of purity is based on certain criteria such as;

i. Microscopically,the microorganisms look alike and stain in the same manner.
ii. When plated, all the colonies formed look alike morphologically and in color
iii. Streaks, stabs are uniform
iv. Several isolated colonies perform identically; ferment the same sugars.

After the isolation of the various pure cultures, the isolates were then subjected to simple and gram staining for identification and characterization. Simple and Gram staining tests are used to study microbial morphology and nature respectively.According to Austrian (1995), when a single staining-reagent is used and all cells and their structures stain in the same manner, the procedure is called simple staining procedure. The staining is useful in the study of bacteria morphology for characterization purposes.For example, bacilliare rod-shaped bacteria, cocci are spherical bacteria while spirillum is a curved bacterium. Cocci may occur singularly, in chain (Streptococcus), or in clusters (Staphylococcus). Crystal violet was used for the staining. The simple staining procedure was conducted as outlined in the General microbiology laboratory manual.

According to Beveridge (2000)Gram stain is essential for separating bacteria in two broad groups;Gram-negative and Gram-positive. Gram-positive bacteria retain the primary dye giving a purple to blue-black appearance. Gram-negative bacteria take-up the colour of the counter stain (i.e red or pale red colour). Thus, gram-positive bacteria stain violet, and gram-negative bacteria stain pink.Crystal violet, Gram’s iodine, 95% alcohol and safranin stains were used. The Gram staining procedure was carried out as illustrated in the General microbiology laboratory manual.

3.1 ISOLATES PRELIMINARY HYDROCARBON ACTIVITY INDUCTION

Each of the different pure isolates was subjected to test for activity on paraffin wax at temperatures ranging from 37^0Cto 50^0C, being the estimated range for the bacteria survival. This was for the purpose of predetermining whether the isolates would have action on the cotton fibres wax, it being a hydrocarbon. The paraffin wax was melted and solidified in sterile petri dishes for the streaking practice.The working area was first sterilized with methylated spirit.About 10mLof wax was melted in each of nine sterilepetri dishes and allowed to solidify. Flame sterilized wire loop was used to pick each pure isolate that was then streaked onto each of the solidified wax plates. Immediately after streaking each plate, it was covered and the plates incubated, at 37^0C, 45^0C and 50^0C for 24 h.

3.2COTTON FIBRES SCOURING AND SOLVENT EXTRACTION

Prior to scouring, cotton fibres were treated with *n*-hexane (30 minutes, 75^0C), as a control for the wax removal to evaluate each procedure's efficiency, since the solvent extracts all the wax contained in the fibre (Agrawal, 2005; Agrawal *et al*., 2008b).

Cotton fibres collected from speed frames at Rivatex East Africa Ltd, were scoured using differently constituted baths; Caustic with and without surfactant and Pectinase with and without surfactant scour procedures were conducted to predeterminetheir efficiencies in cotton fibre wax removal. The isolates, at specified temperatures, pH and incubation timesscour procedures were also conducted to optimise the parameterscombination sets for eventual assimilation into the Pectinase scour bath for optimal fibre wax removal. After each scour procedure, each cotton fibre sample was rinsed and dried completely in an oven at 100^0C and the weight difference calculated to establish the relative performances of each procedure in removing general impurities contained in the fibres.

Each of the scoured fibresfrom each procedure wasthen subjected to solvent extractionusing soxhlet apparatus as specified in ASTM D2257-04 at the rate of six extractions per hour for a total duration of 2 hours (Nalankilli*et al*.,2008). Trichloroethylene solvent was used for the extractions after which the fibres were evaporated at 105^0C in an oven. The weight difference between the scoured and solvent-extracted fibres obtained gave the percentage wax removed by each scouring procedure.Before weighing, every sample was allowed to reach equilibrium under standard conditions with a relative humidity of 65% ± 2%, and a temperature of 25 $\pm 2^o$ C(Saravanan*et al*., 2010; Nalankilli*et al*., 2008).

Caustic in combination with a surfactant scour procedure was performed with caustic soda pellets constituted to 0.25M for use. A non –ionic surfactant (Sodium Lauryl sulphate) was used for the test. According to KiroMojsov (2012b), a liquor ratio of 20:1 and 1% of the surfactant were set in10 beakers for ten tests.Tensamples of cotton fibreseach weighing5.00$\pm$0.001 g were prepared and each put into each of the beaker's contents and boiled for one hour. The Percent removal of the waxes was calculated using the following formulaas modified from Saravanan*et al*. 2010 and Rajendran*et al*., 2011: Percentage removal of waxes= $1.2-[(W_C-W_S/W_C)]*100$, where W_C and W_s are the content of waxes (%) in the caustic scoured and solvent extracted fibres respectively.

Further, caustic without the surfactant procedure was also conducted by repeating the caustic with surfactant procedure but with the exemption of the surfactant in the scour baths constitution.

Petinase in combination with non-ionic surfactant was performedwith alkaline pectinase enzyme (*A. Niger)* as per the following specifications; 4mL, pH 8, 1 hour treatment time,

50:1 liquor ratio, and 50-60^{0}C temperature (KiroMojsov, 2012b). About 2mL of pectinase, 1% of surfactant (Sodium Lauryl sulphate) and 2mL of potassium phosphate buffer, pH 8, were put into ten test tubes. Ten samples of cotton fibres weighing 1.000$\pm$0.001g were put into each test tubeand incubated at 50^{0}C for 1 hour, after which the test tube contents were subjected to boiling to inactivate the enzyme.

The Percentage average waxes removed was calculated using the following formula as adapted from Vigneswaran*et al*. 2012b: Percentage removal of waxes=1.2- [(W_p-W_S/W_p)]*100, where W_p and W_s are the content of waxes (%) in the pectinase scoured fibres and solvent-extracted fibres respectively.

Additionally, the pectinase without the surfactant procedure was also doneusing the procedure usedunder the pectinase with surfactantwith the omission of the surfactant in the scour bath constitution.

3.3OPTIMISATION OF THETEMPERATURE, PH AND INCUBATION TIME FOR COTTON WAX REMOVAL BY THE ISOLATES

Fibre wax degradation process parameters: temperature, pH and incubation time, that are pertinent in pectinase scouring and affect the bacteria isolates activity were sampled to establish the most optimal combination sets formaximum wax degradation. Once the parameters combination sets were identified, they would be used to evaluate the isolates integration with the pectinase enzyme visibility. Optimization of the parameters combination values sets were determined by the Universal Rotatable Design (URD).

In table 3.1, the elements of the URD are illustrated where the maximum value for each design parameter is $Z_2(+\mu)$while the minimum value for each design parameter is $Z_1(-\mu)$

and the average value for the sum of the maximum and minimum values for each parameter is Z_0 (0) and also;

$+1=Z_0 + \sigma$

$-1=Z_0 - \sigma$

Where; $\sigma=\left(\frac{Z_2-Z_0}{\gamma}\right)$, γ is a constant dependent on the number of variable parameters.

γ =1.682 for three variable parameters. From the codes interpretations, the specific values for each of the parameters were worked out and table 3.2 derived.

Table 3.1: Three factors Universal Rotatable Experimental Design

Design No.	Temperature (^{0}C)	pH	Time (minutes)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	0	0	+μ
10	0	0	-μ
11	0	+μ	0
12	0	-μ	0
13	+μ	0	0
14	-μ	0	0
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table 3.2: Code values for the Temperature, pH and Time parameters

Design Parameter	-1	-μ	0	+1	+μ
Temperature	30	30	45	55	60
pH	8	8	10	11	11

Time	50	30	105	160	180

Tables 3.1 and 3.2 show three factor coded Universal Rotatable design for three factors and the respective code values for the temperature, pH and time respectively. Each isolate was used to treat the cotton fibres. Tris-HCL Buffer was used at varied pH ranges (pH 8-11). One ml of specified Tris- HCL pH buffer was put into each of ten test tubes and a flame sterilized wire loop was used to pick each of the isolate and introduce them into the test tubes. Fibre samples weighing 1.00±0.01g were prepared and each put into each test tube's contents, covered and incubated at different temperatures and time periods as guided by the experimental design.The test tubes were then subjected to boiling to deactivate the isolates. The Percent wax removed was calculated using the following formula as adapted from Vigneswaran *et al*. 2012b: Percentage removal of waxes=1.2- $[(W_I-W_S/W_I)]*100$, where W_I and W_s are the content of waxes (%) in the isolates scoured fibres and solvent-extracted fibres respectively.

The pectinase in assimilation with the isolatestest was performed to assess the effectiveness of the isolates in enhancing thepectinase in the fibre wax removalin comparisonwith the pectinase/surfactant procedure. The procedure was as outlined under pectinasewith surfactant scour procedure but the surfactant constituent was replaced with the isolates.Five tests were performed for each type of the isolatefrom each soil sample source.Five cotton fibre samples per gin trash source isolates, each weighing 1.00±0.01g were prepared and each put into test tubes containing, 2mL of pectinase and 1ml phosphate buffer (pH8).Flame sterilized wire loop was used to pick each of the isolates and then introduce it into each of the test tubesand the mixturesshaken vigorously to wet the fibres.All the test tubes were then covered and placed in controlled water baths at 40-50^{0}C for 1 hour, after

which they were subjected to boiling to deactivate the pectinase and the isolates.The Percentage average amount of wax removed was calculated using the following formula as adapted from Vigneswaran *et al*. 2012b: Percentage removal of waxes=1.2- [(W_E-W_S/W_E)]*100, where W_E and W_s are the content of waxes (%) in the enzymatic scoured fibres and solvent-extracted fibres respectively.

