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Aloe vera as Disinfectant for Drinking Water Purpose

by

Nirajan Thapa

A THESIS

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The undersigned certify that they have read, and recommended to the institute of engineering for acceptance, a thesis entitled **"*Aloe vera* as Disinfectant for Drinking Water Purpose"**, submitted by Mr. Nirajan Thapa in partial fulfillment of the requirement for the degree of Master of Science in Environmental Engineering.

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ABSTRACT

Disinfection is an important water treatment process used to remove all bacterial impurities present in water. In this study, the effectiveness of a natural disinfectant derived from *Aloe vera* species for removal of *E. coli* from natural water and synthetic water containing *E. coli* were evaluated using spread plate method and membrane filter technique. Significant reduction in bacterial load was seen in batch test for all the range. For 20, 25, 30 and 35ml dose of *Aloe vera* 94.74%, 93.68 %, 94.17 % and 97.08% removal of *E.coli* concentration were obtained in Batch test. Hence, contact time was considered to be 19, 14, 10 and 6 hour for 20, 25, 30, 35ml dose of *Aloe vera* respectively. For continuous flow, 6 hour detention time was taken to design a sedimentation tank. Effectiveness of *Aloe vera* as disinfectant was analyzed for 0-25, 25-50, 50-75, 75-100 CFU/100ml range at distance of 0.2, 0.4, 0.6, 0.8 and 1m. It was obtained that for flowrate of 9.00, 10.28 and 12.00L/hr there was 84.11%, 91.39% and 93.78% removal of *E. coli* load respectively. For 0-25 CFU/100ml there was 100% reduction in *E. coli* load.

In conclusion, the study indicates that *Aloe vera* has the potential merits for reduction of bacterial contamination in water.

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LIST OF ABBREVIATIONS

Conc.	-	Concentrated
CFU	-	Colony forming Unit
df	-	Dilution factor
DMSO	-	Dimethyl sulfoxide
DW	-	Distilled Water
EMB	-	Eosin Methylene Blue
IOE	-	Institute of Engineering
MF	-	Membrane Filter
N	-	Nitrogen
NA	-	Nutrient Agar
NDWQS	-	Nepal Drinking Water Quality Standard
WHO	-	World Health Organization
^{o}C	-	Degree Celsius
gm	-	gram
mg	-	milligram
ml	-	milliliter
mg/L	-	milligram per liter
%	-	Percentage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The safety and accessibility of drinking-water are major concerns throughout the world. Health risks may arise from consumption of water contaminated with infectious agents, toxic chemicals, and radiological hazards. Improving access to safe drinking-water can result in tangible improvements to health. Safe drinking-water, as defined by the Guidelines, does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. (WHO, 2011).

Water purification is the process of removing undesirable chemicals, biological contaminants, suspended solids and gases from contaminated water. The goal of this process is to produce water fit for a specific purpose. Most water is disinfected for human consumption (drinking water) but water purification may also be designed for a variety of other purposes, including meeting the requirements of medical, pharmacological, chemical and industrial applications. In general the used methods include physical processes such as filtration and sedimentation, biological processes such as slow sand filters, chemical processes such as flocculation and chlorination and the use of electromagnetic radiation such as ultraviolet light.

The purification process of water may reduce the concentration of particulate matter including suspended particles, parasites, bacteria, algae, viruses, fungi; and a range of dissolved and particulate material derived from the surfaces that water may have made contact with after falling as rain. It is not possible to tell whether water is of an appropriate quality by visual examination. Simple procedures such as boiling or the use of a household activated carbon filter are not sufficient for treating all the possible contaminants that may be present in water from an unknown source. Even natural spring water – considered safe for all practical purposes in the 19th century must now be tested before determining what kind of treatment, if any, is needed. Chemical and microbiological analysis, while expensive, are the only way to obtain the information necessary for deciding on the appropriate method of purification. Simple techniques for treating water at home, such as chlorination,

filters, and solar disinfection, and storing it in safe containers could save a huge number of lives each year

Though the chlorination is the most abundant method of disinfection, there are various drawbacks. As an alternative, natural disinfectants are on the way to research. From ancient times, *Aloe vera* was used for the treatment of water, but without the knowledge of dose and effect. *Aloe vera* is a succulent plant species. The species is frequently cited as being used in herbal medicine since the beginning of the first century. The leaves of *Aloe vera* is presented in Figure 1.1

Figure 1.1: *Aloe vera* leaves

1.2 Rationale of the Study

Disinfection of water before consumption is vital for good health. Natural water streams or even water supplied from pipe lines may get contaminated during the course of flow or even in source. Disinfection of water can be carried out by boiling, chlorination, ozonation and UV treatment. Boiling is simplest process and is generally preferred for domestic use. By far till to date chlorination is the most common disinfection used. Chlorine helps to destroy different kinds and number of pathogens that may be present in the water. But Chlorine itself also has limitation. Excess of chlorine is harmful to health and carcinogenic if taken for long time. It produces trihalomethane type of compound which may affect digestive tracks.

Aloe vera being a natural herb having lots of medicinal value may become alternative to the chlorine which is to be imported from outside countries.

1.3 Objective of the Study

The main objectives of the study were to determine the efficiency of *Aloe vera* gel's juice as disinfectant. Other specific objectives are:

- To identify the contact time of *Aloe vera* as a disinfectant.
- To determine the optimum dose of *Aloe vera* as a disinfectant.

1.4 Limitations of the Study

Limitations of the study are:

- Test will be limited for *E. coli* species present in water only.
- Test will not be carried for different parasites present in water.
- Variation in temperature within a day is not considered.

1.5 Organization of the Report

The report is divided into five chapters.

a) Chapter I: Introduction

Introduction includes brief background about background, rationale of the study, objective of the study and limitation of the study.

b) Chapter II: Literature Review

This chapter deals with the brief literature review relating to the area of the study.

c) Chapter III: Methodology

This chapter includes the detail about the methodology followed to perform the study.

d) Chapter IV: Results and Discussions

This chapter shows the results obtained during the study period.

e) Chapter V: Conclusions and Recommendations

This chapter contains all conclusions and recommendations regarding the whole study so that it will help for future research and study of same nature.

The Annex contains sampling data, sampling methods, tables, computations and outputs.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Characteristics of *Aloe vera*

Aloe Vera has succulent, fleshy leaves of a mottled light green color and reaches maturity after four years, with average leaf length between 60 – 100 cm, base width from 7 to 13 cm and leaf weigh from one to two kilos. It produces an average of twelve to thirty leaves. It leafs are rich in gel in comparison to the external cuticle or the skin encasing it (Bassetti and Sala, 2005).

Its thick leaves contain the water supply for the plant to survive long periods of drought. The leaves have a high capacity of retaining water also in very warm dry climates and therefore this plant can survive very harsh circumstances where most other vegetation disappears. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed a clear mucilaginous substance appears that contains fibres, water and the ingredient to retain the water in the leaf. This is called the gel. *Aloe vera* gel consists of 99.3% water. The remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product (Bassetti and Sala, 2005).

2.1.1 *Aloe vera*'s extract composition

Large fluctuations are observed in Aloe Vera gel's composition considered in the literature, but they have been explained by the fact that the gel's composition is significantly influenced by factors like aloe type, season, soil and cultivation routines (Bassetti and Sala, 2005; Hamman, 2008).

Aloe is made up of a vast range of compounds which can be divided into three large groups, but the main ingredient is water (98,5%-99,5%). The first group, complex sugars (with acemannan as primary polysaccharide), are constitutes of the leaf's gel. Next are the anthraquinones, contained in the outermost part of the skin and last are the several chemical substances (Bassetti and Sala, 2005).

The Chemical Compound present in *Aloe vera* is shown in Tabular form as in Table 2.1

Table 2.1: Chemical composition of *Aloe vera* Pulp and excudates. (Hamman, 2008)

Class	Compound
Anthraquinones/anthrones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid
Carbohydrates	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid
Chromones	8-C-glucosyl-(2'-O-cinnamoyl)-7-O-methylaloediol A, 8-C-glucosyl-(S)- aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol, 8-C-glucosyl-7-O methylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoaloesin A
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxidase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, superoxide dismutase
Inorganic Compounds	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc
Phenols and Miscellaneous including organic compounds and lipids	Pyrocatechol, p-Coumaric, 1,8-cineole Arachidonic acid, γ-linolenic acid, steroids (campestrol, cholesterol, β-sitosterol), triglicerides, triterpenoid, gibberillin, lignins, potassium sorbate, salicylic acid, uric acid
Non-essential and essential amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, lysine, methionine, proline, tyrosine, valine
Proteins	Lectins, lectin-like substance
Saccharides	Mannose, glucose, L-rhamnose, aldopentose
Vitamins	B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol

2.1.2 Antibacterial constituents of *Aloe vera*

Aloe vera antimicrobial effect is due to its main gel components which are anthraquinones, phenols and terpenoids, targeting mostly the bacterial cell wall and membrane (Lawrence *et al.,* 2009; Pareek *et al.*, 2013). Similarly, it is considered that the role of specific plant compound such as anthraquinones and

dihydroxyanthraquinones as well as saponins have direct antimicrobial activity (Mariappan and Shanthi , 2012).

