European Masters in Textiles Engineering

E-Team

Bio-active anti-mosquito Personal Protective Equipment (PPE):
Electrospinning and fiber extrusion

Ciera Lucy Wanjiru

Promoter: Prof. Lieva Van Langenhove

Tutors: Prof. Karen De Clerck

Dr. Vincent Nierstrasz

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Masters dissertation submitted in partial fulfillment of the requirements
for the degree of Masters of Science, Textiles Engineering

2009-2010 Academic Year

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Promoter,   Author,

Prof. Lieva Van Langenhove   Ciera Lucy Wanjiru

Signature: Signature:

Date: 14.06.2010   Date: 14.06.2010

Acknowledgment

Summary

Integrating bio-active materials into polymeric structures to add functionality to the existing textile materials is an interesting research subject to study. The aim of our research was to embed bio-active anti-mosquito spores into nanofibrous structure via electrospinning technique and to integrate the same spores in filament fibers via fiber extrusion (melt spinning). Process parameters were optimized in order to maintain stable processes. The end application of these materials was to make Personal Protective Equipment (PPE) to protect against mosquito bites for use by professional travelers who travel to mosquito prone areas in the course of their duty. Light microscope, SEM and EDX techniques were used to analysis the samples for evaluating the quality of the nanofibers and the non woven structure as well as the distribution of spores inside the fibers. Results showed that we succeeded in embedding the spores into the nanofibrous structures as well as in extruded fibers. However, we used a "cocktail" *bacillus sp.* spores that had some floating and sinking spores. The spinning solution was filtered before electrospinning but we were not able to determine how much spores were filtered out.

Keywords; Personal protective equipment, Bio-active anti-mosquito, Electrospinning, fiber extrusion, professional travelers.

Extended Abstract

Bio-active anti-mosquito Personal Protective Equipment (PPE): Electrospinning

Ciera Lucy Wanjiru

Supervisor(s): Prof. Lieva Van Langenhove, Prof. Karen De Clerck, Dr. Vincent Nierstrasz, ir. Sander De Vrieze.

Abstract

This research work was aimed at embedding bio-active anti-mosquito spores into nanofibrous structure via electrospinning technique. The intended results were to add Mosquito repellency functionality in Polyvinyl Alcohol (PVA) nanofibrous structure. These materials were to be used in making Personal Protective Equipment (PPE) to be used by professional travelers. The polymer used in this experiment was (PVA) dissolved in de-ionized water. Spores concentration of 15% was the most optimal for electrospinning. Electrospinning parameters for the experiment were 1 ml h^{-1} flow rate, 15 cm deposition distance, 7 wt% polymer solution and 17 kV. Light microscope, SEM and EDX techniques were used to analysis the samples for evaluating the quality of the nanofibers and the non woven structure as well as the distribution of spores inside the fibers.

Introduction

Electrospinning is a unique technique that produces polymeric fibers with a diameter ranging from several nanometers to a few micrometer. An electric charge is used to draw fibers from an polymer liquid or melt. It is similar in characteristics with electro spraying and conventional dry spinning of fibers. It is a comparatively low cost process with a relatively high production rate (Young-Kyou et al., 2007). Ramakrishna et al. (2005) discuss the simplest form of electrospinning process that involves two electrodes, a polymer dissolved in a suitable solution, a syringe with a pump that holds the polymer solution and a DC voltage supply which is in kilovolts range. The polymer solution drops from the needle tip and a high voltage supply is provided so that the polymer jet is electrified which results to nanofibers (Duan and Xie, 2005). The fibers are then collected in the form of a web on a grounded collector. The stability of the process, fiber formation and fiber morphology are influenced by ambient conditions, polymers solution parameters and process conditions.

Materials

PVA (Mowiol 40-88) obtained from Kuraray in Europe was used. A "cocktail" of Bacillus *sp.* spores were obtained from Devan in Belgium. Filter papers with a pore size of 7 and 50 micrometers (Mogul SB 18 g m^{-2}) were obtained from Mogul in Turkey.

Method

PVA solution was prepared using 20 ml de-ionized water at 40 $^{\circ}$C with gentle stirring for at least 2 hours using a magnetic stir bar at room temperature. After 24 hours cooling at room

temperature, a predetermined amount of spores was added to the polymer solution and gently stirred for one hour at room temperature. Before electrospinning, the solutions were filtered using 50 micrometer filter papers except 2 samples which were filtered with 7 micrometer filter paper and 2 others which were not filtered for the purpose of comparing the distribution of spores.

In all experiments, 7 wt% solution, 15 cm distance and 1 ml h^{-1} flow rate were used. These conditions were determined from the other experiments. Spores concentrations were 0.5, 5, 10, 15 and 20 %. The experiments were carried out at humidity ranging from 25 % to 33.9 % and temperature between 20.1 $^{\circ}$C and 22.7 $^{\circ}$C room temperature. The electrospinning set up was as discussed in 3.1.1.2 and schematic set up is presented in Figure 1 (2.1).

Results and discussion

A "cocktail" of *Bacillus sp.* spores was used in this work for incorporation in the nanofibers. The dry spores formed aggregates of different sizes but when mixed with PVA solution, the big aggregates separated and formed smaller ones. Unfortunately, we didn't receive any information on the size distribution of the spores.
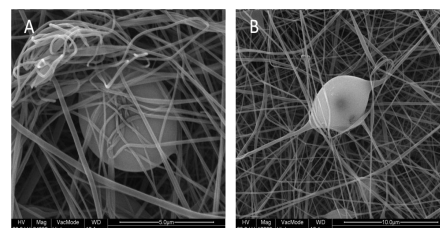
We observed that the spores of the cocktail had different behavior in solution. Some spores sank, others floated while others could have dissolved in the solution. This can be due to the fact that we had a mixture of spores which could have had different densities, thus the more dense spores sank, while the less dense floated.

To solve the problem of the big spores aggregates, we used a filter to remove all the big aggregates and other impurities (medium). After filtering the solution, we observed that no remaining spores aggregates decanted which means that all the sinking aggregates were bigger than 50 µm in diameter. Spores also changed the colour of PVA solution to brownish colour. This change of colour could have been caused by the medium which might have dissolved in the solution. The intensity of the brown colour varied with the spores' concentration. The intensity increased with the spores' concentration. All aggregates bigger than 7 or 50 µm (depending on the filter paper used) were filtered out and the filtered residue contained big aggregates and some PVA as shown in the figure below.
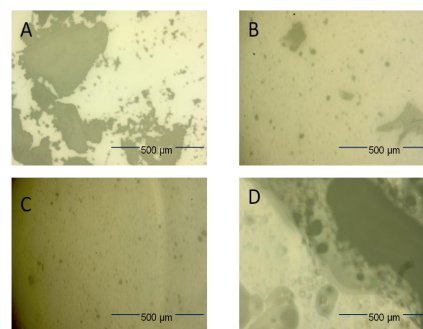
Light microscope images (A) dry spores (B) unfiltered solution (C) filtered solution (D) filtered substrate

The floating spores were electrospun and were inserted in the fiber web as shown in the figure below.
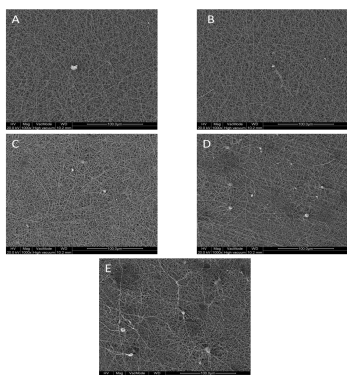
SEM images of nanofibers embedded with spores (A) 24000x (B) 1000x

We were not able to determine the amount of the spores filtered out because we did not find an appropriate method to separate the filtered substance which was a mixture of spores and PVA. PVA could have been separated by burning at high temperatures but this would have burnt out the spores. However, there were differences between the two filter sizes used. There were more spores in the 50 μm filtered solution than in 7 μm filtered solution. This is because 7 μm filter paper filtered all the aggregates bigger than 7 μm thus only few spore were left since the average diameter of dissolved spores was 51 μm. Filter paper sized 50 μm allowed more spores to pass through hence more spores were found on the nanofibers.

Different spore concentrations were electrospun to study the influence of concentration on the amount of spores on the fibers. It was evident that the number of spores on the fibers increased with concentration. The higher the spores' concentration the higher the amount of spores in the solution hence more spores will be electrospun.

SEM images showing different concentrations of spores (A) 0.5% 1000x (B) 5% 1000x (C) 10% 1000x (D) 15% 1000x (E) 20% 1000x

The table below shows diameter data of pure PVA fibers electrospun from 7 wt% polymer solution, 15 cm deposition distance, 17 kV applied voltage and 1 ml h^{-1} flow rate.

Average fiber diameter of pure PVA fibers and 7 wt% with different concentration of spores

	Pure	0.50%	5%	10%	15%	20%
Mean	360.91	274.55	310.66	324.81	272.94	319.50
Max	405.8	424.61	758.33	521.98	503.52	605.71
Min	264.06	101.45	227.34	106.15	95.06	158.26

The spores had an influence on the fiber diameter. From the table 1 above, the difference between the maximum and minimum fiber diameter is quite small for pure PVA while when spores are added the difference is broad.

To analyze the influence of spores on the fiber diameter we conducted a t-test and compared the p- values at different spore concentration with reference to the average diameter of pure PVA nanofibers. We assumed a null hypothesis that the two means are the same and hence we conducted a t-test for each concentration. Our level of significance was 0.05. The results are as shown in the table 2 below.

t-test and p-value to show influence of spores on fiber diameter

| Spores concentration	0.50%	5%	10%	15%	20%
t-test	-8.63	-3.15	-2.98	-6.75	-2.92

	0.00	0.00	*0.00*	0.00	0.00
p-value	01	22	*36*	01	44

From the t-test we found that all the p-values at the different concentrations were lower than our alpha (0.05). This implies that the means for different concentration are significantly different from the mean of the original sample. This means that statistically there were significant differences among the various concentration therefore the spores concentration had influence on the average fiber diameter. This can be due to the fact that spores do conglutinate thus resulting to much thicker nanofibers. Hence the higher the spores concentration, the higher the number of spores aggregates or the bigger the spore aggregates which influences the fibers diameters. From the SEM images, we noticed that the fiber size varied. In specific images the nanostructure was a mixture of large and small fibers, while some fibers didn't have uniform diameter along the length. We noticed some thick sections.
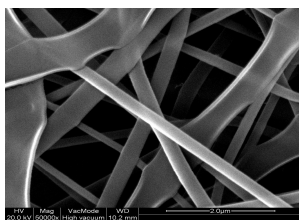
SEM image of fiber with spores showing different fiber sizes

This contributed to the differences in the average fiber diameters. SEM images of fibers with 15% spore concentration show fibers with relatively uniform diameter.
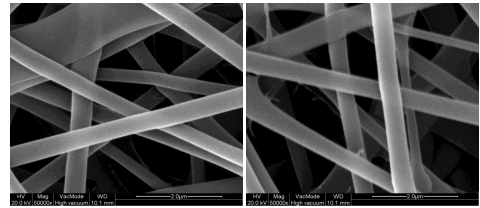
SEM images of fibers with 15% spore concentration

The relatively uniform fiber diameter resulted to the minimum average fiber diameter.

From visual analysis, spores distribution seemed to be homogenous. This was quite evident especially where a higher spore concentration was used.
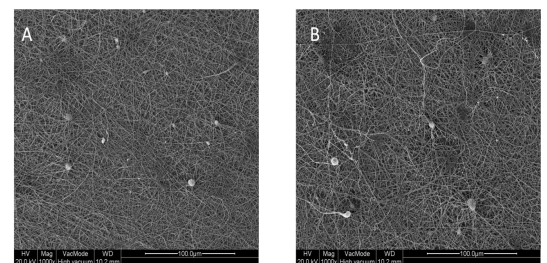
SEM images showing spores distribution at different spores concentrations (A) 15% 1000x (B) 20% 1000x

Conclusion

We succeeded in incorporating spores in nonwoven structures. Different experiments proved that a homogeneous distribution of spores inside the nonwoven was obtained. Incorporation of 15% spore concentration was optimal because for our application we are interested in a combination of small fiber diameters which will give large surface area and a high amount of functional spores. However, future study is required to get a method that can be used to determine the amount of spores that were filtered out. Since at 0.5% and 15% spores concentration recorded low average fiber diameter, more experiments need to be carried out to find out

the mosquito repellency at these concentrations. These experiments will also help in finding out whether the spores survived the process conditions.

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Abbreviations

AFM: Atomic Force Microscope

B.t.i.:   *Bacillus thuringiensis*

C.V: Co-efficient of Variation

DEET: N,N-diethyl-3-methylbenzamide

DSC: Differential Scanning Calorimetry

EDS: Energy Dispersive Spectroscopy

FE-SEM: Field Emission Scanning Electron Microscope

FTIR: Fourier Transform Infra Red

NMR: Nuclear Magnetic Resonance

PET: Polyethylene Terephthalate

PPE: Personal Protective Equipment

PVA: Polyvinyl Alcohol

SEM: Scanning Electron Microscopy

STM: Scanning Tunneling Microscopy

TEM: Transmission Electron Microscopy

WAXD: Wide-Angle X-Ray Diffraction

WHO: World Health Organization

XRD: X-Ray Diffraction

1.0 Introduction

Personal protective equipment (PPE) refers to protective garments, barrier materials and equipment. They are designed to protect people exposed to chemical, biological, mechanical, radioactive, electrical, physical or other workplace hazards (Das, 2005). Besides safety glasses, safety shoes and face shields, PPEs can be in various forms like overalls, vests, gloves, respirators, earplugs, etc. They are aimed at protecting the wearer from hazardous environment at work place (NCI-Frederick, 2008). They guarantee worker's safety which results to high productivity that translates to economic growth for the workers, company and the country as well.

Mosquitoes are small flying insects that belong to *Culicidae* family. The mouthpart of female mosquitoes has long piercing-sucking proboscis suitable for piercing the skin (Janet et al., 2010). They are nuisance and annoying insects which pose health problems to the public. There are more than 2500 different mosquito species worldwide (Fradin, 1998). However, not all mosquito species carry viruses that cause or spread diseases. Some of the diseases that misquote transmits includes yellow fever, malaria, arboviral encephilitides, dengue fever and west Nile fever. Malaria is the severest mosquito-borne disease, infecting more than 300 million people worldwide and killing around three million people each year which includes one child in every thirty seconds (WHO, 2010; Michael *et al.*, 2003).