3.4EFFECT OF THE BACTERIAL WAX REMOVAL ON FIBRE STRENGTH

The strength of the original (untreated) and the bacteria-treated cotton fibre samples was measured at CottonDevelopment Authority (CODA), Kenya, usingHigh-Volume Instrumentation (HVI-1000 M700). The samples were conditioned according to ASTM D 177698and tested as per ASTM D-5867-95 standard testmethod. Six replicate tests per sample were performedand the average calculated.

3.5LYSING OF THE ISOLATES FOR ENZYMEPRESENCE INVESTIGATION

The isolates bacterial cells rupturing tests were conducted to determine if the bacteria contained enzymethat would have had the activity on the wax.Each pure isolate was Sub-divided into 10 eppodorf tubes, labeled and put into a centrifuge.The bacterial cells/isolates were then pelleted by centrifuging at 6000rpm for 5 minutes, supernatants aspirated and the pellets frozen at -30^{0}C for 30 minutes.Five test tubes of pellets of the bacterial cells from each isolate were then re-suspended in 0.75mL of lysis buffer, and then incubated at 30^{0}C for 15 minutes.The other five pellets from each pure culture were re-suspended with nutrient agar as controls.All the tubes were thenvortexed for 1 minute until the cell suspension was homogeneous. The pellet tubes were then centrifuged at 12,000 rpm for 20 minutes at 4^{0}C, supernatants from the lysis buffer-suspended tubes collected to new tubes and pellets re-suspended in 0.75 mL of lysis bufferas described in *http*:

//*wolfson.huji.ac.il/expression/...lysis/Lysis_of_bacterial_cells.html*.Each of the supernatants, nutrient agar and lysis buffer suspended pellets, were then used to test for their activity on wax, by streaking the contents onto solidified pre-melted wax in petri dishes which were then incubated at 37^0C for 24 hours.The activity on the wax was then examined by observing the growths along the streak lines.

CHAPTER 4 RESULTS

This chapter sequentially presents the findings of all tests and procedures conducted; Bacteria cultures from gin trashes, isolation and identification of the pure isolates, Hydrocarbon activity induction tests on all the isolates, Preliminary cotton fibres caustic scour and n-hexane solvent extraction, Caustic and pectinase with and without surfactant scour procedures, the isolates temperatures, pH and incubation time optimisation during cotton fibre wax degradation for eventual assimilation with pectinaseat harmonizedprocess parameters. The chapter also gives the findings for the bacterial enzyme presence in the bacteria cells investigation test.

4.0 GIN TRASH BACTERIA CULTURES, ISOLATION AND IDENTIFICATION

Under the pour plate culture method, the following bacteria culturesobservations were made from the Kibos and Kitui gin trash samples.All the dilutions from the Kibosgin trash sample showed bacterial growth of three different distinctivecoloniesindentified based on Sanders (2012)description of a distinct colony. These colonies comprised of large serrated yellow colonies, small round white colonies and oval shaped transparent blue colonies.The bacterial growth population decreased with the dilution factors. Dilution 10^{-1} showed vast growthof different colonies that was hard to observe segregate colonies while 10^{-5} showedsparsegrowth that clearly showed the distinct colonies. Majority of the colonies constituted of the small, round white colonies, followed by long, serrated yellow colonies and the least were transparent bluish colonies.The Kitui gin trash sample dilution 10^{-1} and 10^{-2}showed bacterial growth decreasing in concentration from dilution 10^{-1} to 10^{-2}.The growth comprised mainly of small round white colonies and few serrated yellow and very tiny transparent bluish colonies. Dilution 10^{-3} and 10^{-4} showed even sparse bacterial growth

comprising of only the white colonies, even smaller in size in dilution 10^{-4}.Dilution 10^{-5} hardly gave any bacterial growth.

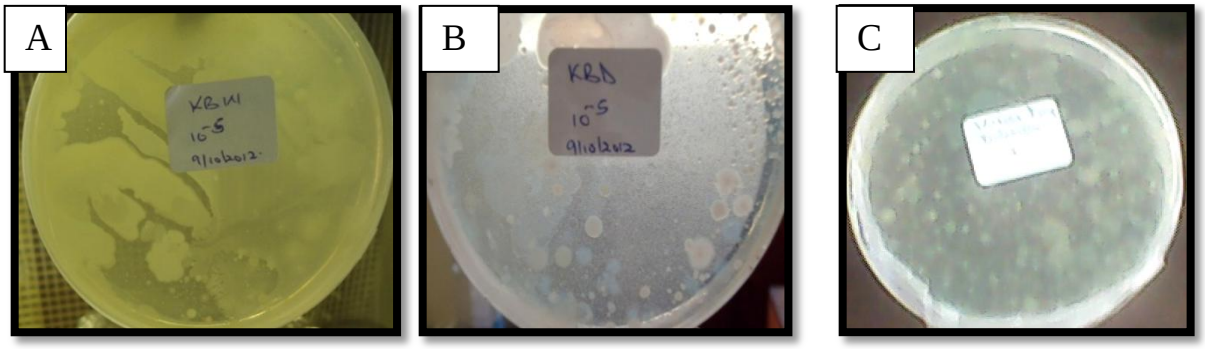

Figure 4.1: Pour plate dilution culture plates for Kibos gin trash sample.

(A) Dilution 10^{-5}distinctive long, serrated yellow and small round white colonies growth.

(B) Dilution 10^{-5}distinctive small round white and the oval transparent blue colonies

(C) Dilution 10^{-1}indistinct vast colonies growth

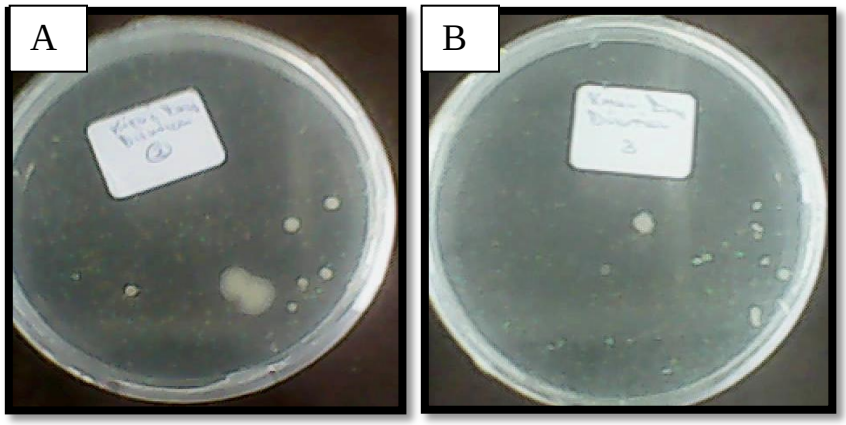

Figure4.2: Pour plate dilution culture plates forKituigin trash sample

(A) Dilution 10^{-2}showing a few small round white colonies growth

(B) Dilution 10^{-3} predominantly tiny round white colonies growth

All the three distinct colonies; white, yellow and blue from the two gin trash samples were further isolated to obtain pure cultures. The isolation of the distinct colonies was done using thestreak and spread plate techniques,whicheventually gave three pure isolates,

characterized by different the colours. The distinct isolates comprised ofthe pure yellow, white and blue isolates.

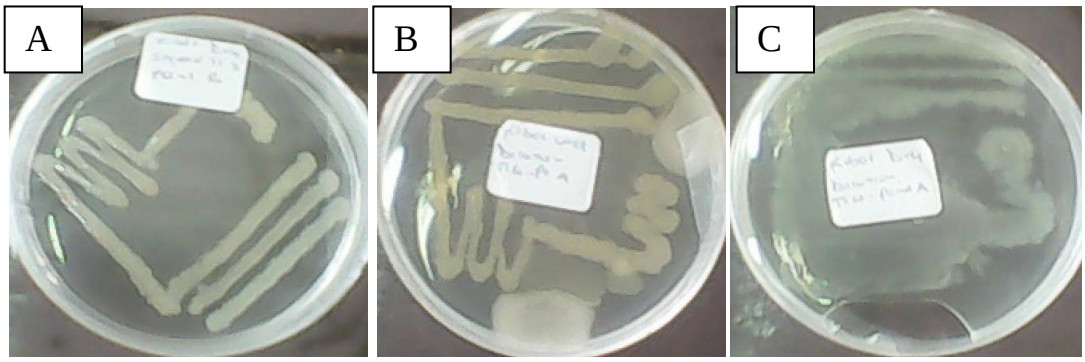

Figure 4.3: The yellow, white and blue streak isolates plates

(A)White colony isolate streak

(B)Yellow colony isolate streak

(C)Blue colony isolate streak

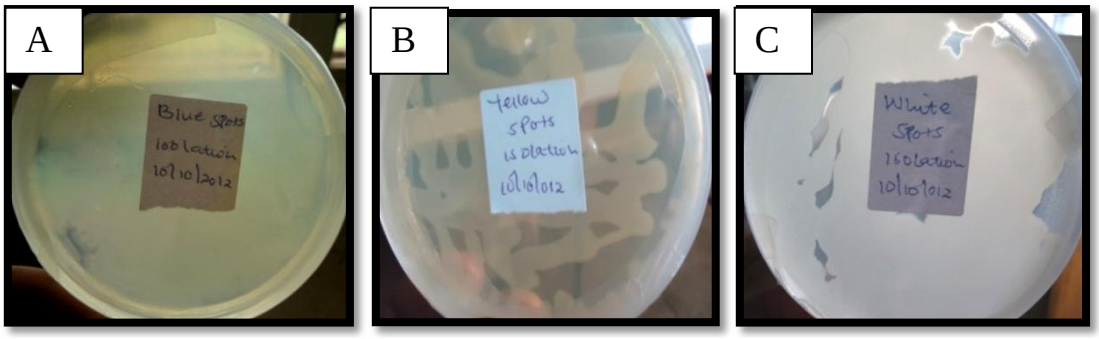

Figure 4.4: The Blue, yellow and white spread platesisolates

(A) Blue colony spread plate

(B) Yellow colony spread plate

(C) White colony spread plate

Plates4.4 A/B/C show the respective photos of the blue, yellow and white pure isolates obtained from the spread plate isolation method.