2.1.3 *Aloe vera* antibacterial activity mechanism

The chemical constitutes that are proposed by the literature to exhibit the antibacterial activity attributed in *Aloe vera* gel are anthraquinones, saponins, phenols, terpenoids and enzymes (Lawrence *et al*., 2009; Pareek *et al.,* 2013). More in specific, 1,8-cineole (monoterpenoid), pyrocatechol (phenol), aloin (anthraquinone) and superoxide dismutase (enzyme) are responsible for a range of antibacterial mechanisms (Lawrence *et al.,* 2009). Anthraquinones antibacterial mechanism is attributed to a similar mechanism as described for phenolic compounds; e.g. increased membrane permeabilization, loss of structural integrity of cell wall and cytoplasmic membrane, and leakage of intracellular contents. Other researchers however, have described a different mechanism. It is stated that anthraquinones produce bacterial photo inactivation through a mechanism that allows their intercalation between the nucleic acid bases. Another proposed mechanism has the same final output as the one before, but this is reached through a photodynamic photosensitization, acting mainly through the generation of reactive oxygen species (ROS). Finally, superoxide dismutase enzyme transforms O_2 into hydrogen peroxide (H_2O_2), known for its antibacterial capability. (Spentzouris, 2015)

2.2 Use of *Aloe vera* as Disinfectant

Research was done by Pareek *et al.* (2013) in the title "Disinfection of Dental Unit Water Line Using *Aloe vera*: In Vitro Study. They considered "*Aloe vera* is considered to have antimicrobial and antifungal properties. It consists of essential oil of E. camaldulensis, characterized by the presence of high concentrations of 1,8-cineole with well-documented antimicrobial activity. Essential oils are capable of affecting biofilm formation. They significantly decrease bacterial adhesion and affect bacterial viability in biofilms. The efficacy of *Aloe vera* liquid as an antibacterial agent is shown to have a wide range of effectiveness against Gram-positive (Gram +ve) and Gram negative (Gram −ve) bacteria due to an extract of the inner gel of the plant Aloe barbadensis Miller or *Aloe vera* (L.). Streptococcus pyogenes and Streptococcus faecalis are two microorganisms that have been inhibited by *Aloe vera* gel. Aloe gel is bacteriostatic or bactericidal against a variety of common wound-infecting bacteria in vitro: *Staphylococcus aureus*, *Streptococcus pyogenes*,

Serratiamarcescens, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*, *Salmonella typhosa*, and *Mycobacterium tuberculosis*."

Similarly, Spentzouris, 2015 conducted a study entitled "Comparative study on disinfection efficacy of Thymus Vulgaris and *Aloe vera* extracts with commercial disinfectants, on bacteria isolated in nosocomial environment" considered that up to 1.5 log reduction was achieved by ethanol dried extract of *Aloe vera* in distilled water of 5 g/l conc. against *E. coli*, *Staphylococcus Aureus* and *Acinetobacter baumanii* after 10 minutes of contact time. The evaluation was performed by broth dilution followed by viable count of the bacteria population after being subjected to different concentrations of the disinfectants.

Another related research was done by Robson *et al.,* 1982 in the research title "Myth, magic, witchcraft or fact? *Aloe vera* revisited" wrote "*Aloe vera* has three major properties that are most beneficial for thermal injuries: either due to aspirin like effect or the high concentration of Mg ion or possibly both, acting synergistically, it can potentiate an anesthetic effect, it has broad spectrum antimicrobial effect, especially against the agents frequently responsible for burn wound sepsis and it has an antiprostanoid. Research also showed *Aloe vera'*s antibacterial effects against gram negative bacteria; *E. coli*, *Citrobacter sp., S. marcescens, Enterbacter sp., Klebsiella sp.* and*P. aeruginosa* and considered that extract was effective as bactericidal agent to conc. as low as 80% for all organisms.

Antibacterial property of *Aloe vera* against *E. coli* was further illustrated by zone of inhibition technique too. It was considered that for ethanol, methanol and acetone extract of *Aloe vera* the zone of inhibition against *E. coli* were 12.66mm, 14mm and 6mm respectively (Lawrence *et al.,* 2009). Similarly for DMSO gel extract of *Aloe vera* zone of inhibition against *E. coli* was 13mm (Renishwya *et al*., 2012).

In practice also, *Aloe vera* juice is used in various medicinal purposes, it has wide range of medicinal properties. Since the researcher considered that *Aloe vera* has medicinal value, I will use it as disinfectant of water.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Sample Collection and Preparation of Synthetic Water Sample

Different drinking water sample was collected from different places of Kathmandu Valley in the sterile bottle. Care was taken while taking the sample to prevent other contamination.

E. coli was preserved from raw water sample collected from cultured from EMB agar plate. Synthetic water sample was prepared by swabbing the colony of *E. coli* bacteria cultured from EMB agar plate in the distilled water with the help of loop. Different sample of synthetic water having different concentration of *E. coli* bacteria was prepared by serial dilution process.

3.2 Material and Its Preparation

Aloe vera plant that is approximately 2 years age at the time harvest was selected. Leaves of plant were plucked and washed with normal tap water 2-3 times and then with distilled water. Inner gel of the leaves was separated using sterile knife. Leaves were then crushed by mortar and pestle and then extracted using distilled water. The extract is then filtered using sterile muslin cloth. For continuous set up as large quantity of juice was required, *Aloe vera* juice was prepared by crushing inner gel of leaves in sterile grinder. All these extraction work was done in sterile environment.

3.3 Experimental Set Up and Procedure

Whole Experiments were done in two stage; one Batch test and other Continuous flow test. Colony count in batch test and continuous flow was done by spread plate technique and Membrane filter technique respectively. Details of these techniques are attached in annex (Shah *et. al.,* 2006).

On Batch process *E. coli* were preserved from EMB agar plate cultured from raw water collected from different drinking water source in Kathmandu valley. Synthetic samples were prepared in beaker of 200ml. To carry out test 10 ml of synthetic water sample was transferred in conical flask. Before adding juice, synthetic water was cultured in NA plate to detect number of CFU in the sample. Prepared *Aloe vera* juice was added in water sample and was well mixed. After mixing *Aloe vera* juice, 0.1 ml

of *Aloe vera* treated sample was transferred to NA plate and was incubated for 24 hours in 44º C temp after regular interval of 1 hour to detect contact time. Dose of *Aloe vera* was detected by treating synthetic water sample with different dose of *Aloe vera* at same contact time that was determined before.

For continuous process, experimental set up consists of three circular water tanks and one rectangular sedimentation tank. Among three water tank; one tank consist of synthetic water sample containing *E. coli* bacteria, second tank consists of *Aloe vera* juice and third tank was meant to be a mixing tank to mix the water sample and *Aloe vera* juice. In mixing tank water sample was well stirred with *Aloe vera* juice before sending mixture to the sedimentation tank at constant flowrate. The Schematic layout for Continuous flow is shown in Figure 3.1

