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Water Disinfection Using Tulsi Leaves

by

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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 **"Water Disinfection Using Tulsi Leaves"**, submitted by Mr. Samit Thapa in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering.

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ABSTRACT

Disinfection is the important water treatment process used to remove pathogens from drinking water. In this study, effectiveness of tulsi leaves juice as a disinfectant were evaluated by spread plate and membrane filter method. Tests were conducted both for batch as well as continuous flow. In batch study, dose and contact time were evaluated. It was revealed that as the dose is increased, there is decrease in contact time and vice-versa. The effective contact time was found to be 17, 12, 9, 5 and 4 hours for the tulsi leaves juice dose 1.5, 2.0, 2.5, 3.0 and 3.5ml respectively. The optimum contact time of 5 hours for 3.0ml dose was considered for continuous flow study. In continuous flow, effectiveness of tulsi was evaluated for 0-25, 25-50, 50-75, 75-100 CFU/100ml range at flow rates 14.40, 12.00 and 10.28 litre/hr and at a distance of 0.2, 0.4, 0.6, 0.8, 1.0 m. *E. coli* removal efficiency of tulsi in continuous flow is 86.67%, 91.11% and 93.33% at lower flow rates 14.40, 12.00 and 10.28 litre/hour respectively. For 0-25 CFU/100 ml range, *E. coli* removal efficiency is highest upto 100% while for other CFU ranges 25-50, 50-75 and 75-100 CFU/100 ml ranges, *E. coli* removal efficiency is maximum upto 93.33%, 93.22%, 93.10% respectively.

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

Keywords: Tulsi, Contact time, Dose, *E. coli* removal efficiency.

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LIST OF ABBREVIATIONS

CFU	-	Colony forming Unit
Conc.	-	Concentrated
df	-	Dilution factor
DW	-	Distilled Water
E. coli		*Escherichia Coli*
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
L	-	Liter
mg	-	milligram
mg/L	-	milligram per liter
ml	-	milliliter
%	-	Percentage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Water is essential for life. The amount of fresh water on earth is limited, and its quality is under constant pressure. Preserving the quality of fresh water is important for the drinking-water supply, food production and recreational water use. Water quality can be compromised by the presence of infectious agents, toxic chemicals, and radiological hazards. The quality of drinking-water is a powerful environmental determinant of health. Assurance of drinking-water safety is a foundation for the prevention and control of waterborne diseases. (source: http://www.who.int/topics/water/en)

About 1.6 million people die every year from diarrheal diseases (including cholera) attributable to lack of access to safe drinking water and basic sanitation and 90% of these are children under 5, mostly in developing countries. Approximately 150 million people are infected with schistosomiasis causing millions of deaths yearly. (approximately 500 million people are at risk of trachoma from which 146 million are threatened by blindness and 6 million are visually impaired). Intestinal helminthes (Ascariasis, trichuriasis and hookworm infection) are plaguing the developing countries due to inadequate drinking water with 133 million suffering from high intensity intestinal helminthes infections, there are around 1.5 million cases of clinical hepatitis every year (Sadul *et al.*, 2009).

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 methods used include physical processes such as filtration, sedimentation, biological processes such as slow sand filters or biologically active carbon, 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.

Though 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, Tulsi was used for the treatment of water, but without the knowledge of dose and effect. Tulsi plant (*Ocimim Sanctum*) is shown in Figure1.1.

Figure1.1: Tulsi Plant

1.2 Rationale of the Study

Tulsi may become alternative to the chlorine, which is expensive and to be imported from other countries. Excess of chlorine is harmful to health and carcinogenic if taken for long time. When chlorine reacts with naturally occurring organic matters, unwanted chemicals known as Disinfection-by-products (DBPs) are generated which are harmful to our health.(Harikumar and Manjusha, 2012). According to recent studies, E. coli was found in normal tap water in Kathmandu valley which indicates the need of water treatment in house-hold level also. (Warner *et al.,* 2008). As Empirical studies of tap water from representative locations of Kathmandu's urban

areas have found that fecal coliform contamination in the water consistently exceeds WHO guidelines for water considered fit for human consumption. As Tulsi plant is easily available throughout the country it would be highly beneficial if it could be used as water disinfectant.

1.3 Objectives of the Study

The main objective of the study is to determine the effectiveness of tulsi plant leaves as disinfectant in raw water for drinking purpose.

The specific objectives of this study are:

- to determine the optimum dose of tulsi as a disinfectant.
- to determine the contact time for disinfection.