Malaria is caused by four main parasites, *p.malarie, p.falciparum, p.knowlesi, p.ovale* and *p.ovale* (Singh et al., 2004). *Plasmodium falciparum and Plasmodium vivax has the greatest prevalence worldwide. However, P.falciparum* is responsible of causing 80% of all malaria cases and 90% of all malaria deaths *(*Mendis et al., 2001). Malaria is only transmitted by bites of female *anopheles* mosquito which bites from sunset to sun rise (Singh et al., 2004; Mendis et al., 2001). Viruses that cause dengue are transmitted through bites of *Aedes aegypti* female mosquitoes which bites during the day (WHO, 2009).

Due to globalization, many travelers from zones without mosquitoes visits tropics and sub-tropics for professional purposes thus being at the mercies of mosquito unless protective measures are taken. Out of 50 million people who travel from developed countries to developing countries annually, 8% come back home when they are ill and 26% infected are professional travelers (WHO, 2010). Dengue causes 2.1% of

the ill travelers with 20.7% of them having travelled for professional duties while malaria accounts for 6.4% with 28.5% of them being professional travelers (WHO, 2010; Freedman et al., 2006). The professional travelers include researchers, business people, volunteers, educationists, peace corps and missionaries and they need to be protected against mosquito bites.

Different strategies to prevent mosquito-borne disease through controlling mosquito bites exist. The most commonly used includes use of mosquito repellents. Repellents controls mosquito bites by reducing mosquito-man contact. They work by either diverting away the arthropods or by disorienting and distracting them thus failing to bite the person (Oyewole et al., 2008; Guptark,1994). Different repellents both synthetic and natural products are been used to control mosquitoes bites. DEET (N,N-diethyl-3-methylbenzamide) is one of the popular synthetic repellent being used today. It has and still is a golden standard for development of many insect repellents (Fradin,1998). It is used by about 200 million people annually and more than 8 billion doses have been used for the past 50 years (Vincent et al., 2009). It has remarkable repellency but is said to be toxic on the skin and the nervous system when used incorrectly and over a long period of time (Xue et al., 2007).

Permethrin is another popular synthetic mosquito repellent. It is a synthetic pyrethroid that originated from crushed daisy *chrysanthemum cinerariifolium* dried flowers. It is a contact insecticide that acts as a neurotoxin that function by affecting the neuron membranes caused by prolonging sodium channel activation resulting to knockdown or death of the insect. It has different mechanisms for fighting insects which includes knockdown, killing, residual activity and hot-feet effect (Xue et al., 2007). Permethrin is incorporated on PPE through impregnation. It has been impregnated on bed-nets, tent walls and clothing. However, its efficacy is limited to a certain period of time after which impregnation is necessary. Dong et al. (2007) advices, exposure to pyrethroid can result to asthma-like reactions and contacting dermatitis. Acute inhalation may result to sneezing, nausea, headache, nasal stuffiness, tremors, itching and burning sensations, swelling and facial flushing. Go (1999) also notes, pyrethroids disrupts endocrine system which can greatly affect the sexual and reproduction development. It can also interfere with the immune system and can increase the chances of breast cancer since they contain man-made xenoestrogens that can lead to an increase of the amount of estrogen in the body (Sarah et al., 2002).

In recent years, use of biodegradable and probably environmental friendly insects repellents of natural origin has achieved a renewed attention for control of vectors that causes diseases. Society is currently demanding for alternative insecticides that will be safe to both human and environment (Weidong and Robert, 2009). These natural repellents come from plants and other biological sources. Plants have different chemicals that are useful in the fight against mosquitoes. These chemicals can be named as toxins, repellents, growth regulators and feeding deterrents (Mattingly, 1965).

Insects, bacteria, virus and other micro-organisms have been proved to repel mosquitoes. Some *Bacillus sp.* are pathogenic to certain mosquitoes. It is used as an effective larvicidal insecticide against *Culex, Psorophora, Aedes vexans* and *Culiseta,* although its effectiveness against the larvae of *Aedes* species varies. However it is not effective against *Aedes aegypti* and *Aedes albopictus* (Lacey, 1986)*. Bacillus* are highly toxic to their target insects, are safe, can be mass produced and they can be easily transported, stored and applied. They bind on the receptor cells of the insect causing disruption in the mosquito's gut (Ffrench-Constant et al., 2003; McCoy, 1987). However, efficacy of natural anti-mosquitoes is much lower than that of synthetic products. Hence a need of a novel anti-mosquito arises.

The intended anti-mosquitoes should provide 100% protection for a sufficient long period of time withstanding about 100 washes without losing its efficacy and must be safe to both man and the environment. In order to develop a successful bio-active anti-mosquito textile based PPE, the chosen anti-mosquito must be integrated on the textile. Different methods can be used to embed the bio-active products on the fabric. This work will involve incorporating bio-active materials on fibers during the process of electrospinning and fiber extrusion. The task requires optimization of various process parameters which will help us achieve our goal.

1.1 Electrospinning

Electrospinning is a unique technique that produces polymeric fibers with a diameter ranging from several nanometers to a few micrometer. An electric charge is used to draw fibers from a polymer liquid or melt. It is similar in characteristics with electro spraying and conventional dry spinning of fibers.

1.2 Fiber extrusion

Fiber extrusion is a technique that produces synthetic fibers by forcing a thick, viscous solution through the spinneret holes to form continuous polymeric filaments.

1.3 Problem statement

Mosquito–borne diseases are putting the public health at risk if no mosquito and mosquito bite control measures are taken. Malaria alone is responsible of about 3 million deaths with 300 million people being infected worldwide, while 2.5 billion people are at risk of dengue with about 50 million dengue infections worldwide annually (Mark et al., 2002; Lynda, 2008; WHO, 2010). About 3 billion more people are expected to be at a risk of dengue by 2085 (Hales et al., 2002). Over 125 million travelers visits more than 100 tropical countries annually for different purposes (WHO, 2010). These traveler are at a high risk of contracting arthropod-borne diseases like malaria and dengue. To fight these vector-borne diseases travelers requires some personal protection equipments to protect against mosquito bites.

Despite the efforts to control mosquito borne diseases through insecticides, vector control and drug treatment, an increase in these cases has been noted. This increase can be attributed to insecticides and drugs resistance and socio-economic development (Chen et al., 2007). Anti-mosquito textiles in form of insecticidal impregnated bed-nets and clothing have proved to be helpful in mosquito bite control. However, the insecticides used to treat these textiles are synthetics products, which are associated with certain toxicity and their efficacy is limited to a certain period of time after which they must be re-treated. This situation leaves a technological as well as a scientific gap for safer anti-mosquito textile products with efficacy that can last longer without any retreatment.

1.4 Objectives

The main goal of this research work was to incorporate bio-active anti-mosquito bacteria spores in fibers through the process of electrospinning and fiber extrusion. However, bacteria with this activity are yet to be identified. To achieve this goal, different experiments were carried out whose objective was the creation of a functionalized anti-mosquito textile material. To complete this objective, following steps were followed:

- Develop optimal parameters for electrospinning polyvinyl alcohol (PVA) dissolved in de-mineralized water with an optimization towards the end application.
- Make a model system for functionalization with electrospinning by using aluminium oxide.
- Analyze the possibility of introducing bio-repellents in the electrospinning solution and the best parameters for the process.
- Explore ways of introducing the bio-repellents in fiber extrusion process with an optimization towards the end application.

2.0 Literature review

2.1 Electrospinning Process

Formhals (1934) patented the process of electrospinning which is a unique and direct method of developing polymeric nanofibers with a diameter that range from nano to a few microns. These fibers are made from a jet of polymer solution or polymer melt that has been charged electrically. It is a comparatively low cost process with a relatively high production rate (Young-Kyou et al., 2007). Ramakrishna et al. (2005) discuss the simplest form of electrospinning process that involves two electrodes, a polymer dissolved in a suitable solution, a syringe with a pump that holds the polymer solution and a DC voltage supply which is in kilovolts range. The polymer solution drops from the needle tip and a high voltage supply is provided so that the polymer jet is electrified which results to nanofibers (Duan and Xie, 2005). The fibers are then collected in the form of a web on a grounded collector.
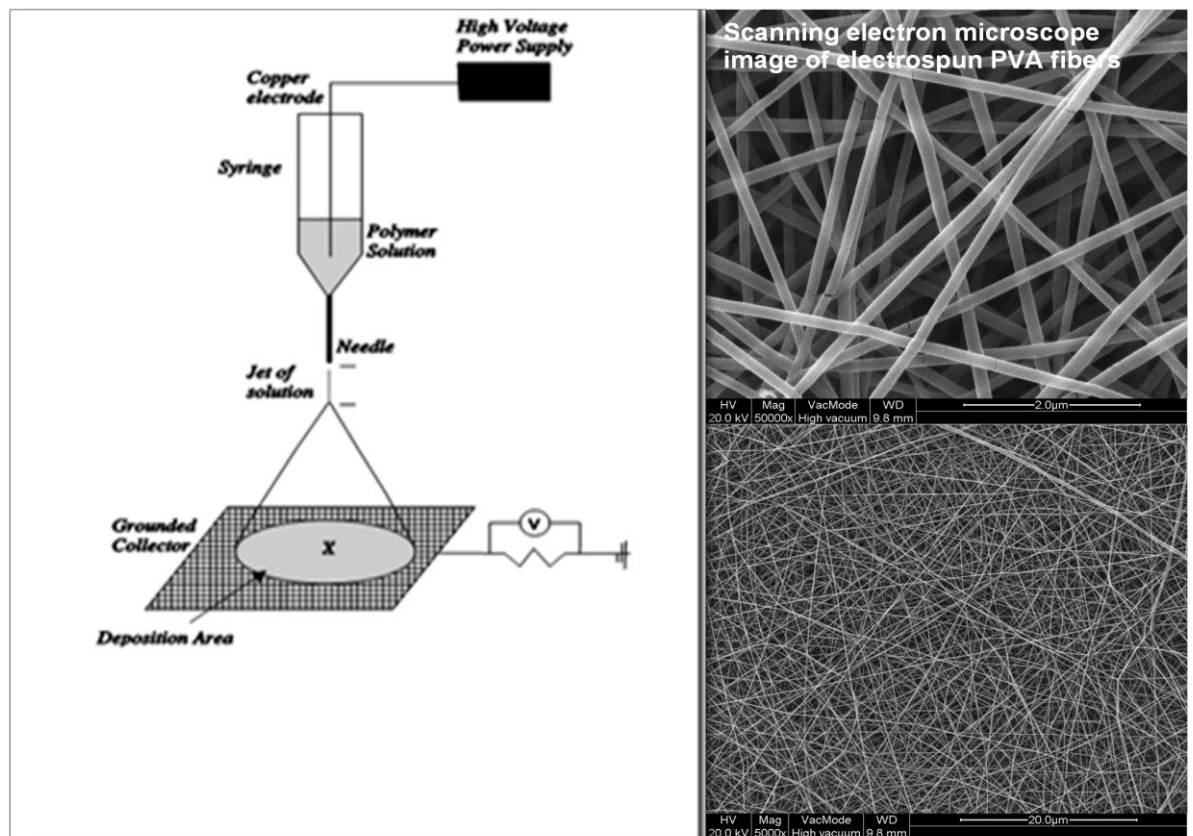
Figure 1 Schematic diagram of the Electrospinning process (Source: Koski et al., 2004)

2.1.1 Parameters that influence Electrospinning process and fiber morphology

The stability of the process, fiber formation and fiber morphology are influenced by three main parameter groups: ambient conditions, polymers solution parameters and process conditions.

2.1.1.1. Polymer solution

For electrospinning to take place, the polymer must be melted or dissolved in a suitable solvent to make a polymer solution. The quality of the polymer solution is influenced by the solvent used, polymer molecular weight, viscosity, surface tension and conductivity. These solution parameters have a great influence on the stability of the process and the fiber morphology. They are so connected such that change in one parameter affects all the other parameters.

Choosing a suitable solvent that can dissolve the polymer is vital for the success of the spinning process. The solvent should evaporate quickly enough so that when the fibers get to the collector they can be able to maintain their integrity. However, the solvent should not evaporate extremely fast in order to give enough time for the fibers to harden up (Ramakrishna et al., 2005). The surface tension as well as the viscosity of the solvent should neither be too small to a point that the polymer solution flows freely from the needle nor be too big to hinder formation of a jet (Duan and Xie, 2005).

Solubility of a polymer in a specific solvent influences the resultant fiber morphology (Wannatong et al., 2004). Molecular weight of polymers influences their solubility. Solubility of high molecular weight polymers is relatively poor due to size difference between polymer and solvent molecules. This can also be attributed to long and strong molecular chains with intermolecular forces between them hence solvent molecules diffuse slowly into the bulk polymer (Seeram et al., 2005).

Polymer solution viscosity has great influence on the stability of electrospinning process and the morphology of the nanofibers. Viscosity is influenced by the extent of entanglement of polymer molecular chain in the solution (Won Keun et al., 2005). Many experiments have proved that low viscosity results to beaded fibers while high viscosity gives smooth and uniform fibers. At low viscosity, entanglement of molecular chain is low which results to formation of polymer particles (beads) instead of fibers (Therona et al., 2004). Every polymer solution requires a given minimum viscosity that can generate beadless fibers. However, there is a certain maximum

viscosity value at which spinning can never occur. Research has proved that there is an influence of initial concentration on the resulting fiber diameter. Increase in concentration results to increase in average fiber diameter. High viscosity can also clog the needle tip as a result of drying out of the droplets at the tip. Solution viscosity can be manipulated by varying polymer solution concentration or polymer molecular weight (Ramakrishna et al., 2005).

Successful electrospinning is guaranteed when the electric forces that electrifiy the polymer solution is high enough to overcome the surface tension of the solution. Different researchers have found out that solutions with low surface tension are good because they produce beadless and uniform fibers (Wongsasulak et al., 2007).

Electrospinning can only happen if the solution has adequate charges such that the solution repulsive charges are able to overcome the solution surface tension. Hence, increase in solution conductivity results in more charges in the solution which generates beadless and uniform fibers with reduced fiber diameter. Alcohols or salt are known to increase solution conductivity (Ramakrishna et al., 2005; Kim et al., 2005; Demir et al., 2002; Zong et al., 2002).

2.1.1.2 Process parameters

The important external features that influence the stability of the process and fiber morphology include flow rate, voltage, deposition distance and type of collector.