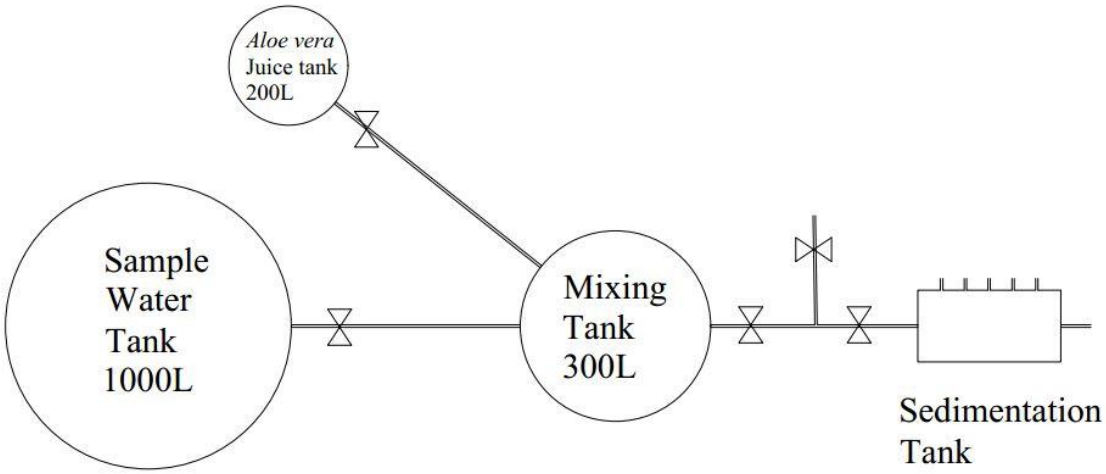


Figure 3.1 Experimental Setup for Continuous flow

The dimension of Sedimentation basin used for analysis was (1.2*0.4*0.4) m^3.It has inlet and outlet at both end. Along its length 5 ports were installed in 0.2m, 0.4m, 0.6m, 0.8m and 1m distance at an interval of 0.2m for collection of *Aloe vera* treated water sample. Details of sedimentation tank is shown in Figure 3.2

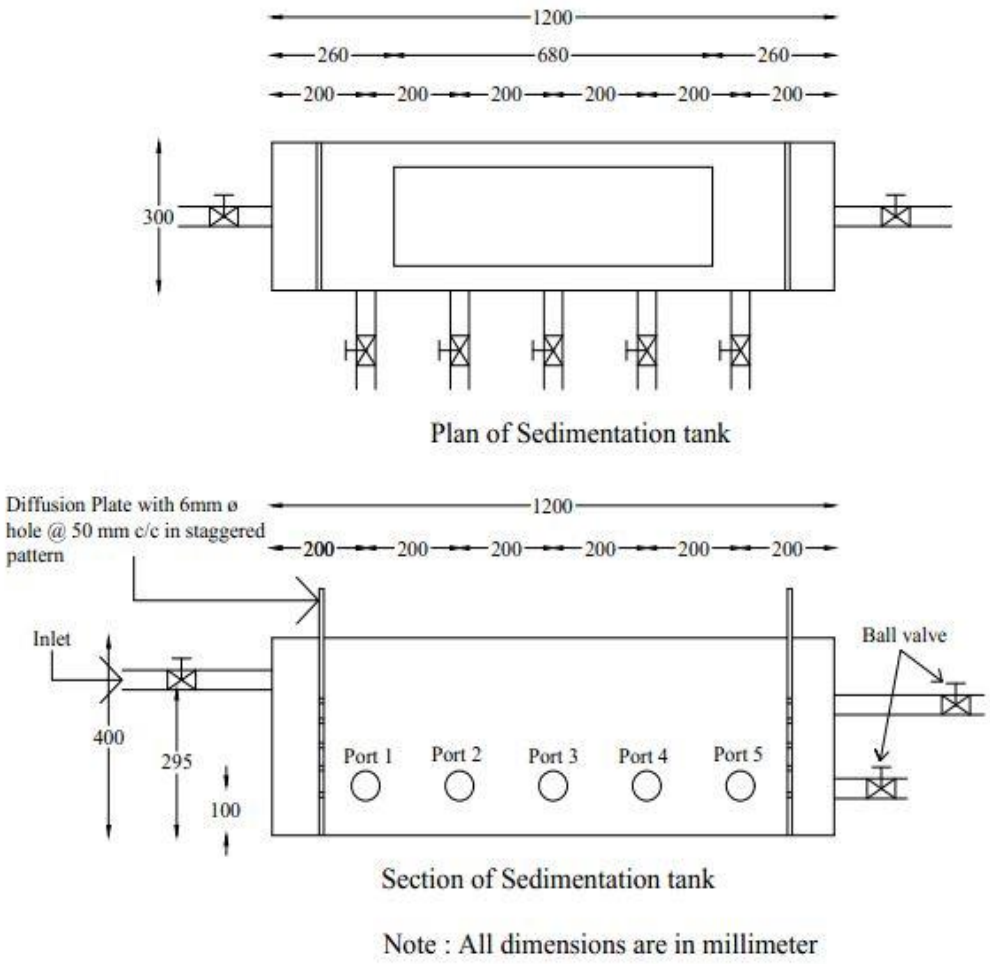


Figure 3.2 Plan and Section of Sedimentation tank

Membrane filter method was used for analysis of bacterial load. For different flowrate bacterial test were conducted. Different flowrate were maintained by controlling the opening of ball valve of pipe suppling mixed sample to sedimentation basin. Samples were collected in sterile bottle through ports along the length of Sedimentation basin. Membrane filter test was conducted in Microbiology lab in sterile environment.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

Among various parameters of drinking water only total *E. coli* present in the water was considered. Hence, the experimental results of *E. coli* removal of water by the use of *Aloe vera* juice are presented in this chapter. The treatment process has to ensure the removal of *E. coli* is in accordance to WHO guidelines of drinking water for Nepal. Analysis was done by spread plate method and membrane filter method. Synthetic water characteristics are presented in Table 4.1.Detail of calculation is attached in the Annex.

Table 4.1 Synthetic Raw Water Characteristics

Characteristics of Synthetic Raw Water	Values
Conductivity (μs/cm)	77.4
pH	7.2
Total Dissolved Solid (mg/l)	39.4
Salinity (ppt.)	0.04
Temperature (ºC)	22.4
Resistivity (10^4Ω.cm)	1.27

4.1 Determination of Contact Time for Different Dose in Batch Test

Before determining contact time, water sample collected from different places and was treated with *Aloe vera* juice to know if the juice has antibacterial property against *E. coli* bacteria. After positive result against different sample of water containing *E. coli* bacteria; test was run for different synthetic water containing *E. coli* bacteria to determine contact time. For determining contact time synthetic water was mixed with *Aloe vera* juice and after each hour the *Aloe vera* mixed water was spread in the plate containing NA and was incubated for 24 hours at 44ºC.

4.1.1 Determination of contact time for 2ml dose

2ml dose of *Aloe vera* juice was thoroughly mixed with 10ml of synthetic water sample and kept in contact in room temperature. For different concentration of *E. coli* load after different contact time the removal of *E. coli* are shown in Figure 4.1

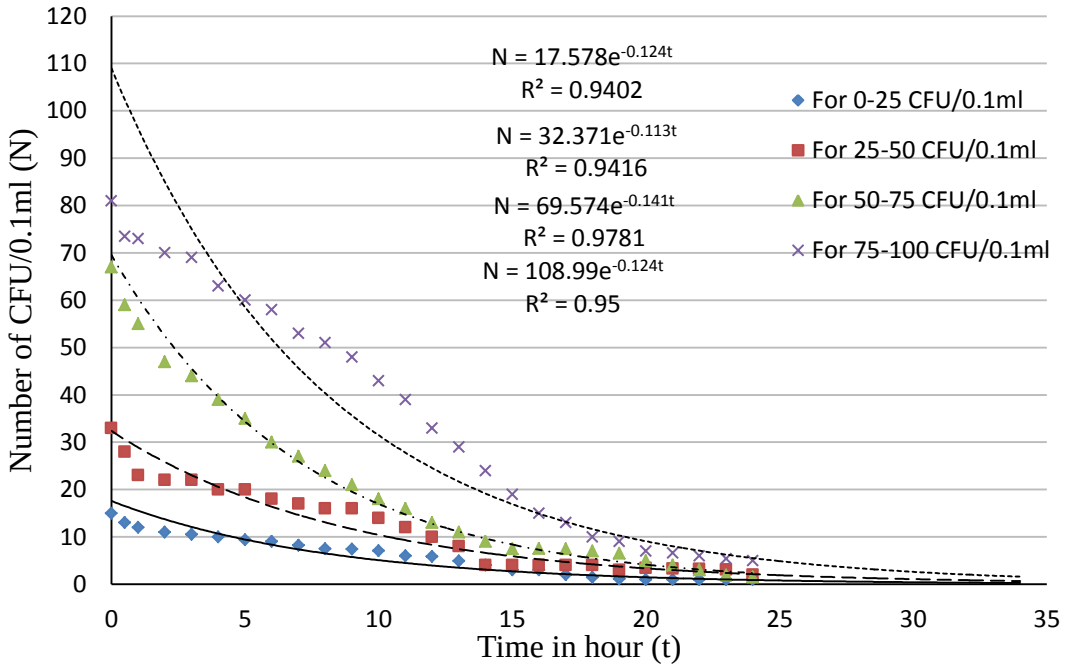


Figure 4.1 Number of *E. coli* (CFU/0.1ml) present in water sample 15, 33, 67 and 81 (CFU/0.1ml) after different contact time in hour after using *Aloe vera* juice.