The different isolates plates photography illustrations from the two gin trashsample sources are shown in Appendix 1a to 1e.

After the isolation of the bacteria cultures from the Kibos and Kitui gin trashes, the pure isolates were subjected to simple and Gram staining tests for the purpose of their morphological and nature study. The simple staining results gave the pure isolates bacteria morphology in terms of their shapes andarrangements asobserved under the microscope from the resultant staining. All the isolates revealed purple stained rod shapes indicating that the bacteria were bacilli. The observations also revealed chained rods occurrence for all the isolates meaning that the bacteria were streptobacilli in nature, based on penney*et al.* (2002) analysis of staining results.

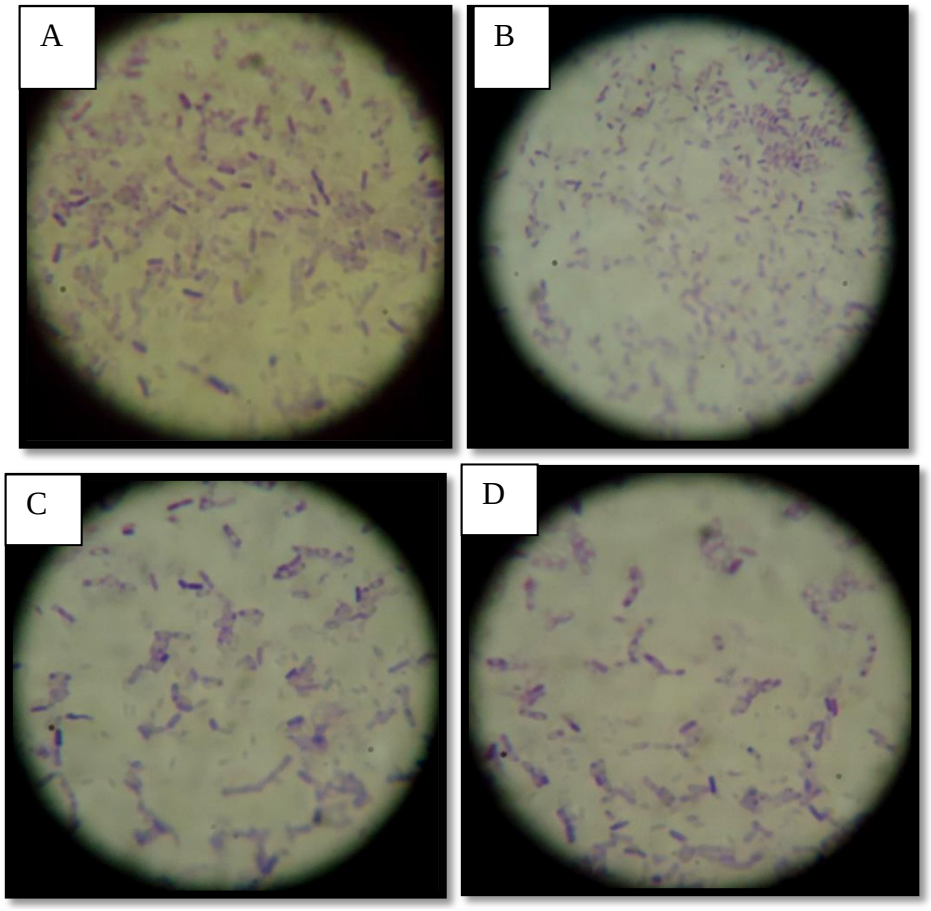

Figure 4.5: The isolates Simple staining results plates

(A/B) The isolates basic rod shapes

(C/D) The isolates chained rods arrangements

Plates A and B demonstrate the basic rod shapes of all the bacteria isolates while plates C and D demonstrate the chainedrodsarrangementsof all the bacteria isolatesas observed under the microscope.

Under the Gram staining test, all the isolates stained purple, the primary colour used, indicating that the bacteria isolates were Gram-positive,based on the staining results analysis outlined by Penney *et al.* (2002).

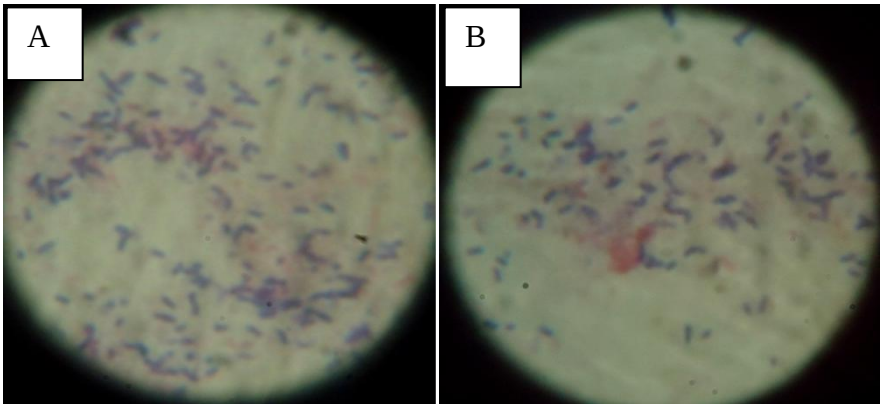

Figure 4.6: The isolates Gram staining results plates

(A) Purple stainedbacillifor the Kibos isolates

(B) Purple stained bacilli for the Kitui isolates

PlatesA and B illustrate the purple colour stained bacteria isolates after the gram staining test, indicating that the bacteria isolates were Gram-positive.

4.1THE ISOLATES HYDROCARBON ACTIVITY INDUCTION

All the wax streaked petri dishes from the three pure isolates, incubated at 37^0C gave a thick bacterial growth along the streak lines. The plates incubated at 45^0C gave a relatively

thick growth along the streak lines while the plates incubated at 50^0C gave the least growth along the streak lines, meaning the isolates survival at that temperature was declining.

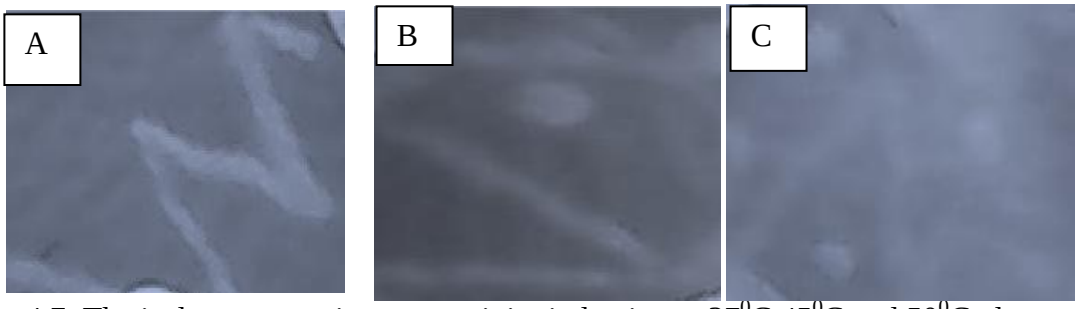

Figure 4.7: The isolates respective wax activity induction at 37^0C,45^0C and 50^0C plates

(A) Wax activity induction at 37^0C

(B) Wax activity induction at 45^0C

(C) Wax activity induction at 50^0C

Plates A/B/C show the thick bacteria growth observed when the isolates were drawn on a cooled pre-melted paraffin wax and incubated at 37^0C for 24h, the relatively low bacteria growth observed at 45^0C and the vague growth at 50^0C incubations for 24h. All the wax inducted isolates plates from Kibos and Kitui regions are shown in appendices 2a to 2f.

4.2COTTON FIBRES SCOURING AND SOLVENT EXTRACTION

The preliminary caustic and solvent fibre treatmentsgave an average of 7.2%and 1.2% of the general and wax impurities contained in the fibres respectively. It is on this basis that the effectiveness of each of the scouring proceduresin wax removal was evaluated.

The cotton fibres were given a caustic with and without a surfactant scour in order to study the role of the surfactant in determining the general impurities and wax removal. Caustic with surfactant boil showed respective high percentage average values of both the overall impurities and the wax removed of 7.2% and 0.72%. Caustic without a surfactant scour on

contrary gave respectively low values of 2.65% and 0.3% as compared to the counterpart process that contained the surfactant.

Pectinase scouring procedure was carried out with and without a surfactant in order to study the effect of incorporating a surfactant in the enzymatic scour in the effectiveness of the general impurities and wax removal. Pectinase with surfactant procedure gave a percentage average impurities removal value of 4.14% and a wax removal value of 0.844% out of the respective 7.2% impurities and 1.2% waxcontained in the raw fibres. Pectinase without a surfactant on the other hand, gave respective percentage average impurities and wax removal values of 2.4% and 0.267%.