The most vital process parameter in electrospinning is the application of high voltage in the polymer solution. The high voltage charges the solution and it also include an external electric field. Electrospinning process is initiated when electrostatic forces in solution overcome the surface tension of the solution (Ramakrishna et al., 2005). In order to have a stable Taylor cone, a high voltage is required which is dependent on the flow rate. High increase in voltage can result to whipping/splitting motions and instability of the process. Supplied voltage together with the resulting electric field influences the acceleration and stretching of the polymer solution jet which has some effects on the morphology of the nanofibers (Kim et al., 2005).

Flow rate determines how much polymer solution will be available for electrospinning. Increase in flow rate results in an increase in fiber diameter and size of beads. This is because there will be a large amount of solution that is will be drawn out from the needle tip hence the solvent will not be fully evaporated before the fibers land on the

collector (Zhong et al., 2002). In order to maintain a stable Taylor cone, applied voltage should match the corresponding feed rate.

Zong et al. (2002) confirmed that the shape of the jet droplet may be changed by an increase in the supplied voltage which may result to a beaded fiber. Different researchers have reported that applied voltage has no significant effect on the fiber diameter. However, high voltage may cause multiple jets which generate fibers with smaller non-uniform diameters. This may be attributed to the polymer jet that is discharged with a high electrostatic repulsion that forces it to go through higher drawing stress (Katti et al., 2004; Demir et al., 2002). Applied voltage also has influence on various factors like, jet morphology (multiple or single), jet elongation level, amount of polymer solution on the needle tip, etc. All these factors affect the morphology of the fiber hence a proper balance between them is required (Kidoaki et al., 2005; Fennessey et al., 2004; Mo et al., 2004).

A lot of work has been done to study the influence of distance between the needle tip and the collector on the fiber properties (Kidoaki et al., 2005; Fennessey et al., 2004; Mo et al., 2004; Katti et al., 2004; Demir et al., 2002). A minimum distance is required to ensure complete evaporation of the solvent. The distance should be big enough to enable the solvent to evaporate in good time for fibers to form (Ramakrishna et al., 2005). Some studies shows that there is no influence of distance on average fiber diameter while others indicates a close relationship between the two. Nevertheless, long distance results to beadless fibers since the long flight time ensures complete evaporation of the solvent (Shengli et al., 2004).

In most electrospinning processes, the collector plate is made from conductive materials and is covered with conductive sheets such as aluminium or copper foil. The plate is grounded to ensure sufficient and stable potential difference between the collector plate and the source (Ramakrishna et al., 2005). In case where a non-conductive collector is used, charges from the spinning jet will accumulate on the collector which will result in the deposition of fewer fibers that are loosely packed together. On the other hand, when a conductive collector is used, charges coming from the fibers are dissipated, which attracts more fibers on the plate and the results is a closely packed nonwoven (Kessick et al., 2004). Both static and rotating collectors are available for use. However, rotating collectors favors better alignment of fibers.

2.1.1.3 Ambient parameters

Humidity, temperature, pressure and atmospheric composition are important ambient parameters that affect the structure of the nanofibers and stability of electrospinning process. However, only few researchers have studied the impact of these parameters on electrospinning process as a whole. The capacity of effect of ambient parameters on the structure of fibers lies greatly on the type of solvent used. Variation of these parameters can generate interesting electrospun nonwoven materials.

Humidity in the environment is a critical ambient parameter which can affect the polymer solution during electrospinning process. Electrospinning in high humidity environment can results to water condensing on the surface of the generated fibers which will have some effects on the fiber morphology. Polymers dissolved in volatile solvents are the most critical to high humidity (Bognitzki et al. 2001). High humidity may result in generation of circular pores on the surface of the fibers. These pores increase in size with an increase in humidity until large, uneven shaped structures are formed. Humidity in the environment also has some influence on evaporation rate of the solvent in polymer solution. At low humidity, the needle tip is likely to clog very fast because there will be no balance between the drying of the volatile solvent and removal of the solvent from the needle nozzle (Ramakrishna et al., 2005).

2.1.2 Characterization of electrospun fibers

Characterization of electrospun fibers requires different instruments. In most literature, Scanning Electron Microscope (SEM) is the most popular instrument used for characterizing fiber diameter and general morphology of the nonwoven structures (Koski et al., 2003; Shengli et al., 2004; Ramakrishna et al., 2005; Duan and Xie, 2005). Other instruments that can be used to characterize fiber diameter and morphology include Atomic Force Microscope (AFM), Field Emission Scanning Electron Microscope (FE-SEM), Scanning Tunneling Microscopy (STM) and Transmission Electron Microscopy (TEM) (Frank, 2003). Although these instruments use different operating principles, they have one thing in common; they all generate a highly magnified image of the fiber surface or the bulk of the sample. Energy Dispersive X-Ray (EDX) is normally used to point out the element composition. They analyse samples near surface element and give an estimate of their proportion at various positions hence giving a general mapping of the sample (Koski et al., 2003).

Degree of crystallinity and thermal properties has been characterized by a number of researchers using Differential Scanning Calorimetry (DSC), TEM and X-Ray Diffraction (XRD). Other techniques of characterizing the molecular structure of nanofibers includes Fourier Transform Infra Red (FTIR) and Nuclear Magnetic Resonance (NMR) techniques (Mohammad et al., 2007).

Few literature records experimental results of elastic properties of nanofibers. However, Kracke and Damaschke (2000) are recorded to have measured elasticity of thin gold films using AFM. Raman Spectroscope and TEM have been used by many scientists to characterize the chemical properties of different nanofibers. They provide images that can be used to determine the size of the pores and their distribution.

2.1.3 Electrospinning of Polyvinyl alcohol (PVA)

Polyvinyl alcohol (PVA) is tasteless, white or cream colored, highly compatible, easy to process, odorless, nontoxic granular powder. It is a water-soluble crystalline, hydrophilic polymer that reacts readily forming a gel by cross-linking with different agents. It is known to have excellent thermal and chemical stability but its high hydrophilicity limits its applications although fiber aggregates can be cross-linked to improve its water resistance (Koski et al., 2003). It is fully degradable with a melting point of 180–190°C and 230°C for the partially hydrolyzed and fully hydrolyzed types respectively. It decomposes very fast at temperatures above 200°C because at high temperature it can undergo pyrolysis.(Hongyu et al., 2003)

Over the past few years, a lot of work has been published on electrospinning of PVA for different applications. Different researchers makes their PVA solution differently but in most published experiments PVA powder was dissolved in distilled water at 80 °C with vigorous stirring until all the powder is dissolved then cooled down at room temperature (Li Yao et al., 2003; Koski et al., 2003; Hongyu et al., 2003). Other electrospinning parameters vary from experiment to experiment. From most experiments, the tested flow rate is between 0.2 and 2 ml h^{-1}, distance between 5 and 20 cm, concentration between 5 and 15 wt% and applied voltage between 7 and 25 kV (Pitt and Surawut, 2008; Xiao-Hong and Shan-Yuan, 2006; Hongyu et al., 2003; Li Yao et al., 2003; Koski et al., 2003; Hongyu et al., 2003).

All the researchers who have electrospun PVA nanofibers confirm that, process parameters are connected to one another such that, a change in one parameter

affects the stability of the process and the fiber morphology (Zheng-Ming et al., 2003). Researchers also agree on the point that an increase in concentration, distance or flow rate leads to a need of higher voltage in order to maintain a stable process which results to an increase in average fiber diameter. It is also evident that a low initial concentration generates fibers with beads (Surawut, 2008; Chunxue et al., 2005; Hongyu et al., 2003; Koski et al., 2003).

2.1.3.1 Characterization of electrospun PVA

Different instruments have been used by different scientists to characterize PVA nanofibers. The most popularly used are discussed below,

Almost all the researchers who have worked with electrospun PVA used Scanning Electron Microscopy (SEM) to characterize the surface morphology and fiber diameter. Working with SEM involves putting a small sample material on SEM sample holder gold coated sputter

Differential Scanning Calorimetry (DSC) has been used to analyse the thermal behavior of electrospun PVA nanofibers by researchers such as (Tong Linet al., 2006; Therona et al., 2004).

A number of researchers have recorded use of Wide-Angle X-Ray Diffraction (WAXD) to analysis crystallinity of PVA electrospun nanofibers. Won Keun et al., (2005) as well as Audrey and Loannis (2003) used Philips diffractometer. It was used together with Geiger counter connected to a computer in room temperature to analyse the crystallinity of PVA nanofibers.

Bin et al. (2002) analyzed the water absorbency of PVA fibers aggregates after swelling with an expression $Wa - \frac{(Wb - Wc)}{Wc}$ where Wa is the water uptake per gram of PVA fiber aggregate and Wb and Wc weights of PVA fiber aggregate after swelling and subsequent drying, respectively. Fibers were allowed to swell for 48 hours.

2.2 Fiber extrusion

Most of the synthetic fibers are generated by "extrusion". Extrusion involves forcing a thick, viscous solution through the spinneret holes to form continuous polymeric filaments. For extrusion to take place, the fiber forming polymers must be converted into a fluid state (Sumesh et al., 2010). This can be achieved by either melting when using thermoplastic synthetic polymers or by dissolving the polymers in a suitable

solvent incase of non-thermoplastic cellulosic (Geller, 2009). A special device known as spinneret is used to extrude polymers into fibers. It looks like a bathroom shower, having several holes through which the spinning solution is forced through. The holes are highly sensitive to impurities and corrosion thus the solution should not be extremely viscous to prevent clogging (Anodar et al., 2003; Geller, 2006).
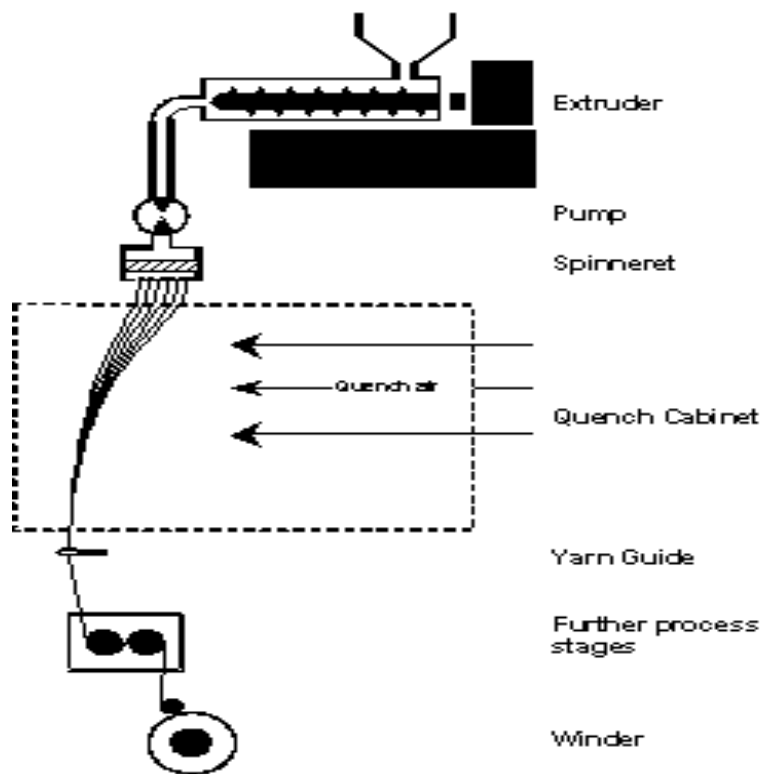
Figure 2 Schematic diagram of fiber extrusion process (Source: www.opos.de/sensors/fibrepd/schematics.gif)

As the filament fibers comes through the spinneret holes, the fibers are first converted into rubbery state and they later solidify. This explains the process of fiber spinning which involves extrusion and solidification of filament fibers (Ravirala et al., 2005). Different fibers spinning methods exists, which includes wet, melt, gel and dry spinning. In wet spinning, the solution is extruded and fibers directed in a chemical bath to solidify. On the other hand, solidification of extruded fibers can be by means of stream of air or inert gases to evaporate the solvent in the case of dry spinning. Gel- spinning exploits both dry and wet techniques since the extruded fibers first pass through a stream of air and further cooling is done in a liquid bath. In melt

spinning, the polymer pellets are melted for extrusion. The fibers solidify by cooling (Esra and Feryal, 2007; Geller, 2006).

2.2.1 Melt spinning of Polyester (PET)

Polyester a petroleum-based synthetic fiber is a common fiber that is used for different application like home furnishings, clothing, industrial fabrics and electrical insulation. Some of its many advantages includes, high tensile strength, abrasion resistant, light weight, resistant to creasing, shrinking, stretching, mildew and sunlight (Sumesh et al., 2010; Geller, 2009; Roland et al., 2007; Anodar et al., 2003). The global annual production of PET fibers and yarns in the year 2008 were estimated to be 30.3 million tones which is quite high as compared to 3.6 and 1.6 billion tones in the case of polyamide and acrylics respectively (Nierstrasz, 2010)

Polyester filament fibers are manufactured through the process of melt spinning. The polymer pellets are first melted and then forced through holes of the die (spinneret) (Geller, 2009). The molten fibers are cooled, solidified and wound on a take-up bobbin. They are later stretched to give orientation of the polymer chains along the axis of the fibers (Sumesh et al., 2010). The process stability depends greatly on the uniformity of the melted polymer and the quality of the spinneret (Esra and Feryal, 2007; Geller, 2006). Geometric parameters of the spinneret holes should be well maintained because they can affect the quality of the extruded fibers (Roland et al., 2007). A number of scientists agrees that processing of PET must be done at low shear stress as well as low temperature levels. The PET pallets must be dried well before extrusion so as to get a sufficient low water content (Ravirala et al., 2005; Walter and Torsten, 2004; Anodar et al., 2003).

2.3 Bio-active anti-mosquitoes

Bio-active anti-mosquitoes are insecticides and repellents that are from natural origins. They can be from plants, insects and microbial origin. They are probably human and environmental friendly as well as biodegradable. However their efficacy against mosquitoes is lower than of synthetic repellants.