From the Figure 4.1 it shows that for different *E.coli* load in synthetic water sample after treating with *Aloe vera* juice there is reduction in bacterial load. Bacterial load was reduced with increase in contact time. For 15CFU/0.1ml, 33CFU/0.1ml, 67CFU/0.1ml and 81CFU/0.1ml 90% removal was obtained after 18 hour, 19 hour, 18 hour and 19 hour respectively. Hence, the contact time was considered to be 19 hour after 90% removal of *E. coli* from synthetic water sample.

4.1.2 Determination of contact time for 2.5ml dose

2.5ml dose of *Aloe vera* juice was thoroughly mixed with 10ml of synthetic water sample and kept in contact in room temperature. For different concentration of *E. coli* load after different contact time the removal of *E. coli* are shown in Figure 4.2

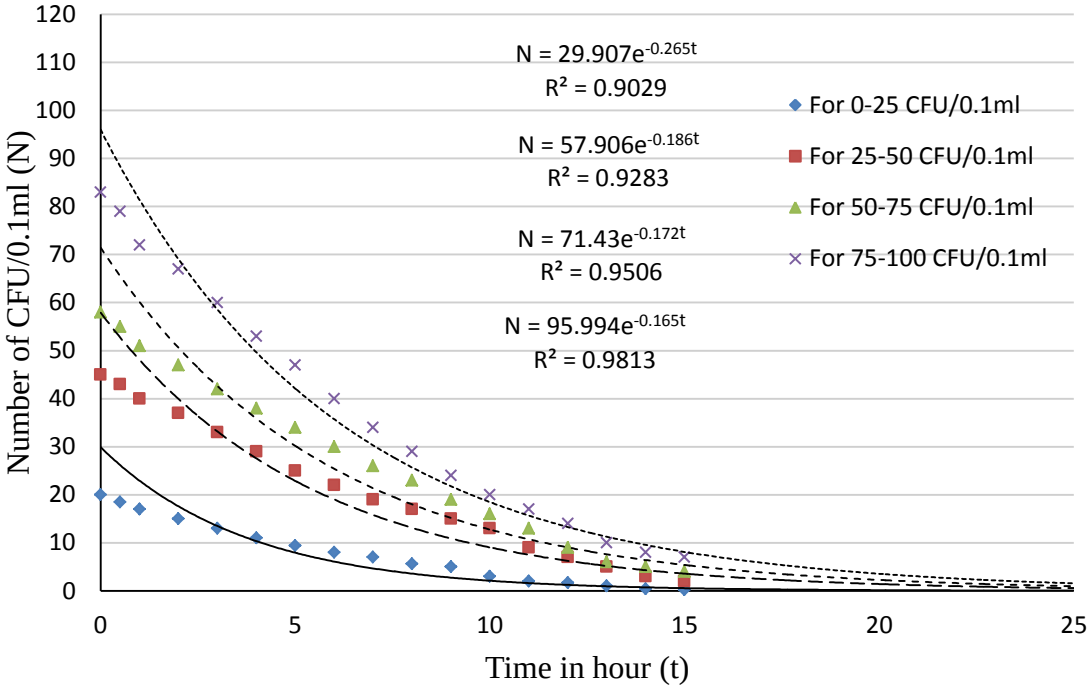


Figure 4.2 Number of *E. coli* (CFU/0.1ml) present in water sample 20, 45, 58 and 83 (CFU/0.1ml) after different contact time in hour after using *Aloe vera* juice.

From the Figure 4.2 it shows that for different *E.coli* load in synthetic water sample after treating with *Aloe vera* juice there is reduction in bacterial load. Bacterial load was reduced with increase in contact time. For 20CFU/0.1ml, 45CFU/0.1ml, 58CFU/0.1ml and 83CFU/0.1ml 90% removal was obtained after 12 hour, 14 hour, 14 hour and 14 hour respectively. Hence, the contact time was considered to be 14 hour after 90% removal of *E. coli* from synthetic water sample

4.1.3 Determination of contact time for 3ml dose

3ml dose of *Aloe vera* juice was thoroughly mixed with 10ml of synthetic water sample and kept in contact in room temperature. For different concentration of *E. coli* load after different contact time the removal of *E. coli* are shown in Figure 4.3

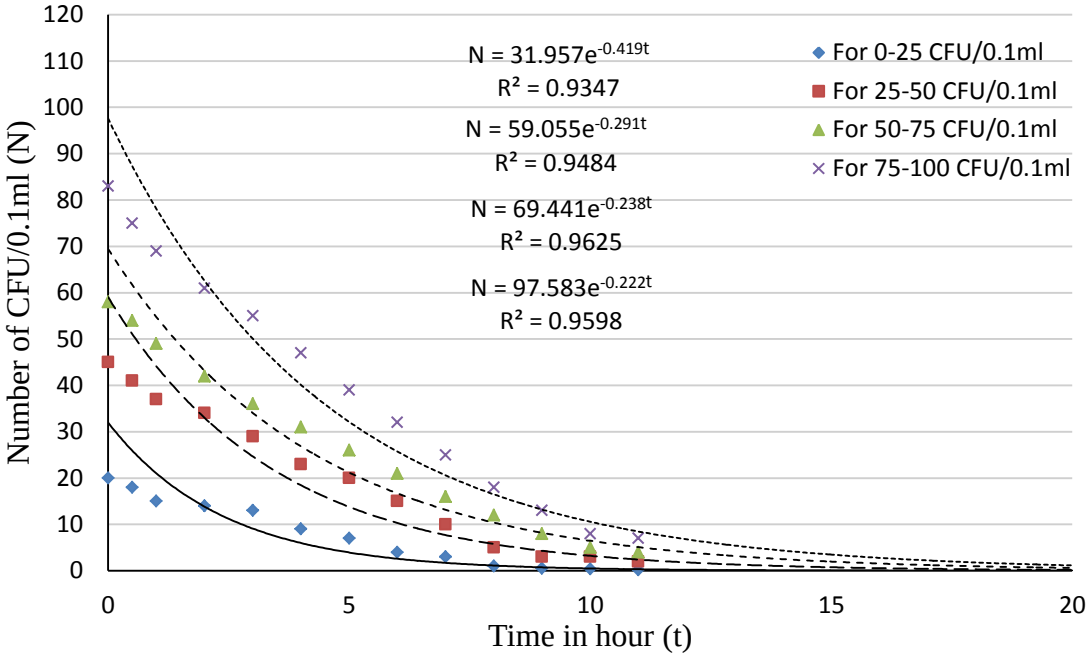


Figure 4.3 Number of *E. coli* (CFU/0.1ml) present in water sample 20, 45, 58 and 83 (CFU/0.1ml) after different contact time in hour after using *Aloe vera* juice.

From the Figure 4.3 it shows that for different *E.coli* load in synthetic water sample after treating with *Aloe vera* juice there is reduction in bacterial load. Bacterial load was reduced with increase in contact time. For 20CFU/0.1ml, 45CFU/0.1ml, 58CFU/0.1ml and 83CFU/0.1ml 90% removal was obtained after 8 hour, 9 hour, 10 hour and 10 hour respectively. Hence, the contact time was considered to be 10 hour after 90% removal of *E. coli* from synthetic water sample.