All the pure isolates from the two gin trash sources were subject to the cotton fibres treatment at different temperatures, pH and incubation time in order to study the most optimized parameters combinations for maximum fibre wax removal. Every isolate, as, already observed under section 4.2, was found to have activity on the cotton fibre wax degradation. However differently sampled temperature/pH/time combinations sets exhibited different levels of the percentage average wax removal.

Universal Rotatable Design was used to devise the three parameters combination sets in order to observe the most optimized parameters combination sets in terms of the percentage average wax removed as shown in Table 4.1.

Table 4.1: Parameters optimization for wax degradation

Temp.(^{0}C)	pH	Time (minutes)	% Bacteria degraded wax
30	8	50	0.370
30	11	50	0.385
30	8	160	0.496
30	11	160	0.485
30	10	105	0.420
45	10	180	0.772
45	10	30	0.494
45	11	105	0.745
45	8	60	0.765
45	10	105	0.695
45	10	105	0.652
45	10	105	0.675
45	10	105	0.720
45	10	105	0.735
40	10	105	0.772
55	8	50	0.154
55	11	50	0.098
55	8	160	0.199
55	11	160	0.195
60	10	105	0.034

Table 4.1 gives the parameters combination sets used to deduce the most optimal parameters set for the maximum fibre wax degradation by the bacteria isolates.

4.3MODELLING AND SENSITIVITY ANALYSIS OF THE ISOLATES WAX DEGRADATION PROCESS PARAMETERS

Modeling of the influence of temperature, pH and time on the amount of cotton fibre wax degraded by the bacteria isolates was done using regression analysis (Minitab. 15) and Monte Carlo techniques (ModelRisk 3.0).

A multiple regression model was developed for the percentage of wax (y) degraded by bacteria isolates as the output while the input parameters were temperature (x_1), pH (x_2) and time (x_3). The input data shown in Table 4.2 were used to design a second order polynomial

equation 4.1, which was used to generate predicted value of the output (percentage wax degraded) and corresponding error for each of the parameters combinations. The model also gave the parameters interactive effects of the independent variables.

$y^* = -4.00 + 0.187\,x_1 + 0.163\,x_2 + 0.00318\,x_3 - 0.00229\,x_1x_1 - 0.0106\,x_2x_2 - 0.000020\,x_3x_3 + 0.00002\,x_1x_2 - 0.000013\,x_1x_3 + 0.000261\,x_2x_3$……4.1.

Table 4.2: input data

x_1	x_2	x_3	x_1^2	x_2^2	x_3^2	x_1x_2	x_1x_3	x_2x_3	y	y*	ε
30	8	50	900	64	2500	240	1500	400	0.370	0.373	-0.003
30	11	50	900	121	2500	330	1500	550	0.385	0.299	0.086
30	8	160	900	64	25600	240	4800	1280	0.496	0.448	0.048
30	11	160	900	121	25600	330	4800	1760	0.485	0.460	0.025
30	10	105	900	100	11025	300	3150	1050	0.420	0.472	-0.052
45	10	180	2025	100	32400	450	8100	1800	0.772	0.646	0.126
45	10	30	2025	100	900	450	1350	300	0.494	0.495	-0.001
45	11	105	2025	121	11025	495	4725	1155	0.745	0.651	0.094
45	8	60	2025	64	3600	360	2700	480	0.765	0.620	0.145
45	10	105	2025	100	11025	450	4725	1050	0.695	0.683	0.012
45	10	105	2025	100	11025	450	4725	1050	0.652	0.683	-0.031
45	10	105	2025	100	11025	450	4725	1050	0.675	0.683	-0.008
45	10	105	2025	100	11025	450	4725	1050	0.720	0.683	0.037
45	10	105	2025	100	11025	450	4725	1050	0.735	0.683	0.052
40	10	105	1600	100	11025	400	4200	1050	0.772	0.727	0.045
55	8	50	3025	64	2500	440	2750	400	0.154	0.170	-0.016
55	11	50	3025	121	2500	605	2750	550	0.098	0.097	0.001
55	8	160	3025	64	25600	440	8800	1280	0.199	0.209	-0.010
55	11	160	3025	121	25600	605	8800	1760	0.195	0.222	-0.027
60	10	105	3600	100	11025	600	6300	1050	0.034	-0.136	0.170

Regression statistics and Analysis of variance (ANOVA) were performed and the model was acceptable since the R^2 value was 0.94 and the F value was more than the F significant value as shown in Table 4.3a and Table 4.3b.

Table 4.3a: Regression statisticsmodel

R	0.969699
R^2	0.940316
Adjusted R^2	0.8866
Standard Error	0.08433
Observations	20

Table 4.3b: ANOVA results for Polynomial regression model

	df	*SS*	*MS*	*F*	*Significance F*
Regression	9	1.12042	0.12449	17.51	0.000
Residual	10	0.07112	0.00711		
Total	19	1.19153			

Table 4.3b gives the output of the ANOVA for the multiple regression model of the relationship between the output (wax degraded) and the independent variables (temperature, pH and time).

The polynomial regression equation exhibited a mean square error of 0.007 and an R^2 value of 0.94. Since the polynomial regression equation gave a higher coefficient of determination value therefore it was adjudged as the optimum equation which was used to explain the mathematicalrelationship between the percentagewaxes degraded(y) and temperature (x_1), pH(x_2) and time(x_3).

Figure 4.8 shows the scatter plot for the predicted value and actual value of the percentage of wax degraded by bacteria. The figure shows a wide spread of values around the regression line, this shows that the model was significant in predicting the influence of temperature, pH and time on the percentage of wax degraded by the bacteria isolates.

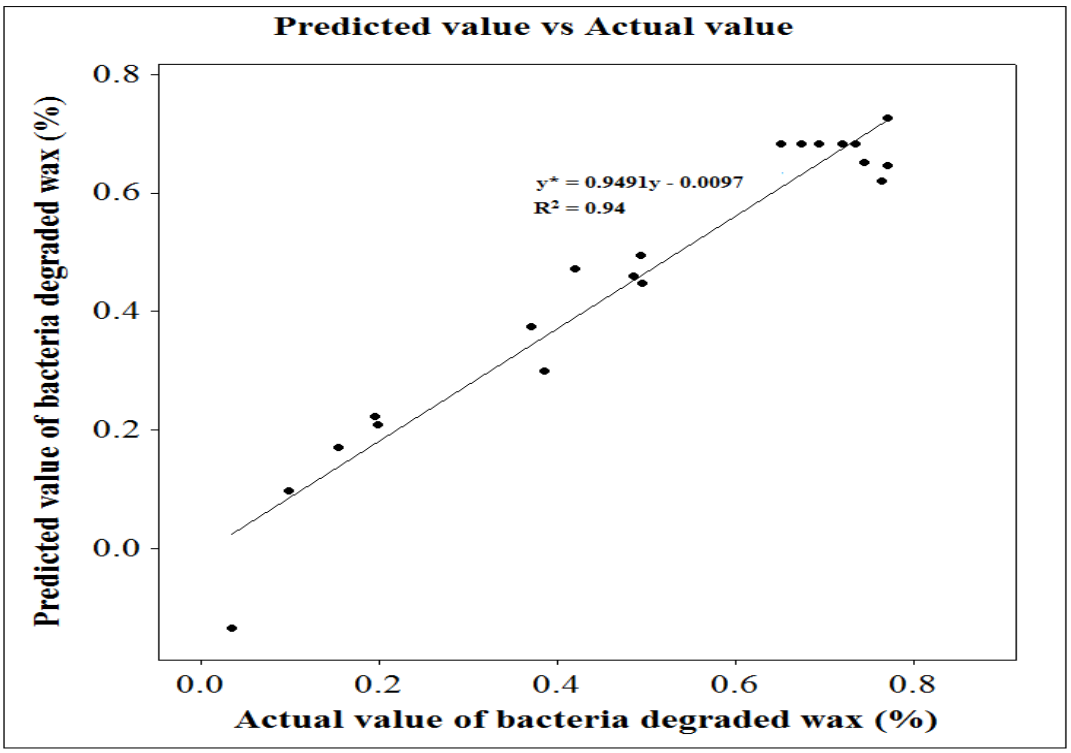

Figure 4.8: Predicted value against actual value of bacteria degraded wax

Figure 4.8 gives the relationship of the predicted(using equation model 4.1) and actual wax degraded by the bacteria isolates.

The prediction of the optimum level of wax degraded by the bacteria isolates was performed by simulation of the regression model (equation 4.1) using Monte Carlo techniques. The predicted value of the percentage wax degraded was evaluated using 100 simulations of the regression model and the cumulative line graph shown in Figure 4.9, was developed, which shows that there is a probability of 0.35 that the amount of wax degraded from the cotton fibres would lie between 0.034% and 0.777% .There is also a probability of 0.36 that the percentage of wax degraded by the bacteria will be more than 0.777%. This shows that according to the model the bacteria actually degrade the cotton fibre wax.