1.4 Limitations of the Study

Limitations of the study are:

a) Test is limited for *E. coli* only.
b) Variation of 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 water treatment and tulsi, rationale of the study, objectives 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 conclusions and recommendations regarding the whole study so that it will help for future research and study of same nature.

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

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Characteristics of Tulsi

Tulsi also known as *Ocimum sanctum*, *Ocimum tenuiflorum*, Holy basil, is an aromatic plant in the family *Lamiaceae* and widespread as a cultivated plant throughout the Southeast Asian tropics. It is an erect, many branched sub-shrub, 30–60 cm (12–24 in) tall with hairy stems and simple green or purple leaves that are strongly scented. Tulsi is cultivated for religious and medicinal purposes, and for its essential oil. It is widely known across the Indian subcontinent as a medicinal plant and an herbal tea, commonly used in Ayurveda, and has an important role within the Hinduism, in which devotees perform worship involving holy basil plants or leaves.' In both the oral and written traditions, knowledge of alternative methods of water treatment is still available. Principal among them are the Charaka Samhita and Sushruta Samhita (300 A.D.) which are the foundations of the Ayurveda, the Indian system of natural healing. According to Shiva the Sushruta samhita, Tulsi (*Oscimum sanctum*) is a water purifier with antibacterial and insecticidal properties. This is virtually costless way to render contaminated water fit for human consumption. (Sadul *et al*.,2009)

Plants are of the important sources of medicine & a large numbers of drugs in use are derived from plants. The therapeutic uses of plant are safe, economical & effective as their ease of availability . Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family *Lamiaceae* are very important for their therapeutic potentials. *Ocimum sanctum* has two varieties i.e. purple (Krishna Tulsi) and green (Rama Tulsi), their chemical constituents are similar . Tulsi is a Sanskrit word which means "matchless one". Several medicinal properties have been attributed to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine . The medicinal use of plants is very old. Literatures indicate that therapeutic use of plants is as old as 4000-5000 B.C and Chinese used first the natural herbal preparations as medicines . Earliest references are available in Rigveda which is said to be written between 3500-1600 B.C . Tulsi (*Ocimum sanctum*), Queen of Herbs, the Legendary, "Incomparable One" is one of the holiest and most cherished of the many healing and health-giving herbs distributed mainly in the oriental region .

Tulsi a widely grown, sacred plant belongs to the *lamiaceae* family. It is called by names like Rama Tulsi, Krishna Tulsi in Sanskrit and Holy Basil in English. It is found growing naturally in moist soil nearly all over the globe . In Nepal, Aryan people grow Tulsi as a religious plant in their homes, temples and their farms. They use Tulsi leaves in routine worship. Three main forms are generally recognized Rama tulsi with stems and leaves of green, Krishna tulsi with stems and sometimes also leaves of purple and Vana Tulsi which is unmodified from its wild form. Variations in soil type and rainfall may also equate to a difference in the size and form of the plants as well as their medicinal strength and efficacy. *Ocimum* genus contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia . Plants have square stems, fragrant opposite leaves and whorled flower on spiked inflorescence . (Kayastha, 2014)

2.2 Antibacterial Property of Tulsi

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. (Joshi *et al.*, 2009).

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. Natural herbs used in this study can be effectively used as a disinfectant. Using these disinfectants, pathogenic bacteria from the water can be killed and it can be made safe to use. (Harikumar and Manjusha, 2012).

Ocimum sanctum is a herbal plant having antimicrobial activity against many of the microorganisms and also has the anticancer, anti-diabetic, anti-ulcer effect, etc. Moreover the water treated with Tulsi extract serve not only as germ free but also as Medicinal water. The essential oil of basil extracted via steam distribution from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances and in traditional rituals and medicines. Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal, nematicidal and fungistatic . These properties can be frequently attributed to predominate essential oil constitutes such as methyl chavicol, eugenol linalool, camphor and methyl cinnamate. A variety of biologically active compounds such as ursolic acid, apigenin and luteolin have been isolated from the leaves. Phytochemical compounds in leaf include eugenol (volatile oil), ursolic acid (triterpenoid) and rosmarinic acid (phenylpropanoid) other active compounds includes caryphyllene and oleanolic acid. Seeds contain linoleic acid and linolenic acid. Nutritional components include vitamin A and C, minerals calcium, iron and zinc as well as chlorophyll. Tannins, alkaloids, glycosides and saponins are abundant in Tulsi. The Tulsi plant is even known to purify or de-pollute the atmosphere and also works as a repellent to mosquitoes, flies and other harmful insects. The major effects of tulsi leaves are anti-fertility effect, anti-diabetic effect, anti-allergic and immuno modulator effects, stress resilience , anti- ageing effects, anti-oxidant activity, immunity tune-up, anti-inflammatory action , antibiotic protection, lung and bronchial support, nutrition, allopathic medicine complement, antimicrobial properties (Sundarmurthi *et al*., 2012)