4.1.4 Determination of contact time for 3.5ml dose

3ml dose of *Aloe vera* juice was thoroughly mixed with 10ml of synthetic water sample and kept in contact in room temperature. For different concentration of *E. coli* load after different contact time the removal of *E. coli* are shown in Figure 4.4

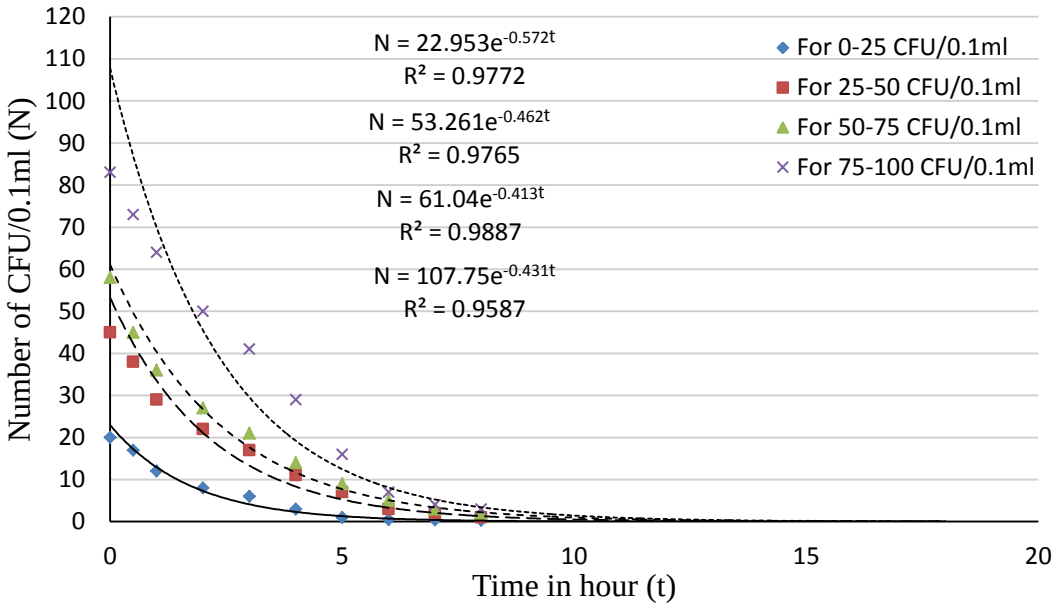


Figure 4.4 Number of *E. coli* (CFU/0.1ml) present in water sample 20, 45, 58 and 83 (CFU/0.1ml) after different contact time in hour after using *Aloe vera* juice.

From the Figure 4.4 it shows that for different *E.coli* load in synthetic water sample after treating with *Aloe vera* juice there is reduction in bacterial load. Bacterial load was reduced with increase in contact time. For 20CFU/0.1ml, 45CFU/0.1ml, 58CFU/0.1ml and 83CFU/0.1ml 90% removal was obtained after 5 hour, 6 hour, 6 hour and 6 hour respectively. Hence, the contact time was considered to be 6 hour after 90% removal of *E. coli* from synthetic water sample. All the findings of batch test to detect contact time is summarized in Table 4.2

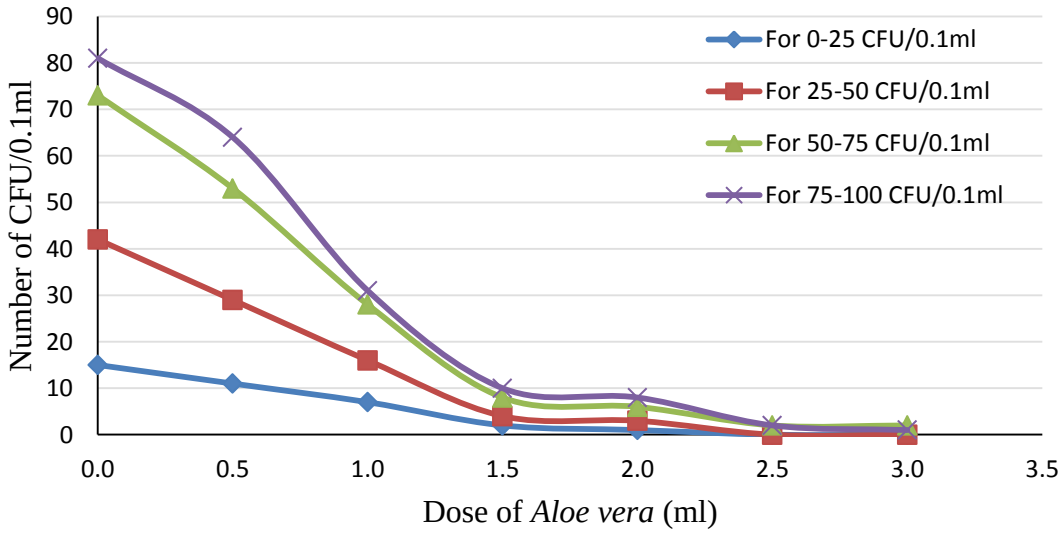
Table 4.2 Summary of batch test.

Dose of *Aloe vera* Juice for 100ml of water	Contact time
20 ml	19 hour
25 ml	14 hour
30 ml	10 hour
35 ml	6 hour

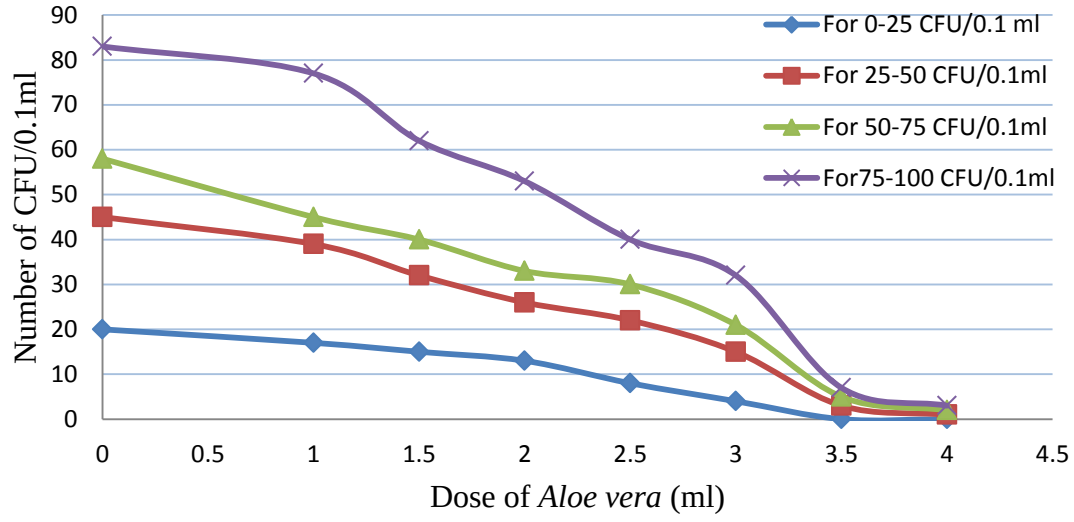
4.2 Determination of Dose for Different Contact Time in Batch Test

After calculation of contact time, dose of *Aloe vera* juice was determined by adding different dose of *Aloe vera* gel as 0.5, 1, 1.5, 2, 2.5 and 3ml in different concentration of 10ml of Synthetic water sample. Contact time taken for every dose was 19 hours and 6 hours. Synthetic water sample of different concentration ranging from 0-25, 25-50, 50-75 and 75-100 CFU/0.1ml was prepared.

For different dose of *aloe vera* juice reduction of *E. coli* is shown in Figure 4.5



(a)



(b)

Figure 4.5 *E.coli* reduction vs different dose of *Aloe vera* for different range *E. coli* concentration of synthetic water sample for contact time of (a) 19 and (b) 6 hour.

From the Figure 4.5 (a) it shows with increase in dose *Aloe vera* juice the bacterial load decreases. For initial concentration of 0-25 CFU/0.1ml it showed almost same number of bacteria load reduction for 2.5ml and 3ml dose of *Aloe vera*. For initial concentration of 25-50 CFU/0.1ml it showed almost same number of bacteria load reduction for 2.5ml and 3ml dose .For initial concentration of 25-75 CFU/0.1ml it showed almost same number of bacteria load reduction for 2.5ml and 3ml dose. For initial concentration of 75-100 CFU/0.1ml it showed almost same number of bacteria load reduction for 2.5ml and 3ml dose. Hence the dose of *Aloe vera* was considered to be 2.5ml for 10ml of water sample for contact time of 19 hour.

Similarly from the Figure 4.5 (b) for initial concentration of 0-25 CFU/0.1ml it was considered zero CFU for 3.5ml dose and 4ml dose of *Aloe vera*. For other range of concentration more than 90 % removal was obtained for 3.5 ml dose and 4 ml dose at 6 hour contact time. Hence the dose of *Aloe vera* was considered to be 3.5ml for 10ml of water sample for contact time of 6 hour.