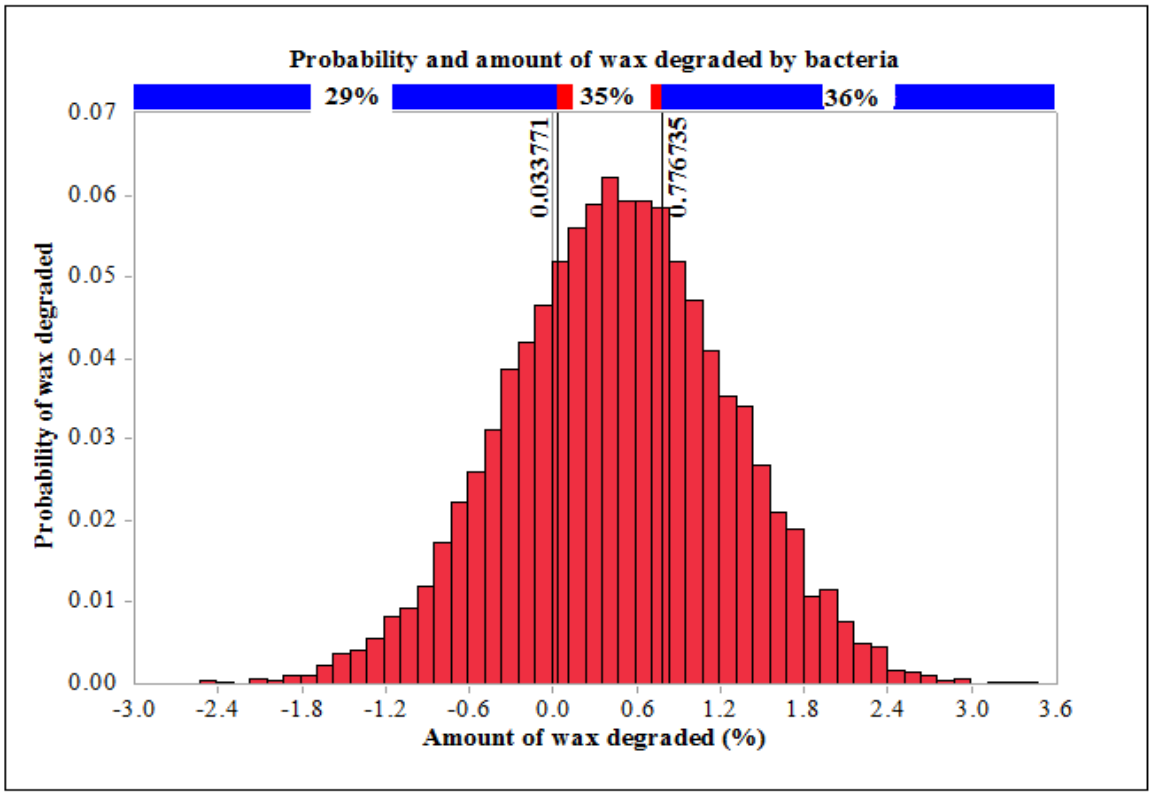

Figure 4.9: Probability of cotton fibre wax degradationby the bacteria isolates

Figure 4.9 illustrates the probabilities of the isolates degrading the cotton fibre wax and the respective degraded amounts.

The sensitivity analysis of the regression model was done using Monte Carlo simulation and was performed to establish the influence of the input parameters (temperature, pH and time) on the percentage of wax degraded by the bacteria isolates. The analysis was evaluated using 100 simulations which gave the sensitivity analysis plot, shown by Figure 4.10.The effect of the input parameters on the amount of wax degraded (%) indicates that the factor with the highest effect is temperature (x_1) with a total range of 1.4, followed by pH (x_2) with a total range of 0.2 and time (x_3) giving a range of 0.1.

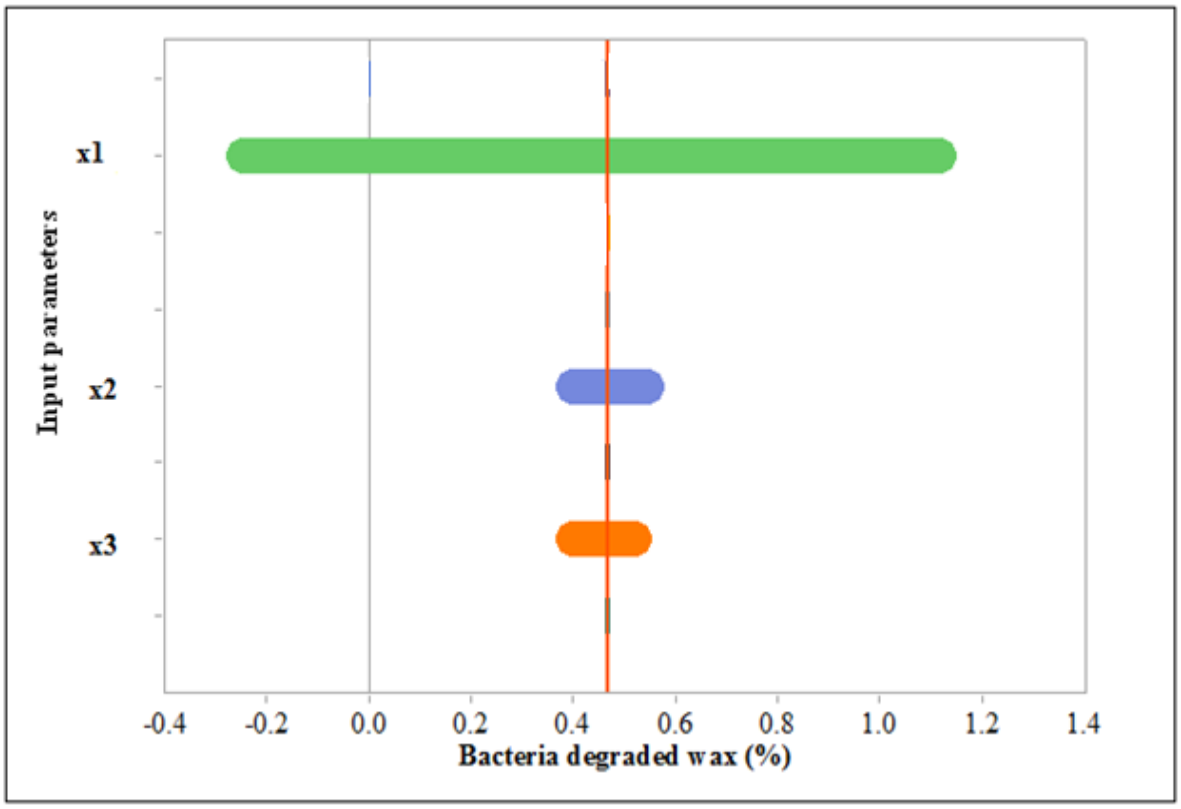

Figure 4.10: Parameters mean sensitivity plot

Figure 4.10 shows each of the variables wax degradation sensitivity range.

The sensitivity of the parameters in relation to the amount of wax degraded was further analysed by plotting a bar graph for the input parameters (temperature, pH and time) against the percentage wax degradedas illustrated in Figure 4.11 which was derived from the parameters coefficients in Table 4.4 as plotted using SPSS t statistic.

Table 4.4: Parameters Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.917	0.256		3.583	0.020
	Temp	0.677	0.006	0.176	3.842	0.000
2	(Constant)	0.327	0.516		2.261	0.005
	pH	0.271	0.053	0.076	3.324	0.013
3	(Constant)	0.414	0.147		2.815	0.000
	Time	0.100	0.010	0.137	0.595	0.590
Dependent Variable:% Wax degraded						

Table 4.4 gives the temperature, pH and time variables significance in influencing the wax degradation by the isolates. It also gives the individual variables level of the wax degradation.

The coefficients show that there is a positive relationship between all the independent variables (Temp, pH and Time) andthe dependent variable (% Wax degraded).The t statistics helps in determining the relative importance of each variable in the model. At 5% level of significance and 95% level of confidence temperature had p value of 0.00, while pHhad 0.013 andtimehad 0.59. Thus temperature and pH are statistically significant determinants of amount of wax degraded as opposed to time.

It was emphasized as shown in Figure 4.11 that temperature caused the greatest change on wax degradation at 67.7%, pH caused 27.1% while time caused 10% change in percentage wax degraded by the bacteria isolates.

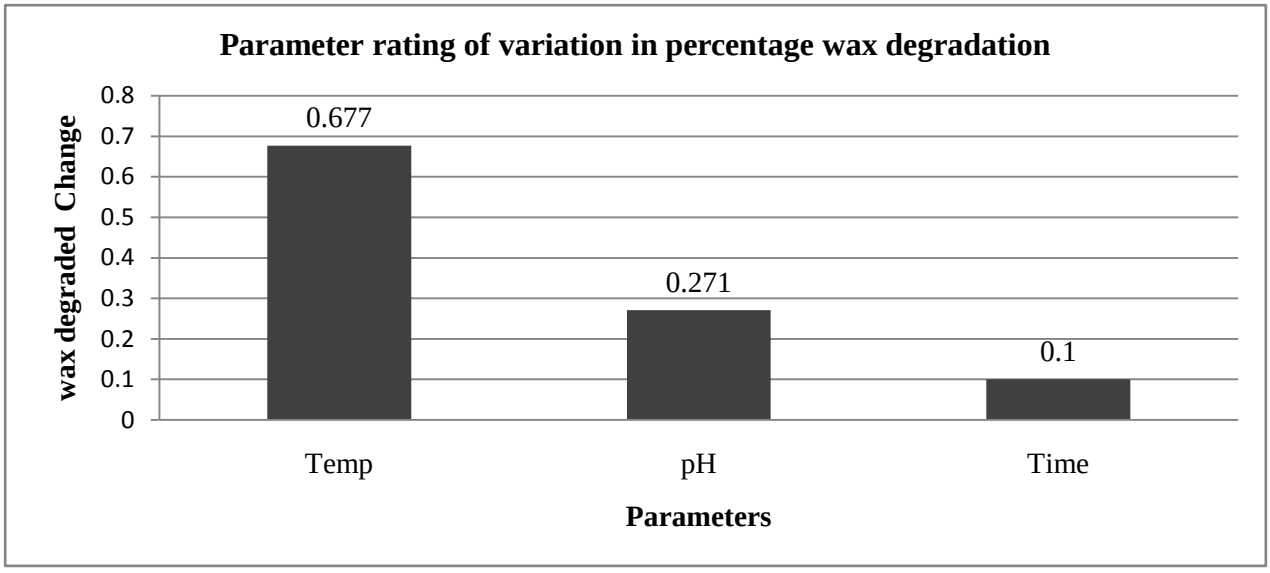

Figure 4.11: Parameter rating of variation in wax degradation

Figure 4.11 demonstrates each of the variables degree of influencing the total wax degraded by the bacteria isolates.