2.3.1 Insects and micro-organism based repellents

Despite the use of synthetic insecticides for the last four decades, their toxicity and environment problems have made researchers to turn to new insect control strategies like use of microbial pathogens that has desirable properties (Murat, 1995). Bacterial insecticides for controlling mosquitoes as larvicide have been in use

for over two decades now. Nevertheless, this insecticides are in limited use due to their moderate efficacy and are expensive. However, bacterium insecticides efficacy have been improved using DNA techniques. This is done by enabling new combinations of endotoxin from various bacteria to be generated within a single strain and increasing synthesis of mosquitocidal proteins. The new strains put together Cyt proteins and mosquitocidal Cry of *Bacillus thuringiensis* with the binary toxin of *Bacillus sphaericus*. These results to increased efficacy against *Culex* species by 10-times (Federici et al., 2003). However, field application of *B. sphaericus* against *Culex* intensively can result to high resistance levels (Margalit, 1990).

Bacterium *Bacillus sp* . is pathogenic to certain mosquitoes. It is a larvicide that has been in use for mosquito control. It is active against *Culex, Psorophora* and *Culiseta* larvae although its effectiveness against *Aedes sp.* larvae varies (Lacey, 1986). *Aedes vexans* larvae is very susceptible, although *Aedes aegypti* (the mosquito that causes yellow fever) and *Aedes albopictus* are not.

Bacillus thuringiensis (B.t.i.) is a gram-positive, aerobic endospore forming bacteria that produces irregular shaped parasporal crystals that are very toxic to specific mosquito species (Murat, 1995; Barjac 1978). They are gut poison and the midgut epithelium of the affected insect larvae (Charles and Barjac 1983). The source of insecticidal properties of these bacteria is insecticidal proteins during sporulation (Federici et al., 2003). However, application of *B.t.i.* as an insecticide especially *B. thuringiensis var.* israelensis which has proved high insecticidal to mosquitoes larvae is disadvantaged by lacks persistence to acidic PH and changes in temperature (Lacey et al., 1978).

Insect neuropeptides have been used to develop bio-active insecticide since they control most functions in insects. Those functions include mating, oviposition, embryonic and post-embryonic development, osmoregulation and homeostasis. These insecticides are neurotoxin, selective and are environmental compatible which are desirable properties that cannot be found in many conventional insecticides (Gade, 2003). However, some of their setbacks include, they don't readily penetrate the cuticle, they degrade very fast in the insect's digestive system, poor solubility in aqueous and organic solutions while their peptides are environmentally unstable (Scherkenbeck, 2009; Edwards et al., 2002). These novel insecticides must be highly

toxic to the target insects, must have possibility of mass production and they must be durable.

2.3.2 Plant-based repellents

Plants have different chemicals that are useful in the fight against mosquitoes. These chemicals can be named as toxins, repellents, growth regulators and feeding deterrents (Choochote et al., 2007). However, so far there is no plant that can compete with DEET in repellency and protection time but concerns on its side effects on environment and life is stimulating further research of possible plants-based repellents. Essential oils have been tested and proved to be potential natural insect repellents (Choochote et al., 2007; Sukumar et al., 1991). Lemon-grass (*Cymbopogon excavatus*), geranium (*Pelargonium reniforme*), citronella (*Cymbopogon nardus*), neem (*Azadirachta indica*), eucalyptus (*Eucalyptus maculata*), peppermint (*Mentha piperita*), soybean (*Neonotonia wightii*) and cedar (*Juniper virginiana*) are plants that have been proved to give essential oils that can be used in plant-based insecticides (Rozendaal, 1997). These plant-based repellents offers very short protection time lasting from few minutes to not more than 2 hours (Weidong and Robert, 2009; Bernard et al., 2004; Fradin, 2002).

Extracts from *Tithona diversifolia* leaves has been used for a *long* time as traditional medicine for treating various ailments. Oyewole et al. (2008) studied its volatile oils to determine their repellency against *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus* at different concentrations. Results showed higher repellency at higher concentration against *A. Gambiae*, but repellency effects on the other tested species at different concentrations was average.

Thomas et al. (2009) studied *Kunzea ambigua*, commonly known as thick bush. From the results obtained, commercial *K. ambigua* oil was found to have a complete protection time of 49 +/- 24 min. Addition of 5% vanillin did not result to any repellency increase for *K. ambigua* oil. The authors advices that *K. ambigua* essential oil should never be advocated as mosquito repellents in areas that are prone to vector-borne disease.

Weidong and Robert (2009) records a study carried out to examine repellency of 33 different essential oils against female specie of *Culex pipiens pallens* adults. Clove leaf, clove bud, majoram and juniperberry showed the best repellency at 0.005 mg/cm^2 concentration. Analysis of Clove leaf and clove bud oils revealed isoeugenol

as the major component in clove bud oil while clove leaf was found to contain eugenol that was also present in clove bud oil. Clove bud oil was found to be the most repellent and its repellency was prolonged by adding vanillin but the authors advises that more studies are required on how to further prolong its repellency before it can replace the synthetic DEET.

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3.0 Materials and methods

3.1 Electrospinning

3.1.1 Suitable parameters for electrospinning water soluble PVA

3.1.1.1 Materials

PVA (Mowiol 40-88) was obtained from Kuraray in Europe. De-ionized water was used as a solvent. The experiment set-up consisted of a stainless steel needle which was positioned vertically on a clamp, a 20-ml syringe, a metal electrode and a grounded collector covered with an aluminum foil.

3.1.1.2 Electrospinning

PVA solution was prepared using PVA (Mowiol 40-88) water-soluble crystalline and 20 ml de-ionized water at 40 °C with gentle stirring with magnetic stir bar for at least 2 hours and then cooled for 24 hours at room temperature. The PVA solution was pumped into a 20 ml syringe; a stainless 24 cm steel needle with 1.024 mm diameter nozzle was screwed on the syringe then clamped on a stand that was above the grounded collector. The solution flow rate was regulated by KD Scientific Syringe Pump Series 100. The collector was covered with a piece of aluminum foil. A Glassman High Voltage Series EH-bron power supply was used to apply high voltage. The power supply was connected to the grounded collector and the stainless steel needle to charge the solution which produced a fine jet. Continuous polymer fibers (nanofibers) were generated as the jet cooled down or dried up, and they were accumulated on a grounded collector to form a nonwoven textile. A schematic set up of electrospinning process is presented in Figure 1.

3.1.1.3 Tested parameters

The concentration of PVA tested ranged from 5 wt% to 9 wt%. The test distances between the needle tip and the collector were 7.5, 10, 15 and 20 cm and the tested flow rates were 0.5 and 1 ml h^{-1} while the voltage ranged from 9 to 22 kV. These parameters were based on a preliminary literature study.

3.1.2 Influence of spinning parameters on fiber diameter

To evaluate the influence of the spinning parameters on the fiber diameters, the PVA solution was prepared using water-soluble crystalline PVA and 20 ml de-ionized water at 40 °C with gentle stirring for at least 2 hours with magnetic stir bar at room temperature. Cooling time and conditions were dependent on the parameter in question.

To determine the influence of cooling time on fiber diameter, the solutions were cooled for 2, 5 and 24 hours at room temperature. In the case of determining the influence of spinning solution temperature, solutions were cooled in a cooling water bath at room temperature. To determine the influence of concentration and distance on diameter, the solutions were cooled for 2 hours at room temperature.

In all the tests, we worked with 15 cm distance, 1 ml h^{-1} flow rate, voltage of 22 kV and a polymer concentration of 7 wt%. Polymer concentrations of 5, 6, 7, 8, and 9 wt% were used in tests for determining the influence of concentration on fiber diameter. The humidity varied between 33 % and 36 % while the temperature ranged from 22.4 °C to 24.3 °C room temperature. The electrospinning set up was as discussed earlier (3.1.1.2) and schematic set up is presented in figure1 (2.1).

3.1.3 Model system with aluminum oxide

3.1.3.1 Materials

PVA (Mowiol 40-88) obtained from Kuraray in Europe was used. Aluminum oxide (BUEHLER-MICROPOLISH II 1.0 MICRON) was obtained from Sigma Aldrich.

3.1.3.2 Method

PVA solution was prepared using 20 ml de-ionized water at 40 °C with gentle stirring for at least 2 hours with magnetic stir bar at room temperature. After 24 hours cooling at room temperature, 1 wt% of aluminum oxide was added to the polymer solution and gently stirred for 30 minutes at room temperature then electrospun. The collector was covered with a piece of copper foil.

Selection of parameters for this experiment was based on light microscope and SEM pictures of the previous experiments. We picked up pictures of samples without beads and with a homogeneous network of fibers. We worked with 7 wt% polymer solution, 1

ml h^{-1} flow rate, 15 cm distance and a voltage of 20 kV. The process was carried out at a humidity of 33 % and 24.2 $^{\circ}$C room temperature.

3.1.4 Incorporating spores in spinning solution

3.1.4.1 Materials

PVA (Mowiol 40-88) obtained from Kuraray in Europe was used. A "cocktail" of *Bacillus sp.* These spores were obtained from Devan in Belgium. Filter papers with a pore size of 7 and 50 micrometers (Mogul SB 18 g m^{-2}) were obtained from Mogul in Turkey.

3.1.4.2 Method

PVA solution was prepared using 20 ml de-ionized water at 40 $^{\circ}$C with gentle stirring for at least 2 hours using a magnetic stir bar at room temperature. After 24 hours cooling at room temperature, a predetermined amount of spores was added to the polymer solution and gently stirred for one hour at room temperature. Before electrospinning, the solutions were filtered using 50 micrometer filter papers except 2 samples which were filtered with 7 micrometer filter paper and 2 others which were not filtered for the purpose of comparing the distribution of spores.

In all experiments, 7 wt% solution, 15 cm distance and 1 ml/h flow rate were used. These conditions were determined from the other experiments. Spores concentrations were 0.5, 5, 10, 15 and 20 %. The experiments were carried out at humidity ranging from 25 % to 33.9 % and temperature between 20.1 $^{\circ}$C and 22.7 $^{\circ}$C room temperature. The electrospinning set up was as discussed in 3.1.1.2 and schematic set up is presented in Figure 1 (2.1).

3.1.5 Characterization

The average fiber diameters were investigated using scanning electron microscopy (SEM) images. Samples were prepared by cutting small pieces of aluminium foil covered with electrospun fibers. Before observing the samples under SEM, they were fixed on SEM stub and gold coated by Balzers Union SKD 030 equipment. At least 50 measurements per sample were recorded to determine the average fiber diameter. To point out element composition in a sample, Energy Dispersive X-Ray (EDX) was used.

3.2 Melting spinning of polyester with spores

3.2.1 Melt spinning PET with *lyophilized* microorganisms spores

3.2.1.1 Materials
The polyester pellets used in this experiment were PET-Arnite thermoplastic polyester from DSM company. Lyophilized microorganisms spores were produced by the team of Johan Mertens (terrestrial biology). Phosphorus pentoxide was from Fuka chemie company.

3.2.1.1 Method
The PET granules were grinded into particles of 0.97 µm average diameter. The grinded particles needed pre-drying to avoid hydrolysis during extrusion. They were dried in an oven at 70 °C for 4 days. They were further dehydrated in a dessicator for another 4 days. Phosphorus pentoxide was put in the space below the dessicator platform. Lyophilized microorganisms spores of 0.84 µm average diameter were homogeneously mixed with the dried grinded PET and the mixture was extruded. For this experiment 300 g of grinded PET was diluted with 0.5% of spores.

The spinning head was combined with a single screw extruder. Other spinning installation included a spin pack with the spinneret, heated godets, spinning pump and a bobbin winder for take-up with velocity of 10 m min^{-1}. The distance between the water bath and die head was kept as small as possible. The temperature of the die was set to 255 °C. The screw speed was 34 m min^{-1}. Temperatures of 239, 309, 275 °C were set for the different heating zones in the extruder. A 5-hole spinneret was used for the production of spore spiced polyester filament fibers. After extrusion, the fibers were dried in an oven at 50 °C for 2 days.

3.2.2 Melt spinning PET with *Bacillus sp. spores*

3.2.2.1 Materials
The polyester pellets used in this experiment were PET-Arnite thermoplastic polyester from DSM company. *Bacillus sp.* spores were from Devan in Belgium. Phosphorus

pentoxide was from Fuka chemie company. Filter papers with a pore size of 50 micrometers (Mogul SB 18 g m^{-2}) were obtained from Mogul in Turkey

3.2.2.2 Method

The PET granules were grinded into particles of 0.97 μm average diameter. The grinded particles needed pre-drying to avoid hydrolysis during extrusion. They were dried in an oven at 70 °C for 4 days. They were further dehydrated in a dessicator for another 4 days. Phosphorus pentoxide was put in the space below the dessicator platform. *Bacillus sp.* were grinded further using mortar and pestle so as to have finer particles. The big particles were filtered out using 50 μm filter paper. The filtered spores were mixed homogeneously with the dried grinded PET and the mixture was extruded. For this experiment 300 g of grinded PET was diluted with 0.5% of spores.

The experiment set up and process conditions were advised by the department of polymer chemistry, Ghent University. They had successfully incorporated bacteria spores in PET although they have not published any work on that. We visited Prof. Sam Verbrugghe, who explained to us their experiment set up they used. They used a twin-screw extruder with temperature distribution of 265, 270, 275, 280, 280, 275, 270 and 265 °C. However, we only had a single screw extruder in the textile department and that is what was used for this experiment.

Other spinning installation included a spin pack with the spinneret, heated godets, spinning pump and a bobbin winder for take-up with velocity of 10 m min^{-1}. The distance between the water bath and die head was kept as small as possible. The temperature of the die was set to 255 °C. The screw speed was 34 m min^{-1}. Temperatures of 239, 309, 275 °C were set for the different heating zones in the extruder. A 5-hole spinneret was used for the production of spore spiced polyester filament fibers. After extrusion, the fibers were dried in an oven at 50 °C for 2 days.

4.0 Results and discussion

4.1 Electrospinning

4.1.1 Suitable parameters for electrospinning water soluble PVA

To choose suitable parameters for electrospinning uniform and thin PVA nanofibers, we conducted several experiments with varying spinning parameters. This included flow rate, concentration of the solution, applied voltage, solution temperature, cooling time and humidity. The experiments were carried out in a span of three days, temperature ranged between 19.6 and 23.2 °C while the humidity ranged was between 33 % and 34.4 % at room temperature.

We made 12 wt% polymer solutions but found out that the viscosity was too high thus it was not possible to spin it. Kameoka et al. (2003) explains that it is normally very difficult to pump solution through the syringe needle when the viscosity is too high. This can also result to drying up of solution at the needle tip even before the spinning process begins. It was however possible to pump solutions between 5 and 9 wt%.