4.3 Determination of Flowrate by Continuous Process

Flowrate was maintained so that 35ml dose of *Aloe vera* juice was thoroughly mixed with 100ml of synthetic water sample in the mixing tank and it was well mixed in tank and then mixed sample was passed to sedimentation basin at different flowrate of 12.00 L/hr, 10.28 L/hr and 9.00 L/hr. For different concentration of *E. coli* load at different flowrate the removal of *E. coli* are shown in Figure 4.6

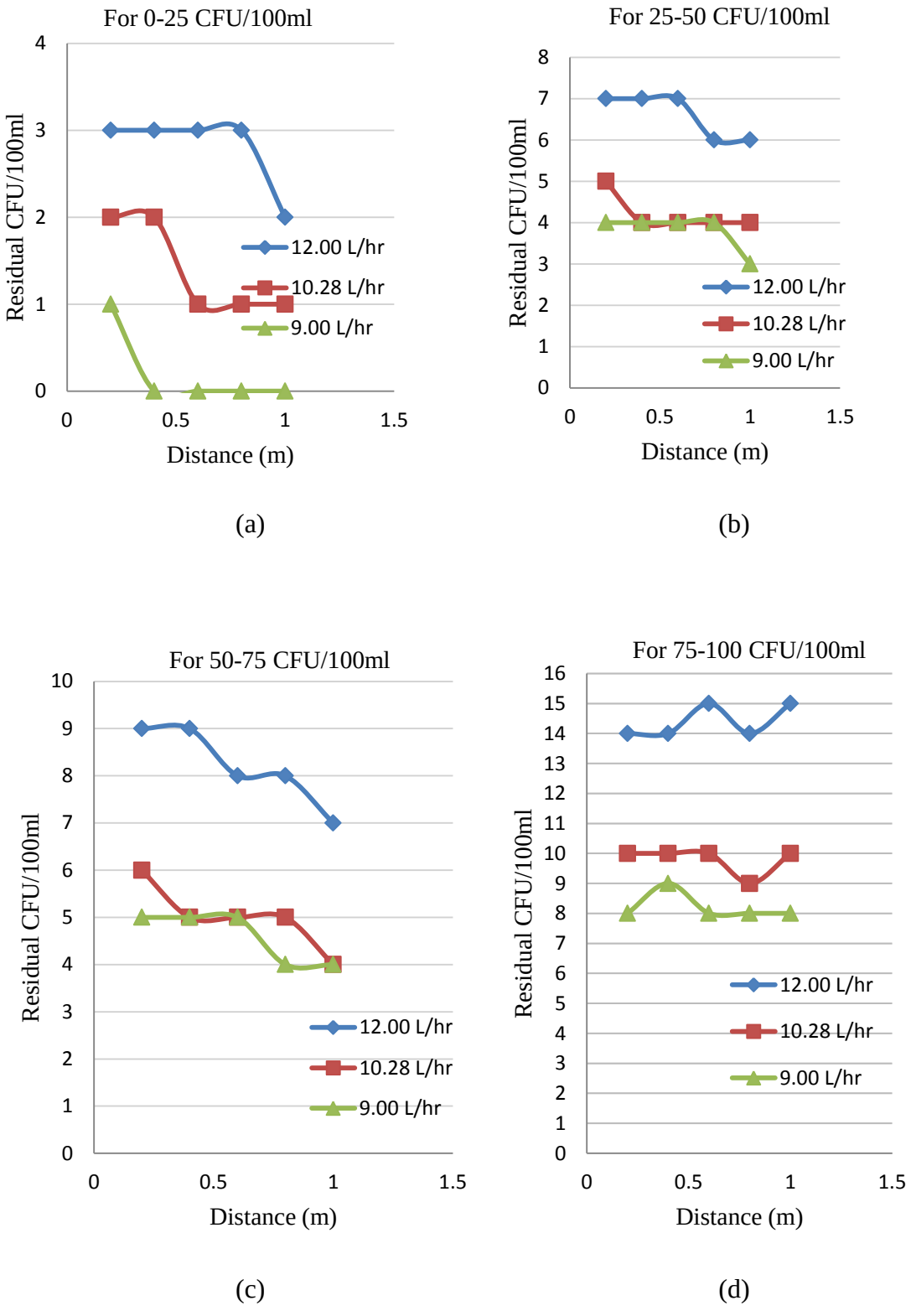


Figure 4.6 *E.coli* reductions at different port at different flowrate for (a) 0-25 (b) 25-50 (c) 50-75 (d) 75-100 CFU/100ml concentration of synthetic water sample.

From the Figure 4.6 it shows with decrease in flowrate the bacterial load decreases. For initial concentration of 0-25 CFU/0.1ml it showed almost all *E. coli* was killed for flowrate of 9.00 L/hr. For initial concentration of 25-50 CFU/0.1ml, it showed almost same number of bacteria load reduction for flowrate of 9.00 L/hr and 10.28 L/hr. For initial concentration of 25-75 CFU/0.1ml it showed almost same number of bacteria load reduction for flowrate of 9.00 L/hr and 10.28 L/hr. For initial concentration of 75-100 CFU/0.1ml it showed almost same number of bacteria load reduction for flowrate of 9.00 L/hr and 10.28 L/hr.

Likewise from the Figure 4.6 it shows there is a gap between flowrate of 12.00 L/hr with 10.28 L/hr and 9.00 L/hr in terms of residual number of CFU. For range of 0- 25, 25-50, 50-75 and 75-100 CFU/100ml the difference in final residual CFU is 1 CFU, 2 CFU, 3 CFU and 5 CFU respectively. For flowrate 9.00 L/hr and 10.28 L/hr there is no significant difference in final residual CFU. It indicates that for lower range of bacteria load efficiency of *Aloe vera* to reduce the bacterial load for different flowrate are almost same but with increase in range of bacteria load the efficiency gradually decreases for higher flowrate. This trend indicates that lower flowrate are suitable for higher efficiency. Hence the flowrate of 10.28 L/hr can be considered as suitable flowrate for the removal of *E.coli* at contact time of 7 hours.

4.4 Cost Analysis

For the cost analysis, Synthetic water sample of specific *E .coli* was treated with *Aloe vera* and Piyush (0.5% Chlorine) available in local market in the batch test at the optimum dose of *Aloe vera* and Piyush respectively. The cost involved in both treatment processes was analyzed. The cost analysis is presented as in Table 4.2

Table 4.3 Cost comparison between *Aloe vera* juice and Piyush

S.N.	Description	*Aloe vera*	Piyush
1	Cost for 100ml	Rs 1.25	Rs 33.33
2	Volume required to reduce 20 CFU/100ml to 0 in 100ml synthetic water sample	25 ml	1 ml
3	Total cost per liter	=Rs0.0125*25*10 =Rs 3.125/ltr	=Rs 0.33*1*10 =Rs 3.3/ltr

From the Table it shows that there is not much difference in cost between *Aloe vera* Juice and Piyush available in local market. *Aloe vera* being a natural herb having lots of medicinal value; it is suggested to use *Aloe vera* Juice as water disinfectant for drinking water purpose as there is not much difference in cost with Piyush.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the study conducted following conclusions can be drawn

- The optimum dose for the *E. coli* removal by *Aloe vera* was considered to be 20ml, 25ml, 30ml and 35ml for contact time of 19, 14, 10 and 6 hour with removal percentage of 94.74, 93.68, 94.17 and 97.08% respectively from batch test.
- It was obtained that for flowrate of 9.00, 10.28 and 12.00L/hr there was 84.11%, 91.39% and 93.78% removal of *E. coli* load respectively.
- The optimum flowrate for continuous flow was considered to be 10.28 L/hr for contact time of 7 hour. Reduction in percentage removal of *E. coli* load in continuous setup was due to short circuiting of flow path in sedimentation tank
- As per result it can be concluded that *Aloe vera* juice can be effectively used as disinfectant for range below 25 CFU/ml both for batch and continuous setup and can be used for drinking purpose. For *E.coli* load greater than 25 CFU/ml *Aloe vera* juice treated water can be used for recreation purpose only.
- High *E. coli* removal determined in this study indicates that *Aloe vera* has the potential to be utilized as disinfectant for lower range of bacterial load for treatment applications.

5.2 Recommendations

The recommendations from my study are

- Need further study to examine the influence of temperature variations.
- These preliminary results showed that *Aloe vera* extract have the potential to be used as disinfectants for lower bacterial load and further studies should be conducted in higher concentrations.
- The test were conducted from aqueous solution of *Aloe vera* juice as a whole, if it is possible to extract antibacterial components of *Aloe vera* and can be used for test; there might be high reduction in contact time and dose as well.

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APPENDICES

A: Spread Plate method

Spread plate technique aids in the isolation as well as enumeration of the organisms in a mixed culture. The mixed culture is first diluted to provide only a few cells as per milliliter before being used to inoculate media. Since the number of bacteria in the specimen is not beforehand, a series of dilutions are made so that at least one of the dilutions will contain a suitably sparse concentration of cells. Usually dilution is done in series of tubes containing a sterile liquid, water or physiological saline. Measured amount of diluted sample is placed on to the surface of agar plate and spread evenly over the surface by means of sterile, bent glass rod. Volumes greater than 0.1ml are rarely used because excess liquid is not soaked in the media and may cause the colonies to coalesce as they form making counting difficult. The number of colonies formed after incubation is counted and total number of organisms is determined as

No. of bacteria= no. of colonies × D. f × 10.