A variety of biologically active compounds such as urosolic acid, apigenin and luteolin have been isolated from the leaves. *Ocimum sanctum* is effective against *E.coli* and shows increase in antibacterial activity with increase in concentration and specified contact time (Sadul *et.al*., 2009).

The major population in our country is living in rural areas, where these natural herbs are easily available. The conventional method of disinfecting water are economically non feasible & also people are reluctant to use chemicals as disinfectants. The effective antimicrobial activity of plants leaf extracts is due to the synergistic effect of the active components present in plant leaves. This technique can be effective for the water obtain from water sources having low degree of contamination or else water can be given prior filtration with charcoal or fine sand to reduce the contamination load. It

remains to be seen if sunlight experiments can be combined with herbal disinfection of water to achieve complete destruction of enteric bacteria (Bhattacharjee *et al.,* 2013). The chemical constitients of Tulsi plant is shown in Table 2.1

Table2.1: Chemical constituents of Tulsi (Bhore and Gatkul, 2012)

Essential oil from leaves	Alcoholic Extract from leaves	Fixed oil form seeds	Mineral Content Per100gm
Thujene	Ursolic acid	Palmitic acid	Carotene(215mg)
Octane	Apgenin	Steric acid	Vit c (83mg)
Noname	Apignin	Linoleic Acid	Ni(0.7)
Benzene	Glucuronide	Sitosterol	Cu(0.4)
Z-(3)-hexanol-Pinene	Luteolin	Linolenodi-Linolin	Ca (3%)
Ethyl 2-methyl	Orientin	Hexouren	P (0.34%)
Butyrate Tolune	Molludistin		V (0.5 mg)
	Rosanilic Acid		Cr (3 mg)
			Fe(2.3 mg)

CHAPTER THREE

3.0 METHODOLOGY

3.1 Sample Collection and Preparation of Synthetic Water

Different drinking water sample was collected from different places of Kathmandu Valley in the sterilized 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 were prepared by swabbing the colony of *E. coli* bacteria 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.

3.2 Material and Preparation

Fresh leaves of Tulsi Plant is plucked and washed with normal tap water 2-3 times and then with distilled water. Leaves are then crushed by mortar and pestle and then extracted using distilled water. The extract is then filtered using muslin cloth. About 100ml juice was obtained from 50gm tulsi leaves.

3.3 Experimental Set up and Procedure

Experiments were conducted for both batch and continuous flow.

3.3.1 Batch study

The batch study was done in microbiological laboratory. Tulsi juice and synthetic water samples were prepared in the laboratory. Water samples were treated with different volume of tulsi juice and treated samples were tested at every one hour by Spread plate method for determining contact time and dose.

3.3.2 Continuous flow study

In this experimental set up, a continuous flow laboratory treatment unit was fabricated and used. It consists of water tank, tank with tulsi dose, mixing tank and sedimentation tank. In the mixing tank, sample water was stirred and sent to the sedimentation tank which maintains the constant flow rate. Water sample containing different concentration of *E. coli* were treated with various dose of tulsi at different flow rates. Flow chart of experimental setup is shown in Figure 3.1

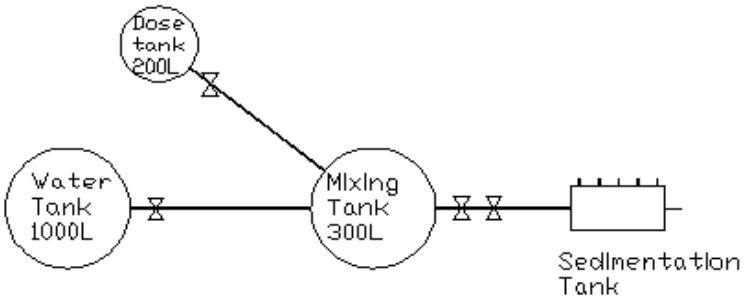


Figure:3.1 Experimental setup for continuous flow

The size of the sedimentation tank is 1.2m*0.4m*0.4m (L*B*H). It consists of seven ports including one inlet, one outlet and 5 test ports at a 20cm interval. The detail drawing of sedimentation tank is shown in Figure 3.2