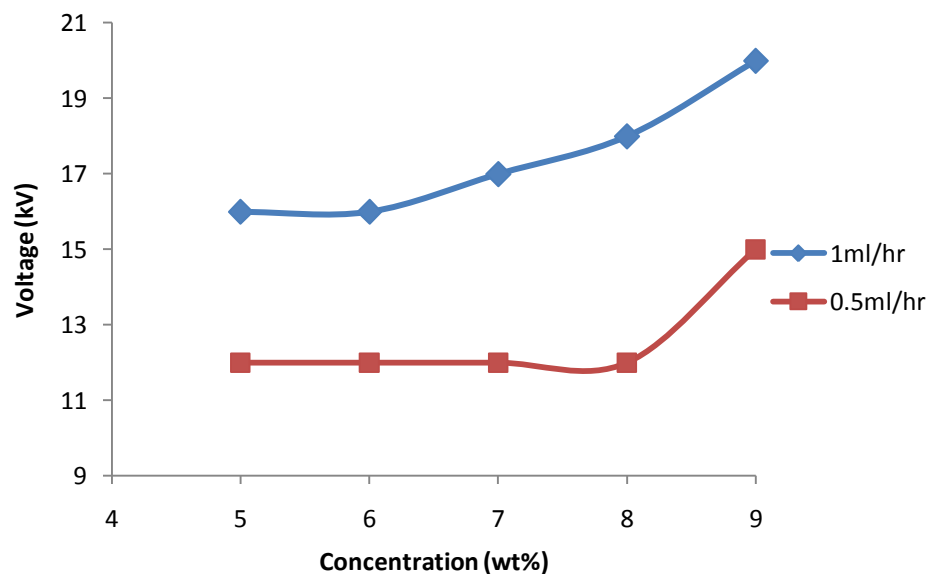The first parameter is the influence of the polymer concentration on the applied voltage.

Figure 3 Relationship between concentration and voltage at distance of 15 cm and flow rate of 0.5 ml h^{-1} and 1 ml h^{-1}

Figure 3 shows the relationship between concentration and voltage levels. At 0.5 ml h^{-1} flow rate, it was observed that the concentration of the solution did not have any influence on voltage from 5 wt% to 8 wt%. However, the 9 wt% required slightly higher voltage which remained constant at a distance of 15 and 20 cm. At 1 ml h^{-1} flow rate, slight differences in voltage were noted at different concentrations but higher voltage was required than in 0.5 ml h^{-1} flow rate. These results confirms the literature, where it is seen that with increasing concentration, the needed voltage also increases. This is necessary to overcome the visco-elastic forces and the surface tension of the polymer solution.

Another process parameter that had an influence on the applied voltage was the distance.
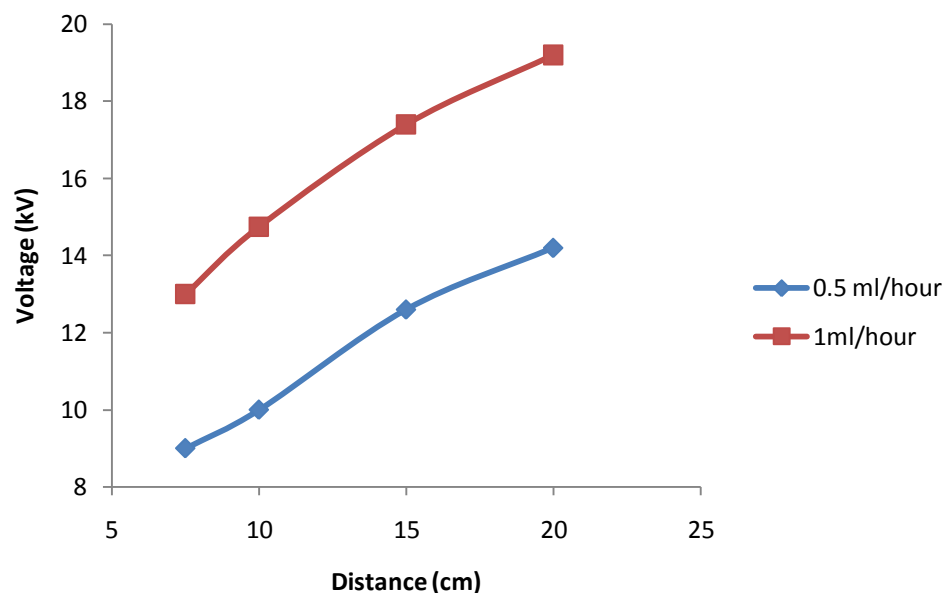
Figure 4 Influence of distance on applied voltage (0.5 ml h^{-1} and 1 ml h^{-1} flow rate)

Figure 4 shows the influence of distance between the tip and the collector on the voltage levels. The distance between the tip and the collector determines the flight time which influences the electrospinning process and the resultant fibers (Ramakrishna et al., 2005). When the distance is varied both the flight time and the electric field strength are

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directly affected. To ensure that individual fibers are formed, the jet must be given enough time to evaporate the solvent.

In both flow rates, the distance quantities showed a positive correlation with voltage at all tested concentrations. The longer the distance the higher the voltage required to stabilize the process. It was not possible to have a stable process with 9 wt% at 7.5 and 10 cm for both flow rates. At both flow rates, the 7.5 cm and 10 cm distances required almost the same voltage. With a short distance, the jet has a short distance to travel before getting to the collector thus low voltage is required. On the other hand, long distances require high voltages since the jet has a long flight before getting to the collector. However, very short distances are not good because the excess solvents on the fibers may bring about merging of fibers resulting to inter and intra layer bonding (Mituppatham et al, 2004).

Flow rate also had an influence on the stability of the process because the results show an increase in flow rate that resulted to higher voltage. Flow rate determines the amount of solution that would be available to make the Taylor cone. For a specific flow rate there is a given voltage required to maintain a stable Taylor cone.

4.1.1.1 Suitable parameters

A polymer solution property has great influence on the fiber morphology and the spinning process. For successful electrospinning, the polymer must have sufficient molecular weight to form a solution of enough viscosity (Mituppatham et al, 2004).
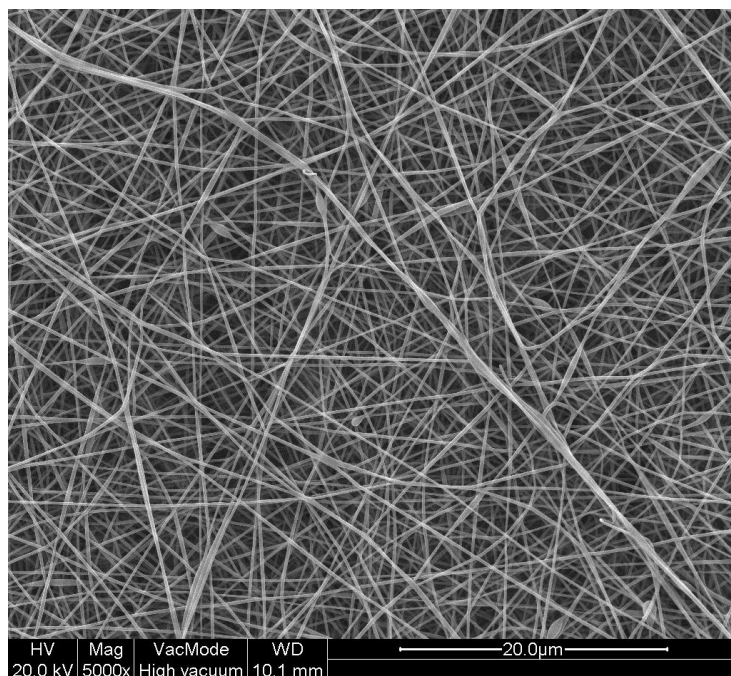
Figure 5 SEM images of 5 wt% Samples at 1 ml h^{-1} flow rate, 12 kV applied voltage and 15 cm distance (5000x)

Figure 5 shows the presence of beads on the images of 5 wt% samples. The presence of the beads is believed to be a result of low viscosity. When the viscosity is too low, not enough chain entanglements are formed to overcome the surface tension. This results to beads along the nanofibers. With increase in viscosity the polymer chain entanglement also increases and the spinning jet's charges will be capable of stretching the solution and the solvent molecules will be distributed well in the polymer chain (Ramakrishna et al., 2005).
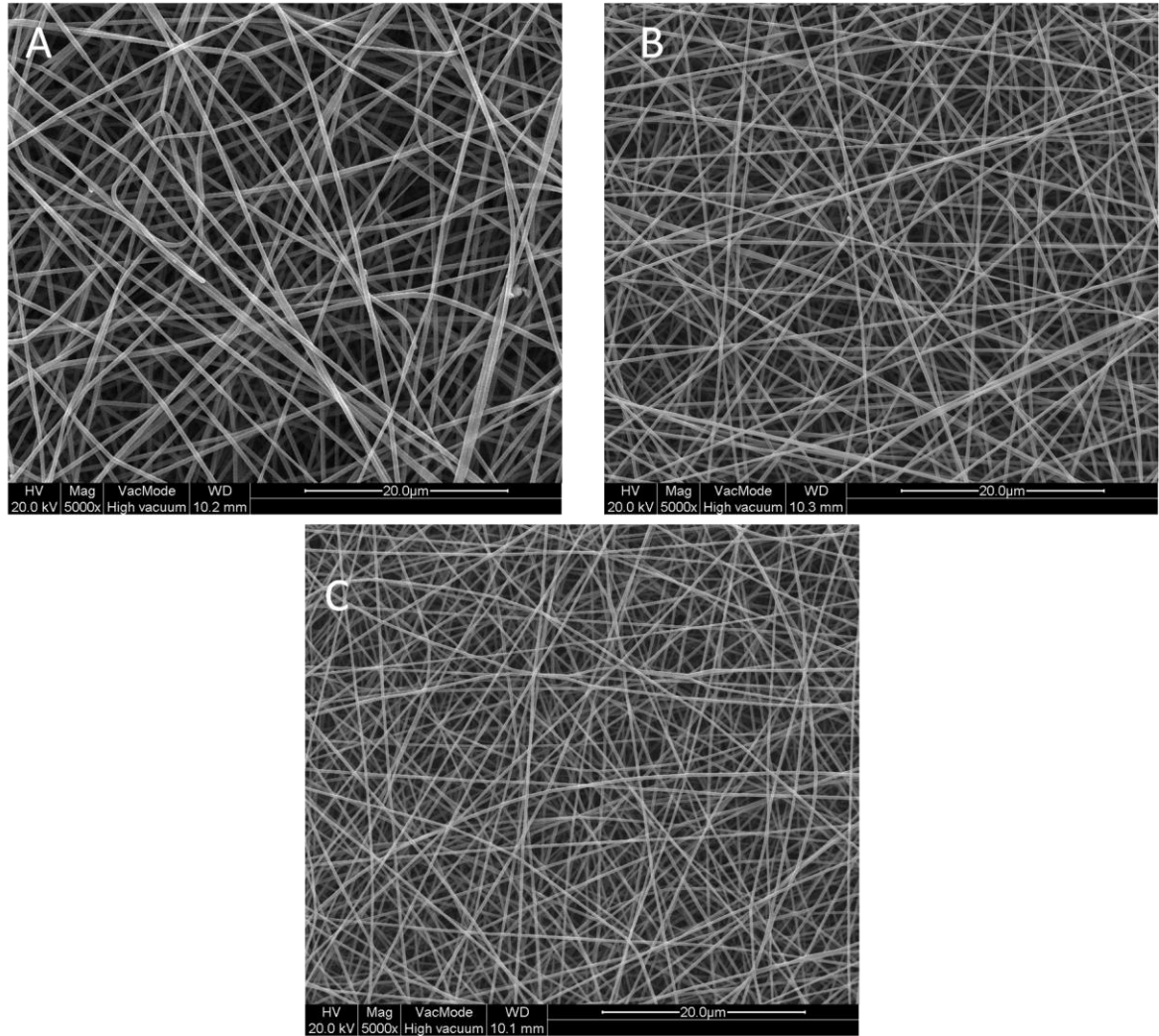
Figure 6 SEM images of 7 wt%, 8 wt% and 9 wt% fibers without beads. Samples electrospun at 1 ml h^{-1} flow rate and 15 cm distance (A) 7 wt% (5000x) (B) 8 wt% (5000x) and (C) 9 wt% (5000x)

For every polymer solution, a minimum viscosity is required to have beadless fibers (Megelski et al., 2002). However, even though viscosity influences the formation of fibers without beads, it may not determine the right concentration at which electrospun fibers are formed (Ramakrishna et al., 2005). From the experiments, samples with 7, 8 and 9 wt% were beadless and the fiber network was good although at some parameters 9 wt% didn't have stable spinning process. Their SEM images are shown in figures 6 above.

4.1.2 Analysis of fiber diameter

The objective for the application of the materials from this experiment is to make personal protective equipment (PPE). The intended PPEs are in form of heavy and light weight clothing that can repel mosquitoes. For this application materials with large surface area to volume ratio are excellent. This is because the large surface area which will be treated with insecticides will always be contact with the environment thus shielding the wearer from mosquito bites. The higher the surface area, the higher amount of bioactive anti-mosquito on the surface which results maximum protection against mosquito bites. The lower the fiber diameter, the higher the surface area.

In literature, fiber diameter increases with an increase in concentration which is influenced by viscosity. At low concentration the solution viscosity is low and there is greater polymer solubility which leads to higher stretching in solution thus having smaller diameters (Demir et al., 2002).