Materials required for Spread plate method

1. 9ml dilution blanks
2. Sterile pipettes
3. Sterile spreader
4. Media plates
5. Bunsen burner
6. Sample

Procedure for Spread plate method

1. First of all 10 ml of distilled water was taken in the sterile 100 ml beaker.
2. 1 loop of *E. coli* cultured in EMB agar plate was swabbed in 10 ml distilled water.
3. Different sample of synthetic raw water sample was prepared by serial dilution to decrease the bacteria load to countable value.
4. After preparation of different concentration of synthetic water sample; 0.1 ml of water sample was transferred in separated dried NA plate to calculate initial number of *E.coli* in water sample.
5. Inoculated sample was spread using sterile L-shaped glass rod throughout the media.
6. The NA plate was incubated at 44ºC for 24 hours.

7. To calculate contact time 2ml of *Aloe vera* gel was well mixed in the beaker containing synthetic water sample and 0.1 ml of water sample was transferred in separated dried NA plate to calculate number of *E.coli* remaining in given water sample and was incubated at 44ºC for 24 hours.

Result and calculation

Number of colonies in the plate was counted.

B: Membrane Filter Method

The use of molecular or membrane filters is a very useful variation on the plate count technique. These filters have a known uniform porosity of predetermined size sufficiently small to entrap the microorganisms. This technique is particularly valuable in determining the number of bacteria in a large sample that has very small number of viable cells. The membrane with trapped organisms is then placed on a special plate containing an absorbent pad saturated with the appropriate liquid medium. Selective media can be used to make it easier to detect certain types of organisms than with the conventional plate count. Upon incubation at appropriate temperature, colonies develop on the membrane filter which is counted and total number of organisms in the sample is calculated as:

No of organisms per ml. = $\frac{No\ of\ colonies}{Amount\ of\ sample\ filtered} \times$ D.f

Fixed amount of sample /diluted sample is filtered through membrane filter with the aid of filtering apparatus (by applying negative pressure) extensively used membrane filter has pore size or diameter of 0.45µm).

The filter paper after enriching few minutes on pad can be transferred aseptically to agar medium and the incubated at appropriate temperature.

The advantages of this method over the multiple tube tests are

(i) High degree of reproducibility of results

(ii) Greater sensitivity since large volumes of sample can be used and

(iii) Shorter time of getting results

Materials required for membrane filter methods are:

- Vacuum pump
- Membrane filter assemblies (sterile)
- Sterile petri plates
- Sterile forceps

Procedures for membrane filter method:

1. First assembling of a membrane filtering unit were done as follows

- Filter holder base was inserted into the neck of a side arm flask

- With a flamed forceps, sterile membrane filter disc was placed on the filter holding base.
- The filter funnel was placed on the top of the membrane filter disc and was secured to the base with the clamp.

2. Rubber hose was attached to a vacuum pump and 100 ml of *Aloe vera* treated sample was poured.

3. Vacuum pump was disconnected, funnel was removed and carefully the filter disc was transferred with sterile forceps to the absorbent pad (keeping the grid side up).

4. EMB plate was incubated at 44˚C for 24 hours.

5. Number of colonies was counted after 24 hour

C: Batch study to determine contact time

Fixed parameters: 10 ml of synthetic water sample of different *E.coli* concentration

Here S-1, S-2 …S-21 represent sample-1, sample-2…sample-21 respectively.

Time in Hour	For 2 ml dose									For 2.5 ml dose				For 3 ml dose				For 3.5 ml dose			
	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15	S-16	S-17	S-18	S-19	S-20	S-21
Initial CFU/0.1 ml	15	27	30	33	38	55	67	73	81	20	45	58	83	20	45	58	83	20	45	58	83
0.5	13	24	25	28	31	48	59	51	73	19	43	55	79	18	41	54	75	17	38	45	73
1	12	21	24	23	31	42	55	48	73	17	40	51	72	15	37	49	69	12	29	36	64
2	11	21	23	22	30	38	47	46	70	15	37	47	67	14	34	42	61	8	22	27	50
3	11	19	23	22	27	33	44	43	69	13	33	42	60	13	29	36	55	6	17	21	41
4	10	16	21	20	23	31	39	41	63	11	29	38	53	9	23	31	47	3	11	14	29
5	9	16	19	20	18	27	35	39	60	9	25	34	47	7	20	26	39	1	7	9	16
6	9	15	19	18	17	26	30	35	58	8	22	30	40	4	15	21	32	0	3	5	7
7	8	14	18	17	14	25	27	31	53	7	19	26	34	3	10	16	25	0	2	3	4
8	7	13	17	16	12	23	24	30	51	6	17	23	29	1	5	12	18	0	1	2	3
9	7	12	14	16	12	22	21	27	48	5	15	19	24	0	3	8	13				
10	7	11	10	14	10	20	18	25	43	3	13	16	20	0	3	5	8				
11	6	10	9	12	8	18	16	23	39	2	9	13	17	0	2	4	7				
12	6	8	8	10	7	17	13	20	33	2	7	9	14								
13	5	6	7	8	6	15	11	19	29	1	5	6	10								
14	4	4	6	4	5	14	9	17	24	0	3	5	8								
15	3	4	4	4	5	12	8	15	19	0	2	4	7								
16	3	3	4	4	5	10	8	13	15												
17	2	3	4	4	5	9	8	10	13												
18	1	3	4	4	4	7	7	7	10												
19	1	3	3	3	4	6	7	7	9												
20	1	2	2	3	3	5	5	7	7												
21	1	2	2	3	1	5	4	6	7												
22	1	2	2	3	1	4	3	6	6												
23	1	2	2	3	0	3	2	6	5												
24	1	1	2	2	0	3	2	6	5												
Final CFU/0.1 ml	15	27	30	33	38	58	70	73	81	20	45	58	83	21	45	58	83	21	45	58	83
	Final CFU/0.1 ml of synthetic water after 24 hours									Final CFU/0.1ml of synthetic water after 15 hour				Final CFU/0.1ml of synthetic water after 11 hour				Final CFU/0.1ml of synthetic water after 8 hour			

Percentage removal were calculated as (Initial – Final) CFU/0.1ml / Initial CFU/0.1ml

For example for dose 2.5 ml initial CFU/0.1 ml is 20+45+58+83=206

Final CFU/0.1ml is 0+2+4+7=13

Removal percentage = (206-13) / 206*100 = 93.68%

D: Batch study to determine dose of *Aloe vera* gel

Different concentrated *E. coli* added synthetic sample was treated with dose of *Aloe vera* for 19 hour contact time. The results obtained are tabulated.

S.N.	Initial Concentration of Sample (CFU/0.1ml)	Final Concentration after treatment with Aloe vera						Final Conc. after 19 hour of sample
		0.5 ml	1 ml	1.5 ml	2 ml	2.5 ml	3 ml	
1	15	11	7	2	1	0	0	15
2	42	29	16	4	3	0	0	41
3	73	53	28	8	6	2	2	73
4	81	64	31	10	8	2	1	81

Similarly, Different concentrated *E. coli* added synthetic sample was treated with dose of *Aloe vera* for 6 hour contact time. The results obtained are tabulated.

S.N.	Initial Concentration of Sample (CFU/0.1ml)	Final Concentration after treatment with Aloe vera							Final Conc. after 6 hour of sample
		1 ml	1.5 ml	2 ml	2.5 ml	3 ml	3.5 ml	4 ml	
1	20	17	15	13	8	4	0	0	20
2	45	39	32	26	22	15	3	1	45
3	58	45	40	33	30	21	5	2	58
4	83	77	62	53	40	32	7	3	83

E: Determination of flowrate by continuous setup

a. Calculation of different flowrate

For time t_1 =6 hour

Here length of sedimentation tank is 1.2m.

v= 1.2/6=0.2m/hr

Area, a= 0.4m*0.15m= 0.06 m^2

Discharge, q= a*v = 0.2*0.06 = 0.012 m^3/hr

for velocity v_1 =0.2m/hr,

Q_1 = 0.012 m^3/hr = 12 L/hr

Similarly, for time t_2=7 hour and t_3=8 hour

Q_2= 10.28 liter/hr and Q_3 = 12.00 L/hr

b. Determining suitable Flowrate

After determining flowrate, 100ml *Aloe vera* treated sample were collected from sedimentation basin from port at 0.2m, 0.4m, 0.6m, 0.8m and 1m in a sterile bottle. Number of Colony formed was analyzed through Membrane filter technique.