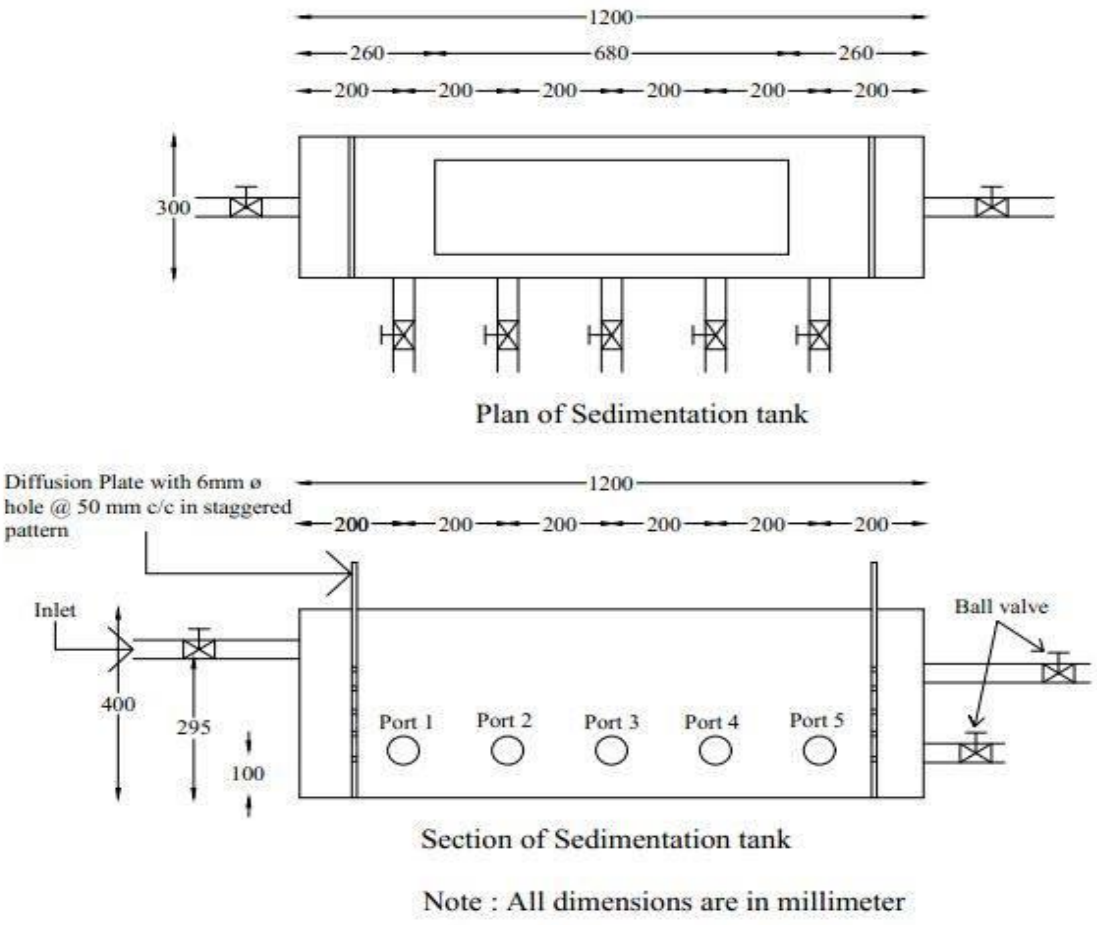


Figure:3.2 Details of Sedimentation tank

Membrane filter method was used for analysis of bacterial load. For different flow rates bacterial test were conducted. Different flow rates were maintained by controlling the opening of valve of pipe suppling mixed sample to sedimentation basin. Samples were collected in sterilized bottle through ports along the length of sedimentation basin. Membrane filter tests was conducted in microbiology laboratory. The details of the spread plate and membrane filter techniques are attached in annex (Shah *et al.*,2006). The synthetic raw water characteristics is shown in Table 3.1

Table 3.1: 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 (Ω.cm)	$1.27x10^4$