To check the effect of concentration, the average diameter was measured and is displayed in figure 7 below.
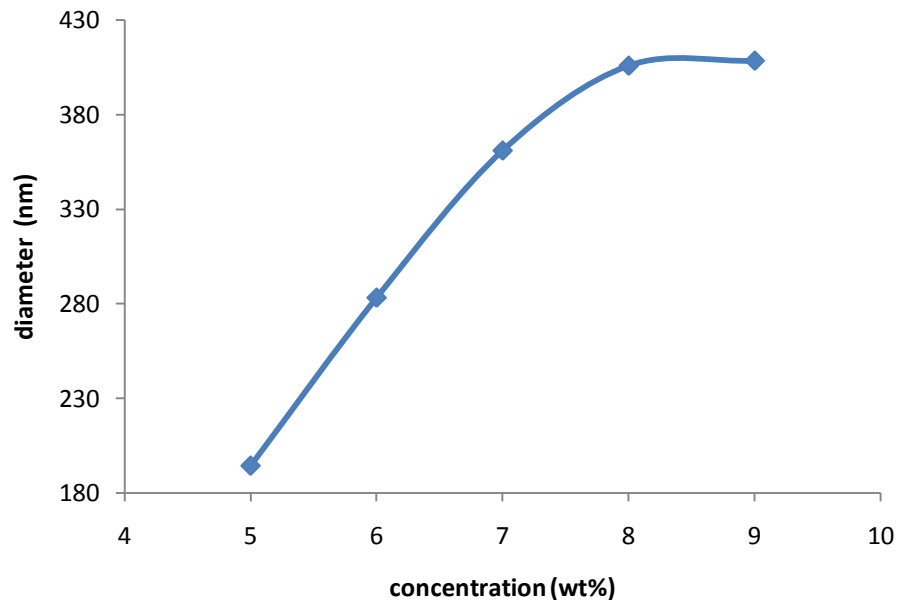

Figure 7 Influence of concentration on average fiber diameter

Figure 7 shows the trends of variation of the concentration level with increase in diameter. The process parameters for this experiment were 5, 6, 7, 8 and 9 wt%, 15cm deposition distance, 17 kV applied voltage and flow rate of 1 ml h^{-1}. The concentration levels showed a positive correlation with the fiber diameter. Between the concentration levels 5 wt% and 8 wt% the diameter was increasing linearly but only a slight increase in diameter was noted at the concentration level of 9 wt%. The average co-efficient of variation of the average fiber diameter was around 15%. The increase of the diameter with concentration was a result of viscosity. At low concentration the solution viscosity is low and there is greater polymer solubility which leads to the solution stretching more thus having smaller diameters (Demir et al., 2002).

Distance is another process parameter that can influence the fiber diameter.

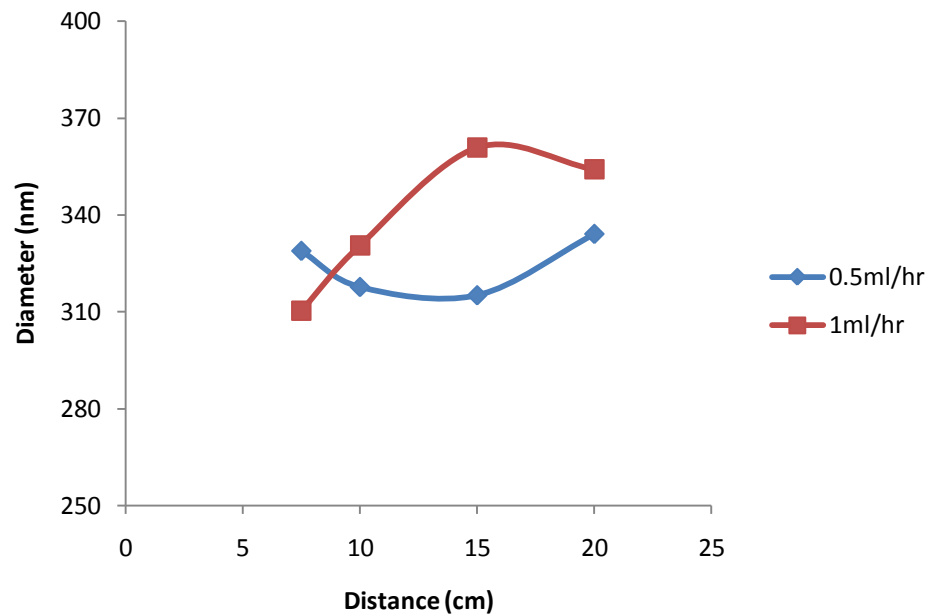Figure 8 Influence of distance and flow rate on average fiber diameter

Figure 8 shows the trends on distance against the flow rate on fiber diameter. The process parameters for this experiment were 7 wt%, 15cm deposition distance, 17 kV applied voltage and flow rate of 0.5 ml h^{-1} and 1 ml h^{-1}. It was noted that the distance had a slight influence on the fiber diameter at both flow rates. When taking the co-

efficient variation (CV) into account, which is 10% and 7.5% at flow rate 0.5 ml h^{-1} and 1 ml h^{-1} respectively, there is no statistical difference between all the samples made at the different distances. This is because the applied voltage and distance compensate each other hence distance doesn't have any significant influence on the fiber diameter. However, in some cases an increase in distance results to an increase in fiber diameter which is caused by electrostatic field strength that leads to minimal fibers stretching (Lee et al., 2004). The longer the distance, the longer the flight time for the solution which stretches it before it lands on the collector (Shengli et al., 2004). With a very high distance it is not possible to deposit fibers on the collector (Shengli et al., 2004). This shows that an electrostatic field strength limit exists. Below this limit a decrease in stretching of the solution will occur which will lead to an increase in the fiber diameter (Ramakrishna et al., 2005).

A specific study was done on the influence of the cooling time on average fiber diameter. The results on the average diameter can be seen in figure 9.
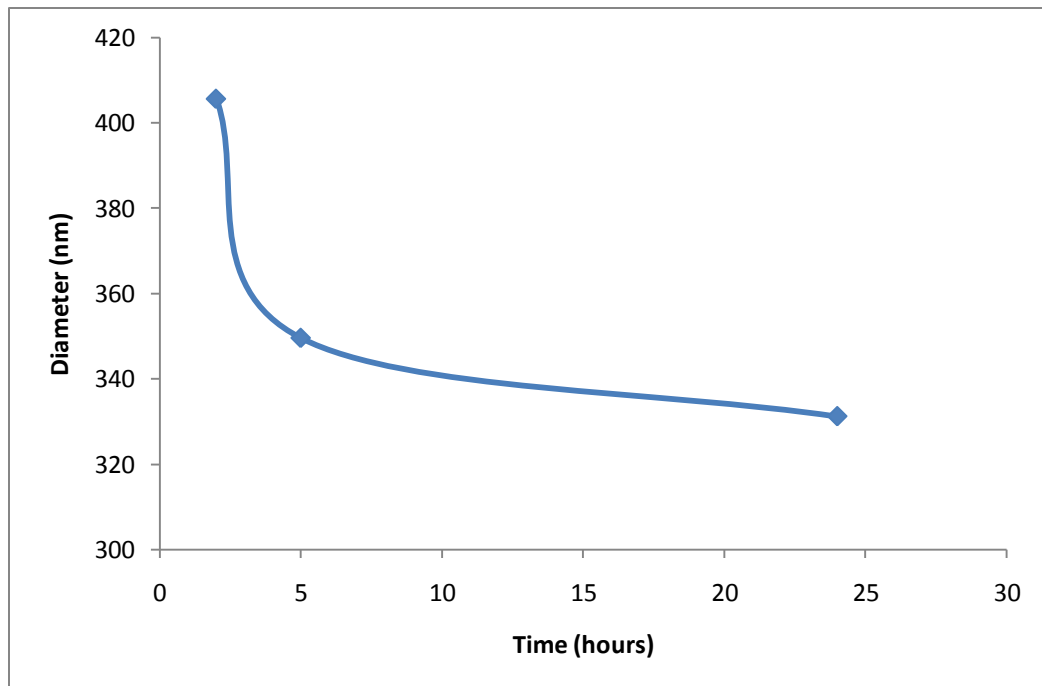

Figure 9 Influence of cooling time on fiber diameter

The results show that the longer the cooling time the smaller the diameter and vice versa. The co-efficient of variation for the fiber diameters was 10 %. Cooling for 2 hours results in a statically thicker fiber diameter than cooling for 24 hours. After 2 hours, the polymer solution has not taken an equilibrium, with the resulting fiber diameter. Therefore, we choose a cooling time of 24 hours for the other experiments since at 24 hours the diameter seems to have already taken equilibrium.

The effect of the solution temperature itself on the average fiber diameter was also studied, figure 10.
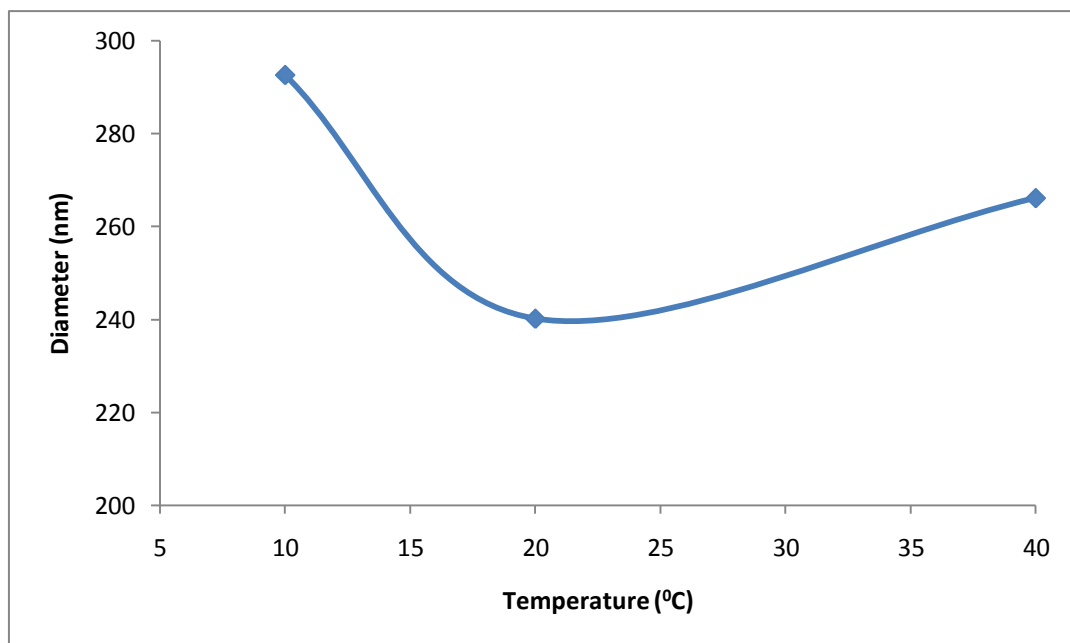

Figure 10 Influence of solution temperature on average fiber diameter

The process conditions used for this experiment were an initial concentration of 7 wt%, 15 cm tip-collector distance, 1 ml h^{-1} flow rate and an applied voltage of 17 kV. The coefficient of variation was around 12%. This means that there was no statistical significant influence of the solution temperature.

4.1.3 Conclusion

From this and previous experiments we can conclude that 7 wt% polymer solutions was the optimal polymer concentration. This is based on the fact that 7 wt%, 8 wt% and 9 wt% polymer solutions formed fibers without beads. The solution with an initial

concentration of 7 wt% have the smallest average fiber diameter hence having the largest surface area. Such materials will not only offer protection against insects but will be resistant to biological and chemical compounds in aerosol form as well (Phillip et al., 2001). The flow rate of 1 ml h^{-1} and 15 cm distance are a good set because the solvent will have enough time to evaporate and at these parameters we had uniform fibers structure. The nanofibers made with these conditions have an average fiber diameter of 361±31 nm.

4.1.4 Model system with aluminum oxide

The main objective of this experiment was to model a system to determine the possibility of electrospinning a polymer solution that has bacteria spores. Aluminum oxide was a perfect choice for the model system because it is an inert chemical and when dissolved in PVA solution it formed aggregates of almost the same diameter as the spores in solution. Aluminum oxide particles have an average diameter of 45.4 micrometer while the bacteria spores have one of 51 micrometer.

From the EDX and SEM analysis it was observed that the nanofibers contained aluminum oxide as shown by the figures 11 and 12 below.
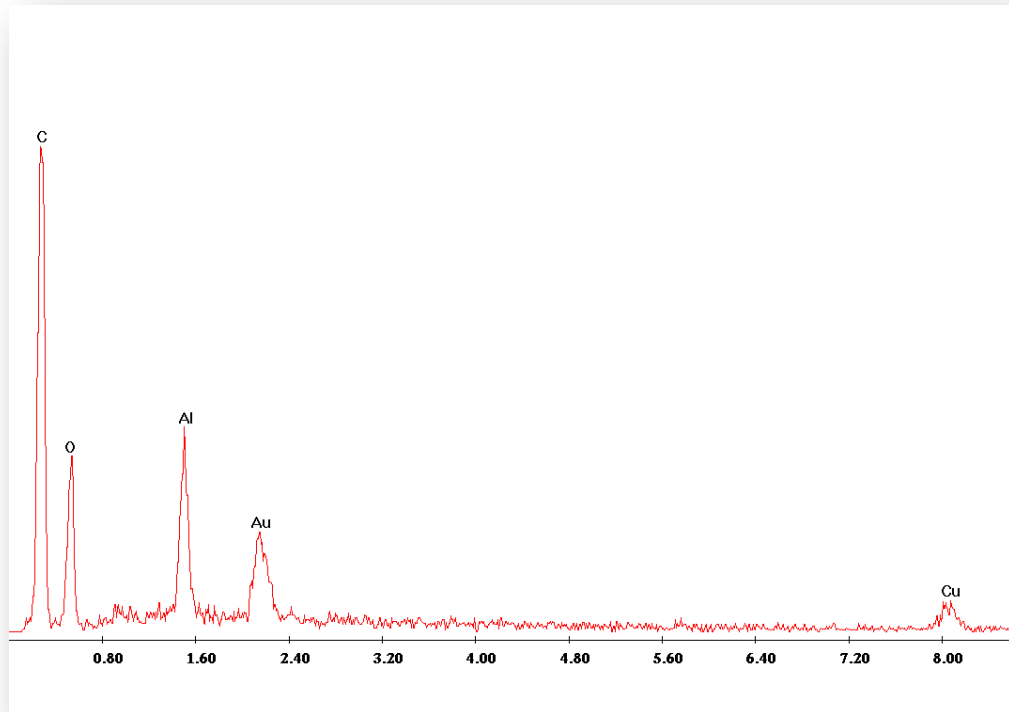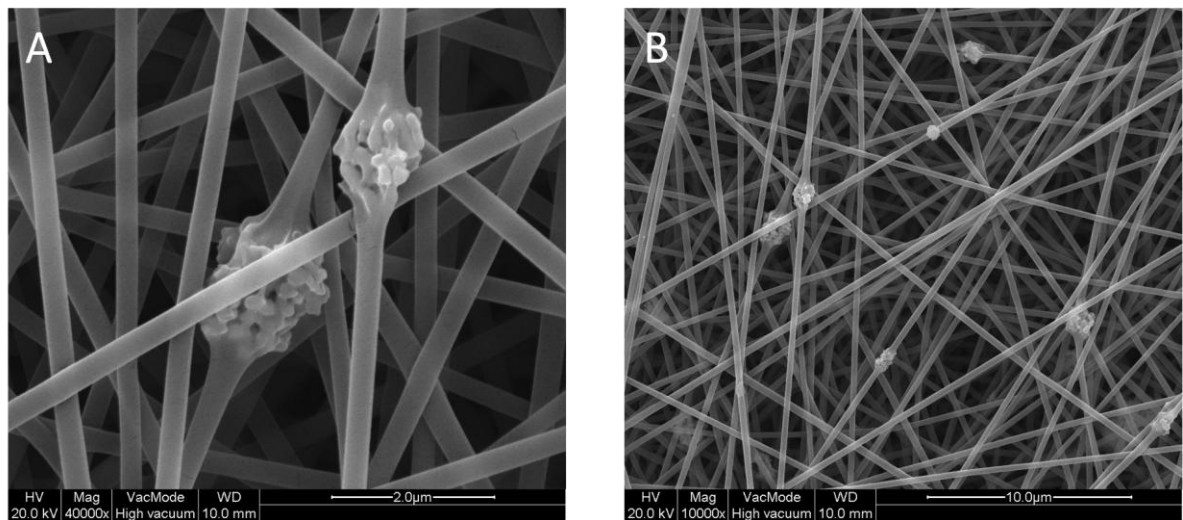
Figure 11 EDX spectrum

Figure 12 SEM images of nanofibers incorporated with aluminum oxide (A) 40000x (B) 10000x

However, from the experiment, aluminum oxide did not work out as a perfect model system because during spinning process some aggregates broke down into small sizes

unlike the spores which maintained their aggregates even in spinning. From out of this experiment, it was observed that it is possible to create a nonwoven with a homogeneous distribution of integrated micro particles.