Dose of aloe vera gel: 35ml

For 0-25 CFU/100ml range

S. N.	Flow rate (L/hr)	Initial Conc. of Synthetic Water Sample	Contact time (Hour)	Final Conc. at different port after treating with *Aloe vera*					Final Conc.. of Synthetic water at tank after 8 hour
				0.2m	0.4m	0.6m	0.8m	1m	
1	12.00	18	6	2	3	2	2	2	18
2	10.28	18	7	1	2	1	1	1	19
3	9.00	18	8	1	0	1	0	0	18

For 25-50 CFU/100ml

S. N.	Flow rate (L/hr)	Initial Conc. of Synthetic Water Sample	Contact time (Hour)	Final Conc. at different port after treating with *Aloe vera*					Final Conc.. of Synthetic water at tank after 8 hour
				0.2m	0.4m	0.6m	0.8m	1m	
1	12.00	45	6	7	7	7	6	6	45
2	10.28	45	7	5	4	4	4	4	45
3	9.00	45	8	4	4	4	4	3	45

For 50-75 CFU/100ml

S. N.	Flow rate (L/hr)	Initial Conc. of Synthetic Water Sample	Contact time (Hour)	Final Conc. at different port after treating with *Aloe vera*					Final Conc.. of Synthetic water at tank after 8 hour
				0.2m	0.4m	0.6m	0.8m	1m	
1	12.00	59	6	9	9	8	8	7	59
2	10.28	59	7	6	5	5	5	4	60
3	9.00	59	8	5	5	5	4	4	59

For 75-100 CFU/100ml

S. N.	Flow rate (L/hr)	Initial Conc. of Synthetic Water Sample	Contact time (Hour)	Final Conc. at different port after treating with *Aloe vera*					Final Conc.. of Synthetic water at tank after 8 hour
				0.2m	0.4m	0.6m	0.8m	1m	
1	12.00	87	6	14	14	15	14	15	87
2	10.29	87	7	10	10	10	9	10	87
3	9.00	87	8	8	9	8	8	8	87

F: Effect of pH

The pH of 10ml of synthetic water of different concentration was measured and it was applied to the *Aloe vera* dose 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml. Then the final pH was measured.

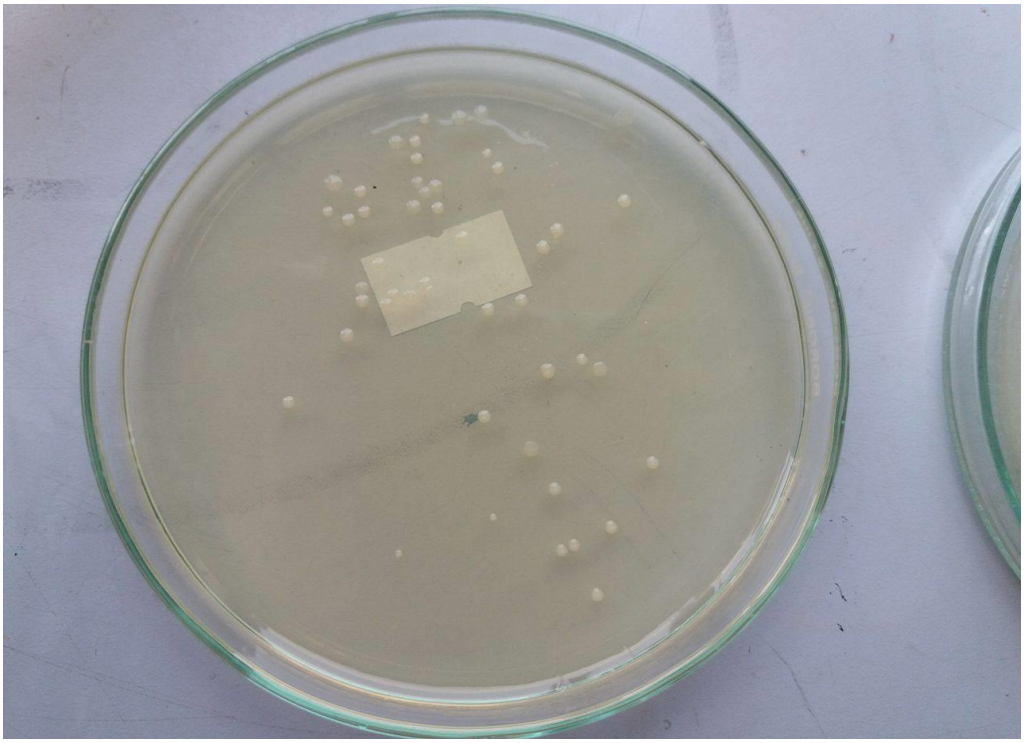
Dose of *Aloe vera* in ml	For sample 1	For sample 2	For sample 3	For sample 4
0	6.9	7.2	7.3	7.5
0.5	6.9	7.2	7.3	7.5
1	6.9	7.2	7.3	7.5
1.5	6.9	7.1	7.2	7.4
2	6.8	7.1	7.2	7.4
2.5	6.8	7.1	7.2	7.4
3	6.8	7.1	7.2	7.4
3.5	6.7	7.1	7.1	7.3
4	6.7	7.1	7.1	7.3

From table it shows that there is slight decrease in pH for different bacterial concentration load of synthetic water after addition of *Aloe vera* juice. Indeed, pH decreases from 7.5 to 7.3, 7.3 to 7.1, 7.2 to 7.1 and 6.9 to 6.7 of synthetic water after treating with 3.5 and 4 ml dose of *Aloe vera.* This decreases shows *Aloe vera* solution is slightly acidic.

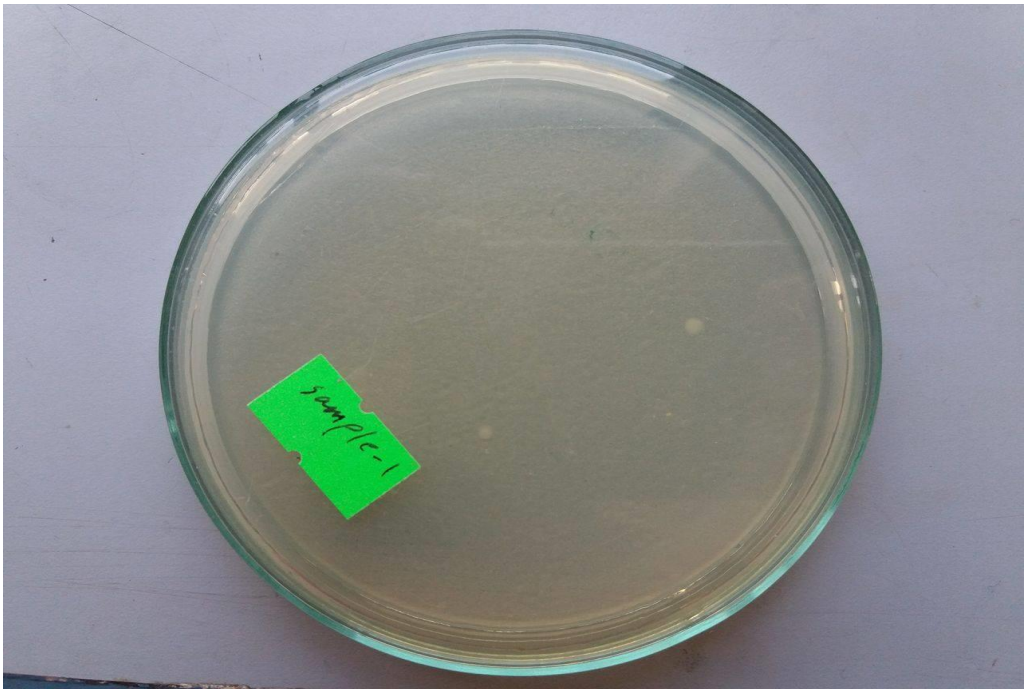
PHOTOGRAPHS

P1: Preparation of NA plate

P2: Preparation of Synthetic water sample



P3: Number of Colonies in given water Sample in NA plate



P4: Number of Colonies in given water sample after treating with *Aloe vera* juice

P5: Experimental setup for continuous process

P6: Sedimentation tank

P7: Conductivity test

P8: Membrane Filter test