In order to establish the direction of influence of temperature (x_1), pH (x_2) and time (x_3) on the amount of wax degraded (y), the sensitivity analysis plots shown in Figure 4.12 was developed from the regression model. The analsyis indicated that x_1^2 had the highest negative influence on the model implying that an increase in x_1^2 would decrease the amount of wax degraded, while x_1(temperature) had the dominant positive influence on the model so that as x_1 increases the amount of wax degraded would increase, this suggest that the temperature would increase the amount of wax degraded by the bacteria upto an optimum point and then beyond the optimum temperature the amount of wax degraded would decrease with increase in temperature. The sensitivity analysis also shows that x_3^2 and x_2^2 both have a negative influence on the model, while x_3, x_2 and an interaction between x_2 and x_3 willhave positive impact on the model. This shows that both the pH (x_2) and time (x_3)

will also have a positive influence on the amount of wax degraded, Interaction between x_1 and x_2 gives almost a neutral influence on the amount of wax degraded implying that temperature and pHhas a high correlation towards determining the amount of wax degraded by the bacteria isolates.

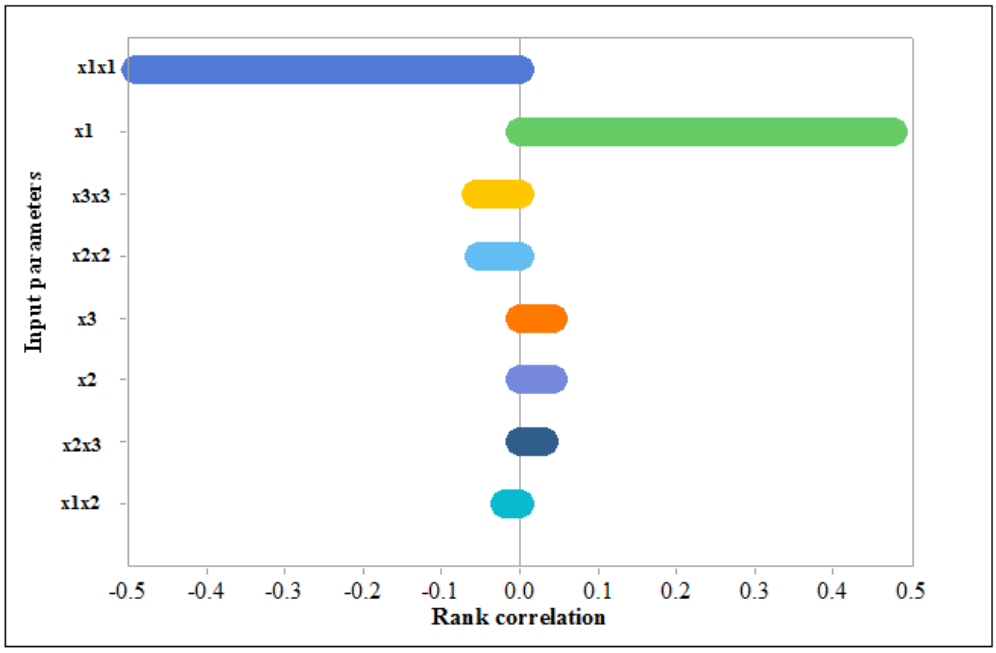

Figure 4.12: Parameters rank sensitivity plot

Figure 4.12 shows the ranking of the temperature, pH and time and their possible interactions sensitivity towards determining the amount of wax degraded.

The temperature, pH and time interaction effects can further be demonstrated by Figure 4.13 plotted using minitab.15. The figure demonstrates that there exist strong interactions between pH with either temperature or time, relatively low levels of interactions between temperature with either pH or time and minimal interactions between time with either temperature or pH due to the nonparallel, relatively nonparallel and almost parallel lines respectively. This implies that pH has the highest impact followed by temperature and time

in defining the nature of parameters interactions effects and respective amount of wax degraded by the bacteria.

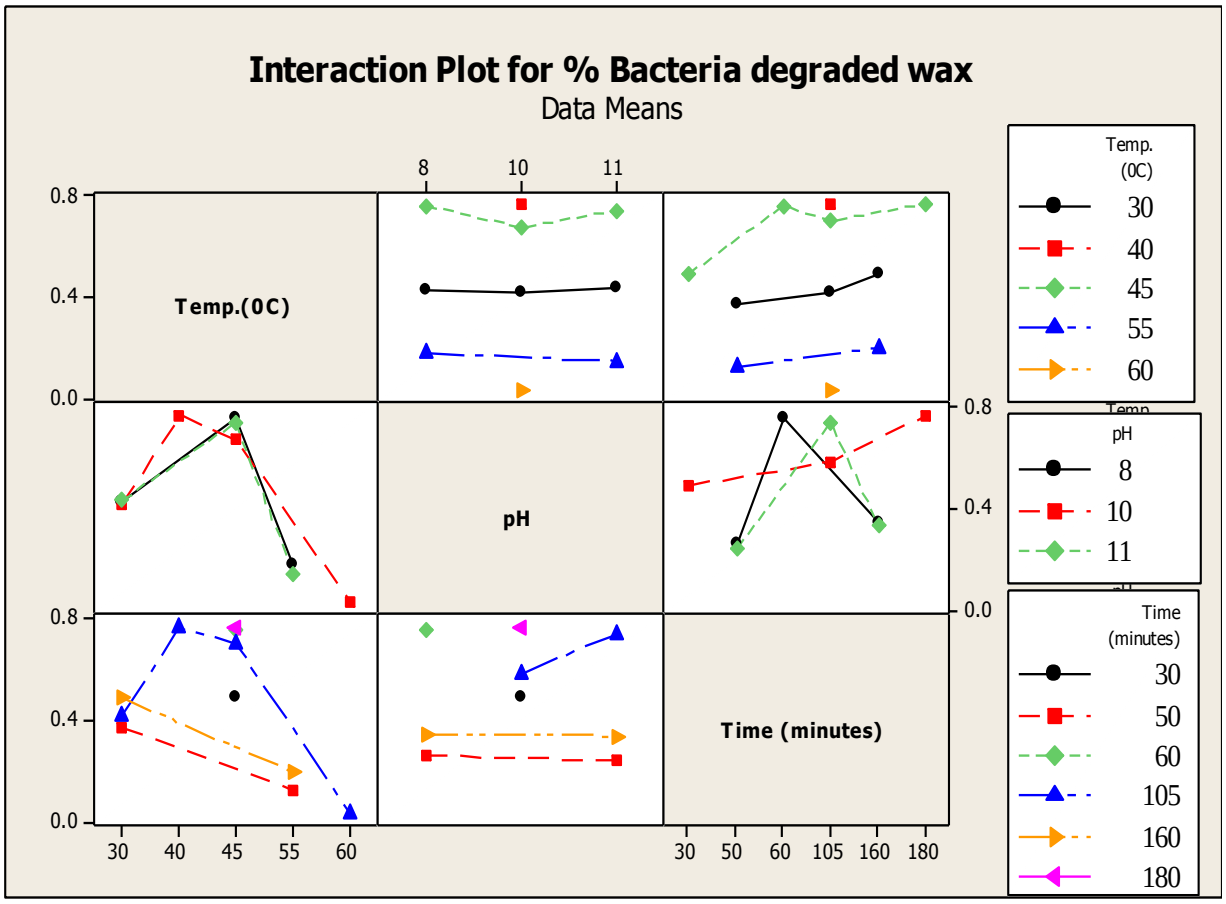

Figure 4.13: Parameters interactions plot for wax degraded

Figure 4.13 shows the interaction effects of the wax degradation process parameters (temperature, pH and time).The figure demonstrates the influence each variable has on another in determining the amount of wax degraded.

Pectinase with incorporation of the bacteria isolates scour procedure wasconducted in order to study the effect of integrating the isolates into pectinase scour bath in terms of wax removal efficiency. The results were also compared with those obtained from the pectinase/caustic with surfactant procedures. The average wax removal values obtained

from the pectinase with all the two different gin trashbacteria isolates present,as shown in Table 4.5,were comparable to those obtained from the pectinase/caustic in combination with the surfactant;0.844% and 0.72% respectively. The pectinase/isolates assimilation scour procedure was carried out at temperatures of 40^0C, 45^0Cand 50^0C, at pH 8 for a period of 1 hour, in order to harmonize the enzymes optimal parameters with the isolates optimized parameters as drawn from Table 4.1.

Table 4.5: Summary of the percentage average wax removed by the pectinase /isolates scour

Temperatures	Colony isolate	Percentage Wax removed
40^0C	White	0.735
	Yellow	0.731
45^0C	Yellow	0.747
	Blue	0.736
	White	0.737
50^0C	White	0.726
	Yellow	0.710
	Blue	0.710

Table 4.5 gives the summary of the percentage average wax removed as obtained from each of the isolate/pectinase scour procedure.