4.1.5 Incorporating spores in spinning solution

A "cocktail" of *Bacillus sp.* spores was used in this work for incorporation in the nanofibers. The dry spores formed aggregates of different sizes but when mixed with PVA solution, the big aggregates separated and formed smaller ones. Unfortunately, we didn't receive any information on the size distribution of the spores.

We observed that the spores of the cocktail had different behavior in solution. Some spores sank, others floated while others could have dissolved in the solution. This can be due to the fact that we had a mixture of spores which could have had different densities, thus the more dense spores sank, while the less dense stayed in the solution.

To solve the problem of the big spores aggregates, we used a filter to remove all the bigger aggregates and other impurities (medium). After filtering the solution, we observed that no remaining spores aggregates decanted which means that all the sinking aggregates were bigger than 50 μm in diameter. The spores also changed the colour of the PVA solution to a brownish colour. This change of colour could have been caused by the medium which may have dissolved in the solution. The intensity of the brown colour varied with the spores' concentration. The intensity increased with the spores' concentration. All aggregates bigger than 7 or 50 μm (depending on the filter paper used) were filtered out and the filtered residue contained big aggregates and some PVA as shown in the figure 13 below.
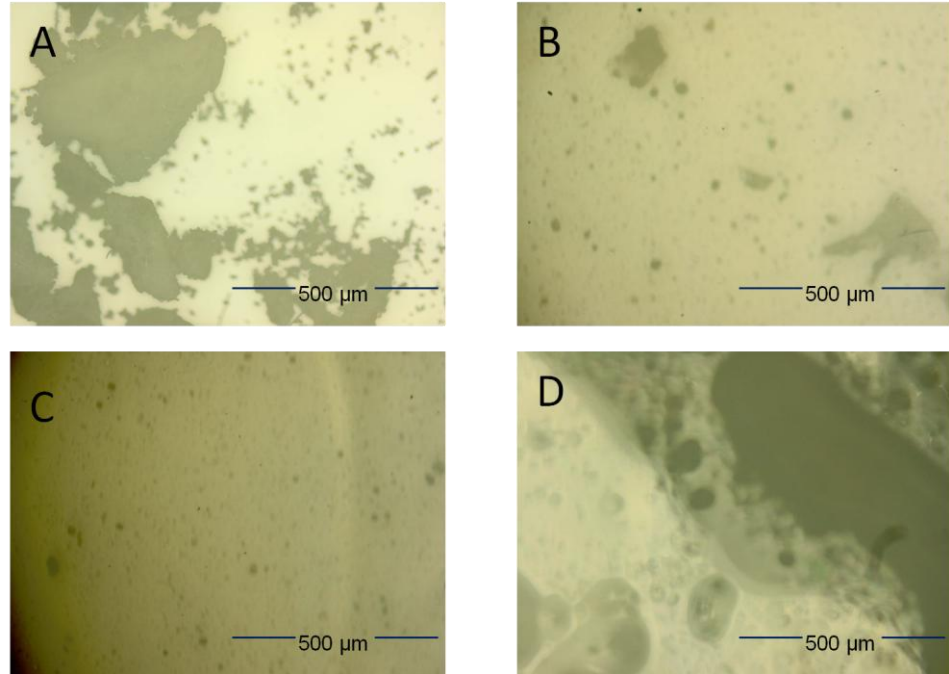
Figure 13 Light microscope images (A) dry spores (B) unfiltered solution (C) filtered solution (D) filtered substrate

The floating spores were electrospun under the conditions given above. The resulting nanofibrous nonwoven contained the spores, figure 14.
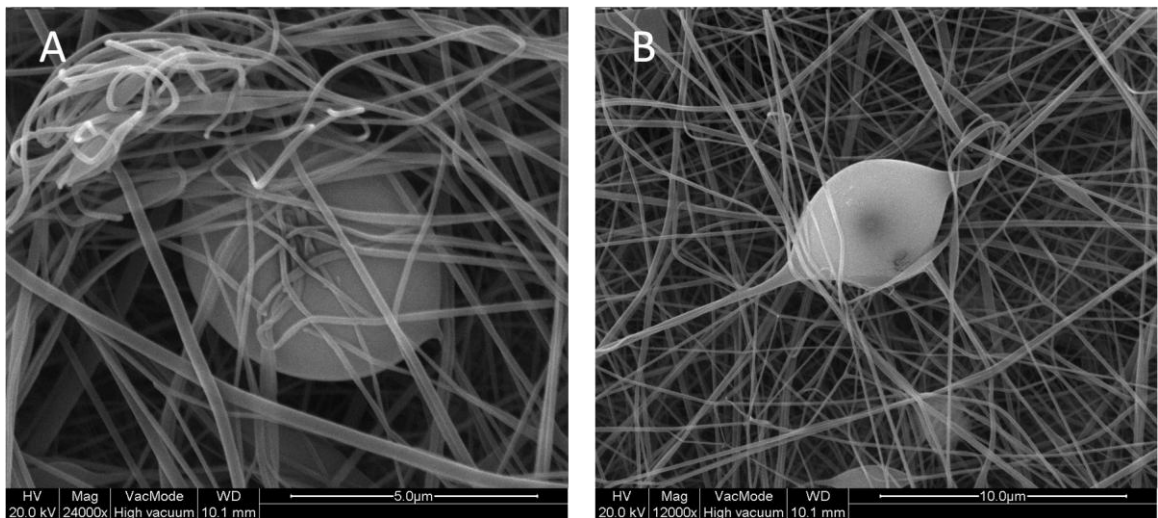

Figure 14 SEM images of nanofibers embedded with spores (A) 24000x (B) 1000x

We were not able to determine the amount of the spores filtered out because we did not find an appropriate method to separate the filtered substance which was a mixture of spores and PVA. PVA could have been separated by burning at high temperatures but this would have burnt out the spores. However, there were differences between the two filter sizes used. There were more spores in the 50 μm filtered solution than in 7 μm filtered solution. This is because 7 μm filter paper filtered all the aggregates bigger than 7 μm thus only few spore were left since the average diameter of dissolved spores was 51 μm. Filter paper sized 50 μm allowed more spores to pass through hence more spores were found on the nanofibers.

Different spore concentrations were electrospun to study the influence of concentration on the amount of spores on the fibers. It was evident that the number of spores on the fibers increased with concentration. The higher the spores' concentration the higher the amount of spores in the solution hence more spores will be electrospun.
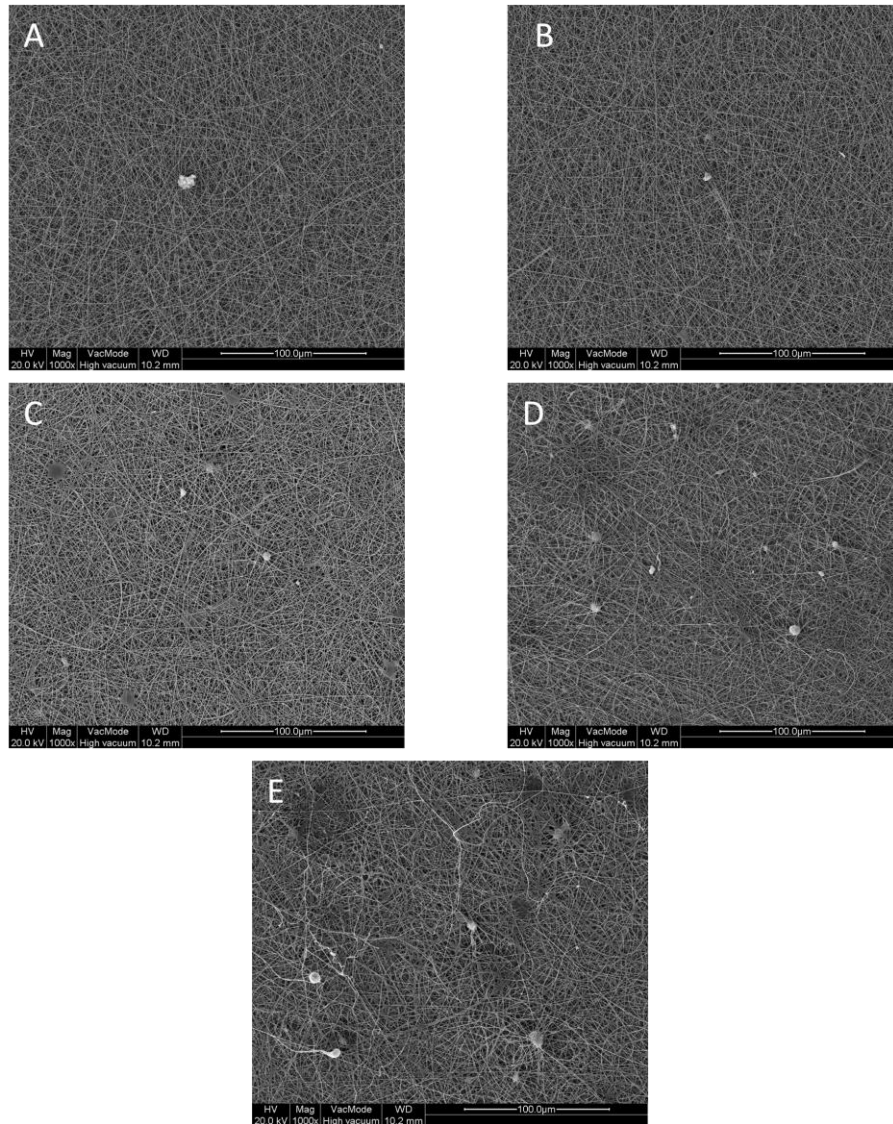
Figure 15 SEM images showing different concentrations of spores (A) 0.5% 1000x (B) 5% 1000x (C) 10% 1000x (D) 15% 1000x (E) 20% 1000x

Table 1 below shows diameter data of pure PVA fibers electrospun from 7 wt% polymer solution, 15 cm deposition distance, 17 kV applied voltage and 1 ml h^{-1} flow rate.

Table 1 Average fiber diameter of pure PVA fibers and 7 wt% PVA solution with different concentration of spores

	Pure	0.50%	5%	10%	15%	20%
Mean	360.91	274.55	310.66	324.81	272.94	319.50
Max	405.8	424.61	758.33	521.98	503.52	605.71
Min	264.06	101.45	227.34	106.15	95.06	158.26

Incorporating spores on PVA fibers had an influence on the fiber diameter. From table 1 above, we can see the range between the maximum and minimum fiber diameter for pure PVA is quite narrow while when spores are added the range broaden.

To analyze the influence of spores on the fiber diameter, we conducted a t-test and compared the p- values at different spore concentration with reference to the average diameter of pure PVA nanofibers. We assumed a null hypothesis that the two means are the same and hence we conducted a t-test for each concentration. Our level of significance was 0.05. The results are as shown in the table 2 below.

Table 2 t-test and p-value to show influence of spores on fiber diameter

Spores concentration	0.50%	5%	10%	15%	20%
t-test	-8.63	-3.15	*-2.98*	-6.75	-2.92
p-value	0.0001	0.0022	*0.0036*	0.0001	0.0044

From the t-test we found that all the p-values at the different concentrations were lower than our alpha (0.05). This implies that the means for different concentration are significantly different from the mean of the original sample. This means that statistically there were significant differences among the various concentration therefore the spores concentration had influence on the average fiber diameter. This can be due to the fact that spores conglutinated thus resulting to much thicker nanofibers. Hence the higher the spores concentration, the higher the number of spores aggregates or the bigger the

spore aggregates which influenced the fibers diameters. From the SEM images, we noticed that the fibers size varied. In specific images the nanostructure was a mixture of large and small fibers, while some fibers didn't have uniform diameter along the length. We noticed some thick sections, figure 16.
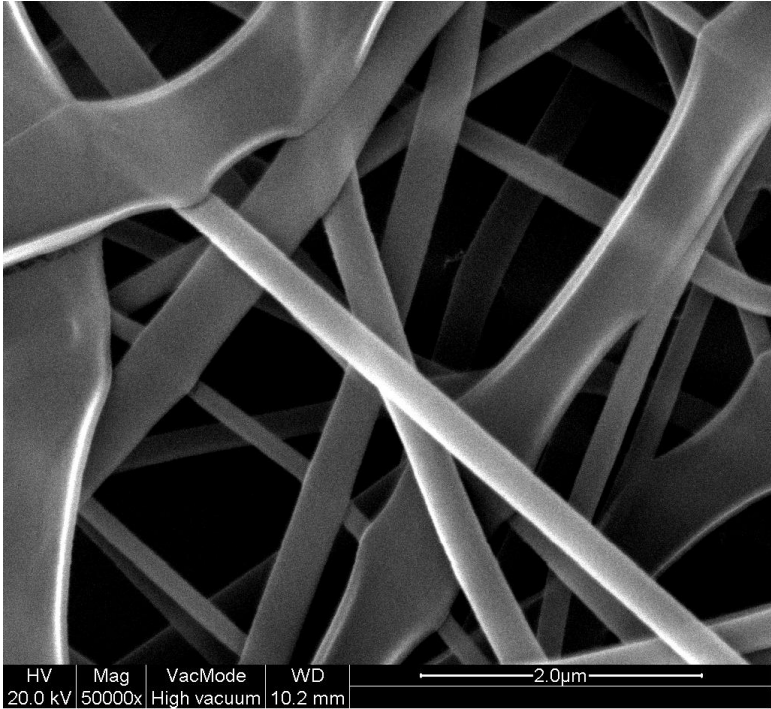

Figure 16 SEM image of fiber with spores showing different fiber sizes

This contributed to the differences in the average fiber diameters. SEM images of fibers with 15% spore concentration shows fibers with relatively uniform diameter (figure 17).
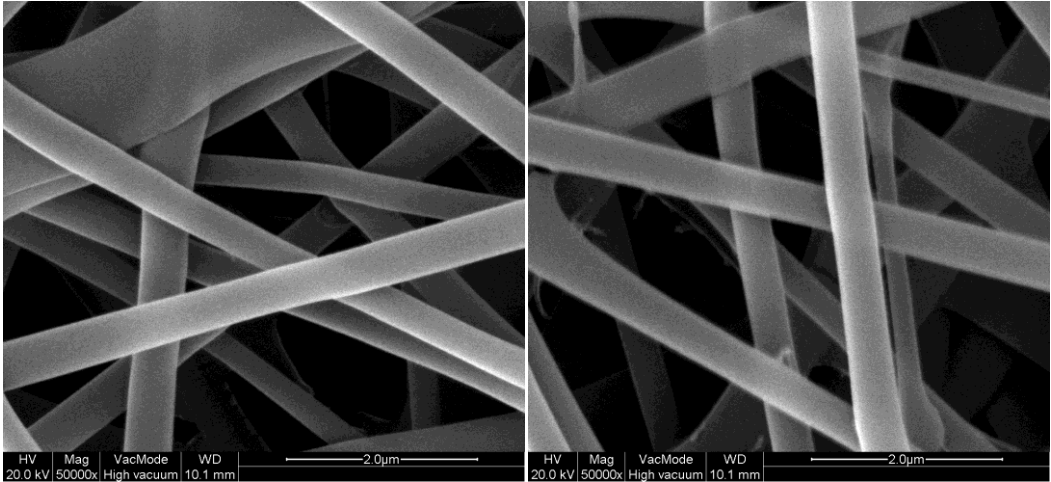
Figure 17 SEM images of fibers with 15% spore concentration

The relatively uniform fiber diameter resulted to the minimum average fiber diameter.

From visual analysis, spores distribution seemed to be homogenous. This was quite evident especially where higher spore concentration was used, figure 18.
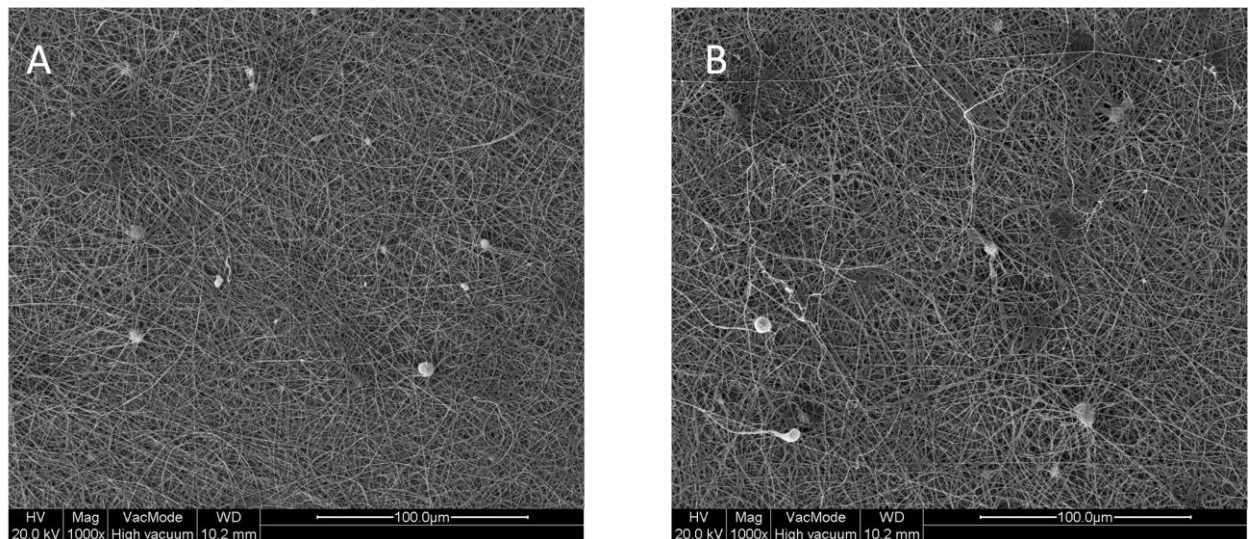
Figure 18 SEM images showing spores distribution at different spores concentrations (A) 15% 1000x (B) 20% 1000x

4.1.5.1. Conclusion

We succeeded in incorporating spores in nonwoven structures. Different experiments proved that a homogeneous distribution of spores inside the nonwoven was obtained. Incorporation of 15% spore concentration was the most optimal because for our application we are interested in a combination of small fiber diameters which will give large surface area and a high amount of functional spores. However, Future study is required to get a method that can be used to determine the amount of spores that were filtered out. Since at 0.5% and 15% spores concentration recorded low average fiber diameter, more experiments need to be carried out to find out the mosquito repellency at this concentrations. These experiments will also help in finding out whether the spores survived the process conditions.

4.2 Melting spinning of polyester with *Bacillus sp.* spores and *lyophilized* microorganisms

Same process parameters were used in extruding melt solution with 0.5% *Bacillus sp.* spores and *lyophilized* microorganisms. The parameters are, 255 $^{\circ}$C die temperature and 34 m min^{-1} screw speed. Temperatures at different heating zones in the extruder were 239, 309, 275 $^{\circ}$C and take-up velocity of 10 m min^{-1}. Change from *Bacillus sp.* spores to *lyophilized* microorganisms didn't not affect the stability of the process at these parameters. The distribution of spores and microorganisms were good as shown in the light microscope images below.
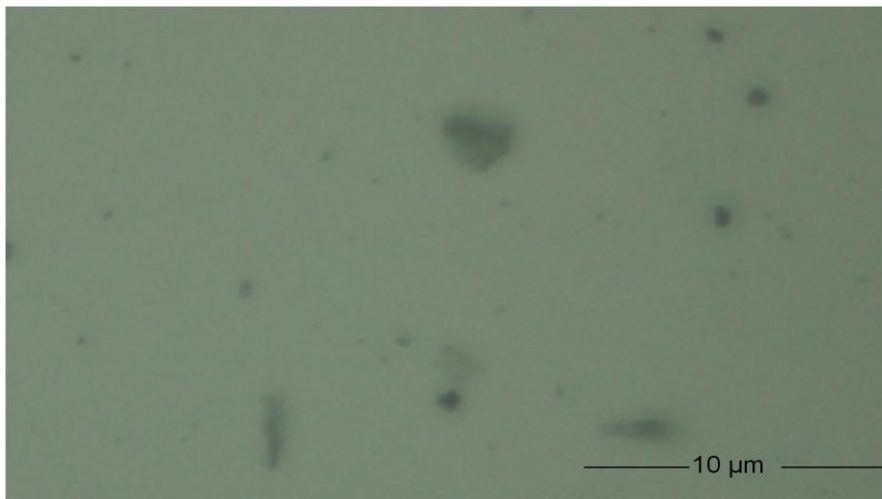
Figure 19 Light microscope image showing the distribution of *lyophilized* microorganisms along the extruded fiber length (20 x)

Filtered *Bacillus sp.* also gave fibers with good distribution of spores as shown in the image below.

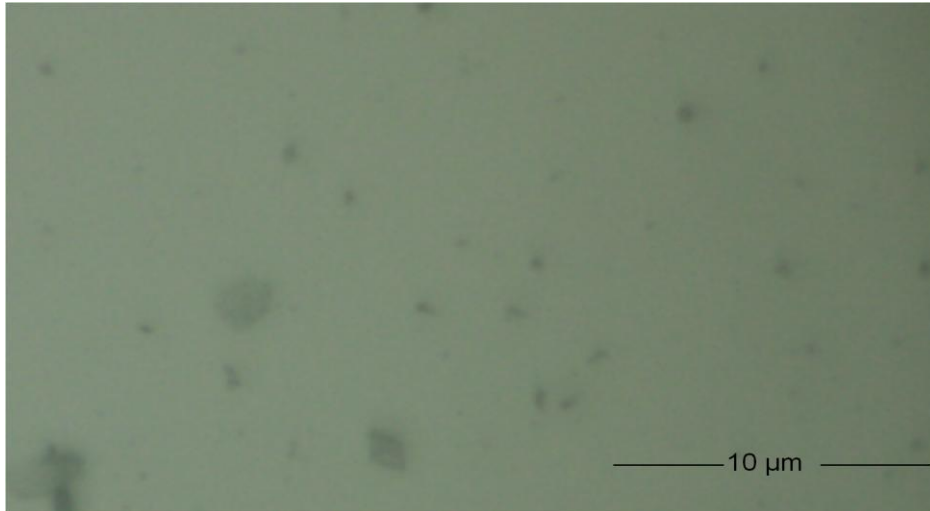Figure 20 Light microscope image showing the distribution of *Bacillus sp.* spores along the extruded fiber length (20 x)

Change of spores neither affected the stability of the process at selected parameters nor the spores distribution along the fiber length. This means that, type of spores don't have any influence on the process as long as they are of similar particle size and their concentration is same.

5.0 Conclusion

From the experiments we conducted, we can come to a conclusion that 7 wt% was the optimal polymer concentration for electrospinning pure PVA polymer solution. We found that lower initial concentrations resulted in beads, and higher concentrations resulted in an increase of fiber diameter. It is only at 7 wt% that we got beadless fibers with the smallest average fiber diameter hence having the largest surface area. We assume that the large surface area will contain sufficient bio-active repellents hence maximizing protection against mosquitoes bites.

We found that it was possible to incorporate biological spores into a nanofibrous nonwoven. Experiments that we carried out proved that it was possible to achieve a homogeneous distribution of spores in the nanofibrous nonwoven. The possibility of incorporating bio-active materials in a polymeric nanostructure may results to increased opportunities of application of such materials in various fields.

Spores concentrations of 0.5%, 5%, 10%, 15% and 20% were examined. We found that incorporation of a spore concentration of 15% was the most optimal because for our application we are interested in a combination of small fiber diameters which will give large surface area and a high amount of functional spores. However, 0.5% concentration of spores also gave small average diameter but it offered small amount of functional spores. Hence, further work need to be carried out to study efficacy at 0.5% and 15% spores concentration.

In extrusion experiment, we succeed to extrude 0.5% *of Bacillus sp.* and lyophilized microorganisms. The processes were stable at discussed parameters and we got a recommendable distribution of spores and microorganisms along the fiber length. However, we cannot give the most optimal spore concentration since we are yet to experiment with different spores concentrations. Hence, we recommend further experiments to be carried out in order to get the most optimal spores concentration. The process conditions were, 239, 309, 275 $^{\circ}$C set temperature at different heating zones in single screw extruder, screw speed was 34 m min^{-1}, take-up velocity of 10 m min^{-1} and 255 $^{\circ}$C die temperature.

6.0 Recommendation

The following future work is recommended

- ➤ More information on the spores being incorporated should be provided so that the electrospinning parameters can be optimized with reference to the condition under which the bio-active organisms can survive.
- ➤ More work should be carried out to determine if the bacteria survived the spinning process.
- ➤ Future study on a method to determine the amount of spores being filtered is required.
- ➤ More characterization techniques to investigate the influence of spores on the fiber structure and surface properties could be used.
- ➤ We recommend further work to be carried out to study the mosquito repellence effectiveness at 0.5% and 15% spores concentration. This will provide interesting results that will help produce the most effective anti-mosquito nanofibers at a minimum cost. Since we might be having same efficacy level at both concentrations.
- ➤ Further work on extrusion experiments should be carried out in order to optimize the spores concentration.

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8.0 Tables

Table 3 Determination of process conditions for a stable electrospinning of PVA solution at 0.5 ml h^{-1} flow rate

Distance (cm)	Voltage (Kilovolts)					
	Concentration					
	5 wt%	6 wt%	7 wt%	8 wt%	9 wt%	Average
7.5	9	9	9	9	-	9
10	10	10	10	10	-	10
15	12	12	12	12	15	13
20	14	14	14	14	15	14

Table 4 Determination of process conditions for a stable electrospinning of PVA solution at 1 ml h^{-1} flow rate

Distance (cm)	Voltage (Kilovolts)					
		Concentration				
	5 wt%	6 wt%	7 wt%	8 wt%	9 wt%	Average
7.5	12	13	13	140	-	13
10	14	14	15	16	-	15
15	16	16	17	18	20	17
20	17	18	19	20	22	19

Table 5 Influence of concentration on average fiber diameter

Concentration (wt%)	Min (nm)	Max (nm)	Diameter (nm)	CV (%)
5	76	326	195±47	±24
6	208	361	283±35	±12
7	264	406	361±31	±9
8	334	500	406±40	±10
9	227	532	408±57	±14

Table 6 Influence of distance and flow rate on average fiber diameter

	0.5 ml hr^{-1}		1 ml hr^{-1}	
Distance (cm)	Diameter (nm)	CV (%)	Diameter (nm)	CV (%)
7.5	329±39	12	310±23	7
10	318±31	10	331±23	7
15	315±32	10	361±31	8
20	334±31	9	354±29	8

Table 7 Influence of cooling time on average fiber diameter

Time (hours)	Diameter (nm)	CV (%)
2	406±40	10
5	350±32	10
24	331±34	10

Table 8 Influence of solution temperature on average fiber diameter

Temperature (^{0}c)	Diameter (nm)	CV (%)
10	293±43	15
20	240±28	12
40	266±27	10