4.3 EFFECT OF THE BACTERIAL WAX REMOVAL ON FIBRE STRENGTH

There was no significant change in strength between theoriginal and the bacteria-treated cotton samples. Theaverage strength of the original sample was 25.7 g/tex,while that of the treated sample was 26.5 g/tex. Therewas a slight 2.7% moisture regain of the treated sample,probably due to the wax removal, which might havecaused the small change in strength. Preservation of thefibre strength in the treated sample confirms that thebacteria action did not affect the cellulose structure ofthe fibre.

4.4BACTERIA CELLSENZYME PRESENCE INVESTIGATION

This test was conducted to investigate if the activity on hydrocarbons exhibited by the bacteria isolates was due to the bacterial cells or a bacterial enzyme contained in the bacteria. All the bacteria isolates were subjected to cells rupturing in order to establish if the cells contained an enzyme and if the activity on the hydrocarbon was due to the cells or the enzyme. All the plates streaked with the supernatants from all the isolates did not show any growth along the streak lines. The plates streaked with lysis buffer- suspended pellets from all the isolates gave comparatively distinctive growths along the streak lines. The plates streaked with nutrient -suspended pellets gave relatively thick and clear growths along the streak lines. This indicates that there was no bacterial enzyme contained in the bacteria and it is the bacterial cells that had the activity on the wax.

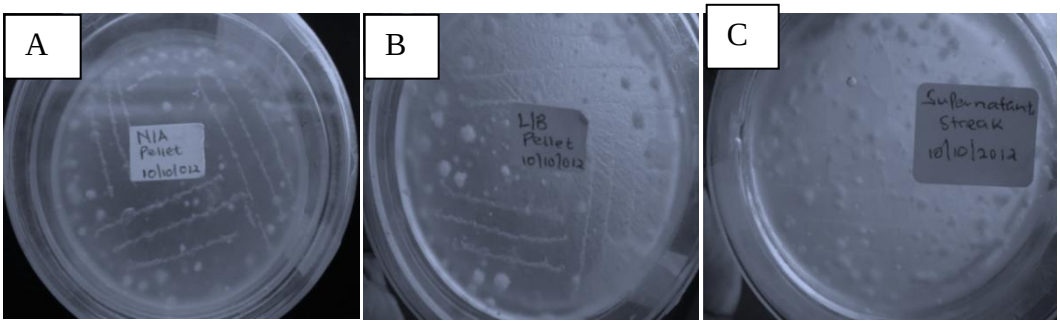

Figure 4.14: Nutrient agar suspended pellet, Lysis Buffer suspended pellet and supernatant wax activity induction streaks plates.

(A) Nutrient Agar suspended bacteria pellet waxactivity induction streak growth

(B)Lysis buffer suspended bacteria pellet wax activity induction streak growth

(C)Supernatant wax activity induction

PlatesA/B/C show the different streak growths observed from the nutrient agar and lysis buffer pellets suspensions as well as the blank supernatantstreak on the wax.Each of the sreaks activity on the wax is notable by the streak lines thickness differences and the complete absence of any growth on the supernatant streak lines.

CHAPTER 5DISCUSSION

This chapter gives the interpretation of the research findings in relation to the stipulated objectives. The results are further compared, related and /or contrasted with the previous research works findings, from which the research's relevance and importance in the cotton fibre bio-scouring implementation enhancement is drawn.

5.0GIN TRASH BACTERIA CULTURE AND WAX ACTIVITY INDUCTION

The successful hydrocarbon degrading bacteria culture and isolation from the cotton gin trash samples collected Kibos and Kitui regions supported the report given by ZoBell (2005). The report accounted of presence of vast hydrocarbon degrading bacteria in the gin trash upon mixing it with water and incubating for 16-24h. Accordingly the bacteria were found to have a preliminary action on a hydrocarbon source, paraffin wax, used for the activity induction, as demonstrated by photos 4.7 A/B/C.The ability of the isolates to consecutively degrade the cotton fibre wax at different temperature, treatment time and pH combination sets as shown in table 4.1, can be related to the bacteria action test on the paraffin wax, the two being hydrocarbon sources that provided the bacteria with nutrients.

5.1 COTTON FIBRES SCOURING

The preliminary caustic and solvent extraction tests carried out on the cotton fibres to establish the general and wax impurities contained in the fibres gave respective values of 7.2% and 1.2%. These values relate to those given by Anita (2004),Agrawal (2005),Qiang*et al*. (2006) and Mohamed (2007), who reported an average value of 10% general cotton fibre impurities out of which a maximum of 1.0-1.3% is the wax constituent.

The values for the general impurities and wax removed by the caustic with surfactant procedure; 7.2% out of 10% and 0.72% out of 1.2%respectively are equally comparative to

those reported by Bahrum (2012); 6.87-7.27% weight loss and Tzanko (2001); 6.3% weight loss . The high impurities values of extraction by this procedure can be attributed to its non-specific nature in the impurities removal. However this is contrary to the low values recorded from the same procedure in the absence of the surfactant; 2.65% and 0.3%, which can be explained by the ability of the hot caustic soda solution with the aid of the surfactant to remove the impurities by breaking, releasing and emulsifying them effectively as argued by Tzanko (2001).

The relatively lower value of the general impurities recorded from the pectinase with surfactant procedure; 4.14% can be explained by the substrate specific nature of the enzyme inimpurities removaland hence the inability of the enzyme to destabilize the cuticle layer containing the wax. Comparatively, under pectinase cotton scour process conditions of 45^0C, reaction time of 60 minutes and a pH of 8.5, Vigneswaran*et al.* (2012a) obtained the best opted test results of overall impurity removal efficiency of 4.80% which can be related to the 4.14% obtained.

However, the relatively higher value for the percentage average wax removed by the same procedure; 0.844%can be explained by the emulsification of the waxes boost given to the enzyme by the surfactant.This can be supported by the argument given by Qiang*et al*. (2006) and Kiromojsov (2012b) that, the mechanism of pectinase scouringreportedly assumes that the degradation and eliminationof pectins makes the loosened waxes moreeasily removable with the help of surfactants andmechanical agitation; this allows the cotton to achievesuperior hydrophilicity without fibre deterioration. However, the same procedure devoid of the surfactant recorded lower values of general impurities and wax

degraded of 2.4% and 0.267% respectively, which again emphasizes on the very significant role of the surfactant in the impurities removal enhancement.

5.2 WAX DEGRADATION PROCESS PARAMETERS OPTIMIZATION AND SENSITIVITY ANALYSIS

When the fibres were subjected for treatment with the isolates at varied temperature, pH and treatment time, different values of the wax degraded were arrived at as shown in Table 4.1. The relevance of the parameters in influencing the amount of wax degraded by the isolates have been emphasized in Figure 4.8 which is derived from model equation 4.1 with predicted degraded wax coefficient of determination of 0.94. As it has been demonstrated by Figures 4.10 and 4.11, temperature has the greatest influence on amount of wax degraded, followed by pH and finally time.Extremely high values of each independent variable(x_1^2, x_2^2 and x_3^2) would have a negative influence on the amount of wax degraded while any two of the variables interaction counteracts the effects of each variable's influence on the wax degraded as shown in Figure 4.12.These findings can be related to those reported by Vigneswaran (2012a), emphasizing on the impacts of process variablesinteractions on the scoured fabric weight loss (impurities removal). This argument is further backed by the illustration in Figure 4.13.

From the data in Table 4.4, it can be observed that the percentage cotton fibre wax degraded by the isolates, 0.765% at 45^0C, pH 8 within a period of one hour is comparable to that degraded by the caustic and pectinase in the presence of a surfactant; 0.72% and 0.844%. This amount wax degraded by the isolates isalso notably higher than that reported by Vigneswaran*et al.* (2012a) of an average loss in wax content of 0.525% at 60^0C, pH 8.5-9.0 and treatment time of 60 minutes using 6% pectinase enzyme concentration.

Vigneswaran (2012b) and Vigneswaran (2013) had also determined that, 3 % protease and and lipase (0.2%, 0.4%, 0.6% and 0.8%) cotton organic fibre treatment at 60^0C, pH 8.5-9 and 45 minutes shows 0.19, 0.29, 0.44 and 0.51 wax loss respectively, which they attributed to proteolytic hydrolysis of wax and protein components in the organic cotton fibre. A percentage wax of 0.772% was degraded at 45^0C, pH 10 for a period of three hours, which means that prolonged time has no significant effect on the percentage amount of wax degraded by the bacteria even though the temperature and pH be favourable.This finding relate to that of Rajendran*et al*. (2011), that a positive correlation between incubation time of fabric in pectinase enzyme and the fabric weight loss existed for a period of 50 minutes but the absorbency (wax removal) reduced inversely with increase in incubation time for the bio scouring process. This finding can further be supported by the illustration shown in Figure 4.9 which shows that there is a probability of 0.35 that the amount of wax degraded from the cotton fibres would by the isolates lie between 0.034% and 0.777% and a probability of 0.36 that the percentage of wax degraded by the bacteria will be more than 0.777%.

Integration of all the isolates from the two soil sample sources into the enzyme scour bath gave considerably high values of the degraded wax as shown in Table 4.5. The values, ranging from 0.710% to 0.747% can be related to those recorded under caustic/pectinase with surfactant, 0.72% and 0.844% respectively. These results can also explain that the isolates played a similar role as the chemical surfactant as explained by Tzanko (2001).The isolates can hence be treated as bio-friendly and economical bio-surfactants that canbe substitute to the chemical surfactant(Jaysree*et al*., 2011).

5.3 BACTERIAL CELLS ENZYME PRESENCE INVESTIGATION

The bacteria cells lysis tests indicated that the nutrient agar suspended bacteria pellets control streaks,gave relatively thick lines of action on the paraffin wax indicating that the bacteria only acted on the wax as a supplement and not as a source of food for the bacteria. The lysis buffer suspended bacteria pellets however gave comparatively thin lines of action on the paraffin wax, meaning that the bacteria cells relied solely on the wax for their nutrients hence the slow rate of action. Consequently, the supernatants from all the isolates did not exhibit any action on the wax, explaining the absence of any protein/enzyme in the bacteria isolates as shown in figure 4.10.

CHAPTER 6CONCLUSION AND RECOMMENDATIONS

This chapter gives the summary of the entire research in relation to the results that were obtained. It also gives the recommendations in affiliation with the related previous research works as a way forward in the cotton bio scouring field.

6.0 CONCLUSION

It can be concluded that, bacteria isolates cultured and isolated from cotton gin trash has activity on cotton fibre wax as a source of hydrocarbon. It was also demonstrated that the isolates can feasibly be integrated into the pectinase, which would enhance the enzyme's capacity to destabilize the cuticle waxy layer from 0.267% to 0.747% of the percentage average wax degraded as discussed above.It can also be drawn that the isolate's action on the cotton fibre wax is highly dependent on the optimal temperature, treatment time and pHcombinations as illustrated by Figure 4.8.

6.1 RECOMMENDATION

Environmental consciousness being one of the major concerns for the textile industry, there is increasing need of replacing the chemical processes with bio- preparation techniques. As it has been demonstrated, chemical surfactants, which have been found to be very important in the cotton fibre impurities/wax removal can successfully be replaced with hydrocarbon degrading bacteria isolates from cotton gin trashes. These isolates can therefore be recommended for integration with the alkaline pectinase, to enhance its capacity to remove cotton fibre wax as their process parameters have been found to match. Such integration would in turn result into more absorbent fibres which would facilitate easier downstream processing of the fibres.

Cutinase have been recommended for pectinase enzyme integration for enhanced cotton fibre wax removal based on the argument that it can degrade wax within fifteen minutes. However due to the enzyme's cost implications, the isolates can be more economical for the integration with the pectinase, given that they are cultured from cotton gin trash wastes hence less expensive.

The following can be future's prospective research areas in this line.

i. Study the bacteria isolates strain type for characterization.
ii. Evaluate the isolatesconcentrations optimization during their integration with the pectinase enzyme and the impact on the amount of cotton fibre wax degraded
iii. Evaluate the effect of pectinase and the isolates assimilation on fibre properties such as strength, absorbency and amount pectin removed.
iv. Evaluate the effect of introducing mechanical action on the pectinase/isolates cotton fibre scour process treatment time and establish the impact on the amount of fibrewax degraded.

This thesis research publication articles are given in Appendices 3 and 4.

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APPENDICES

1. KIBOS AND KITUI BACTERIA ISOLATESPLATES

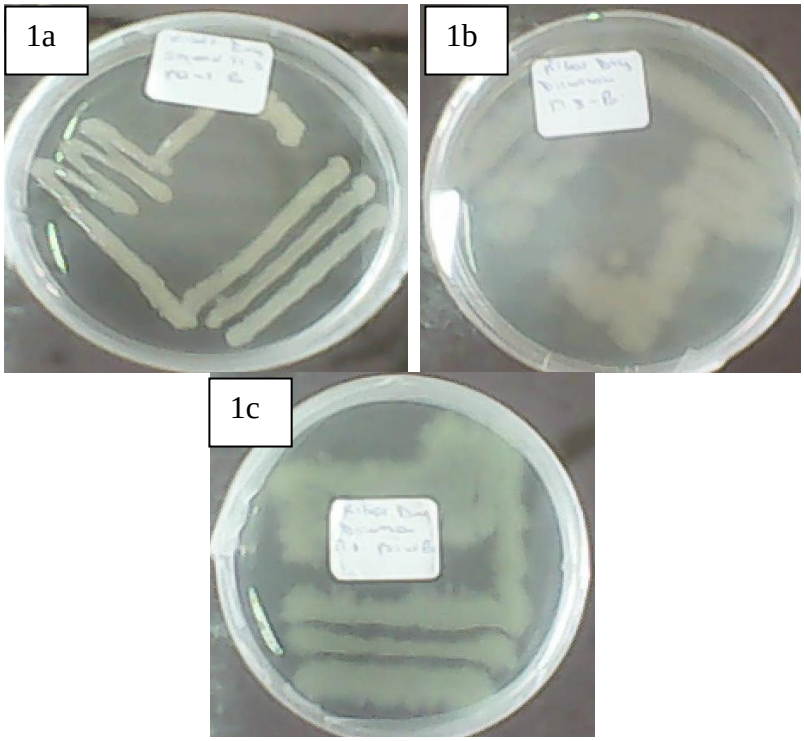

Figure 1a/1b/1c: Respective Kibosgin trash white, yellow and blue bacteria isolates plates

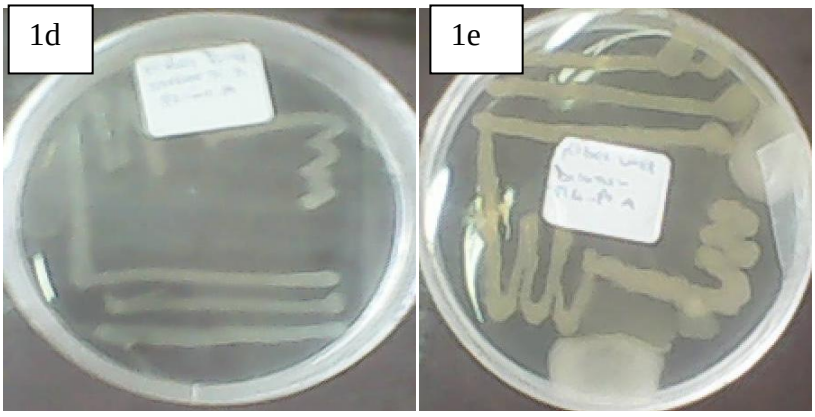

Figure 1d/1e: Respective Kitui gin trash white and yellow bacteria isolates plates

2. BACTERIA ISOLATES WAX ACTIVITY INDUCTION PLATES

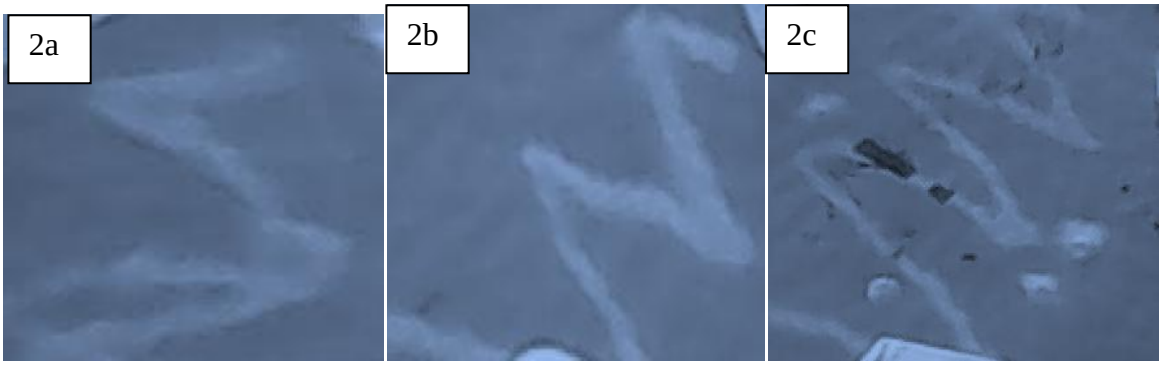

Figure2a/2b/2c:RespectiveKibos white, yellow and blue isolates wax activity induction plates

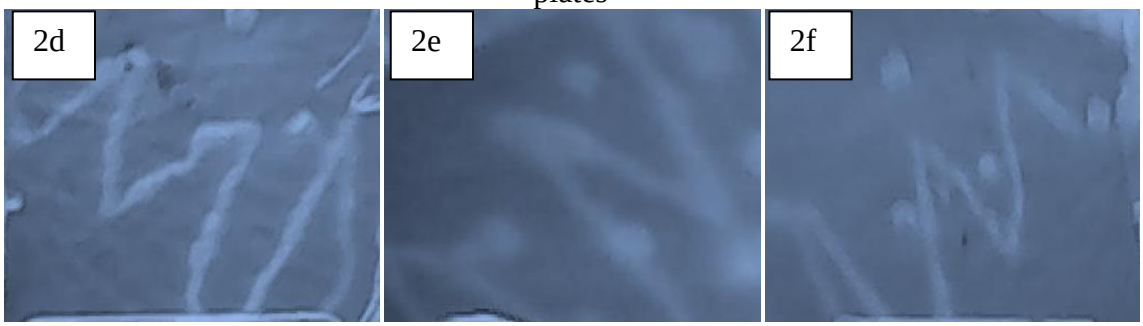

Figure 2d/2e/2f: Respective Kituigin trash white, yellow and blue isolates wax activity inductionplates

3. THESIS PUBLICATIONS ATTACHMENTS