CHAPTER FOUR

4.0 Results and Discussions

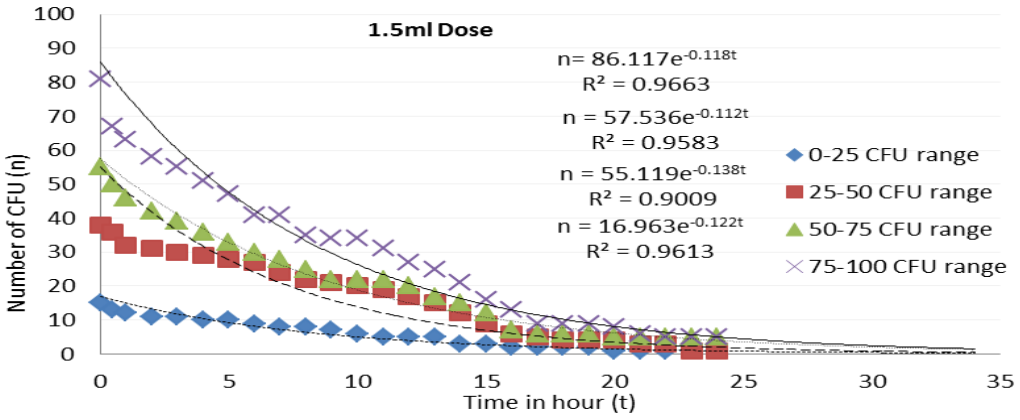
Among various parameters of drinking water only *E. coli* present in the water was considered. Hence, the experimental results of *E. coli* removal of water by the use of Tulsi juice are presented in this chapter. The treatment process has to ensure the removal of *E. coli* is in accordance to WHO guidelines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

4.1 Batch Study

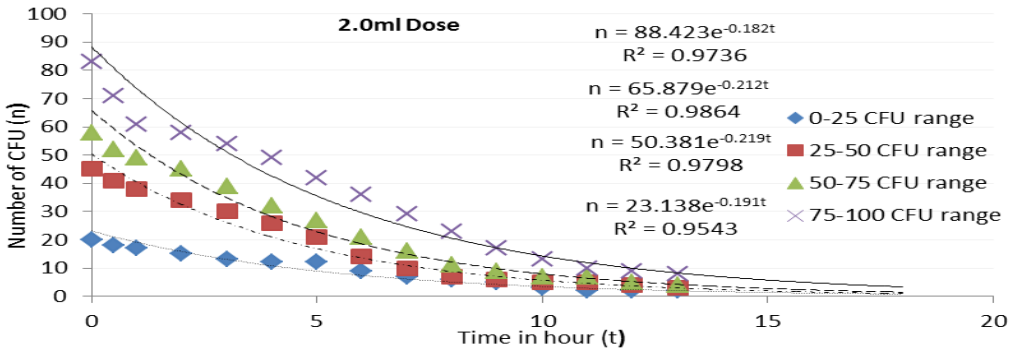
Before proceeding to continuous flow setup, first the contact time and optimum dose were found out in batch reactor in the microbiological laboratory.

4.1.1 Determination of Contact Time

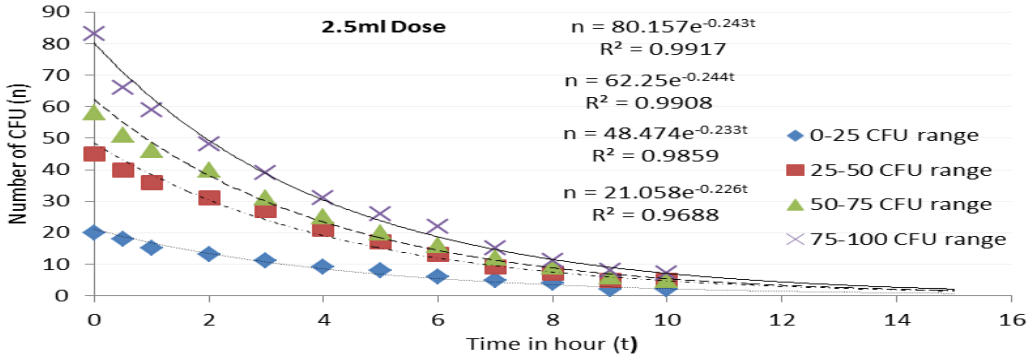
Before determining contact time, water sample collected from different places was treated with Tulsi juice to know if the juice has antibacterial property against *E. coli* bacteria. After positive result against different sample of water containing *E. coli* bacteria; test was run for different synthetic water containing *E. coli* bacteria to determine contact time. For determining contact time synthetic water was mixed with Tulsi juice and after each hour the mixed water was spread in the plate containing NA and was incubated for 24 hours at 44ºC. For different concentration of *E. coli* load at different contact time the removal of *E. coli* are shown in Figure 4.1 (a),(b),(c),(d) and (e).



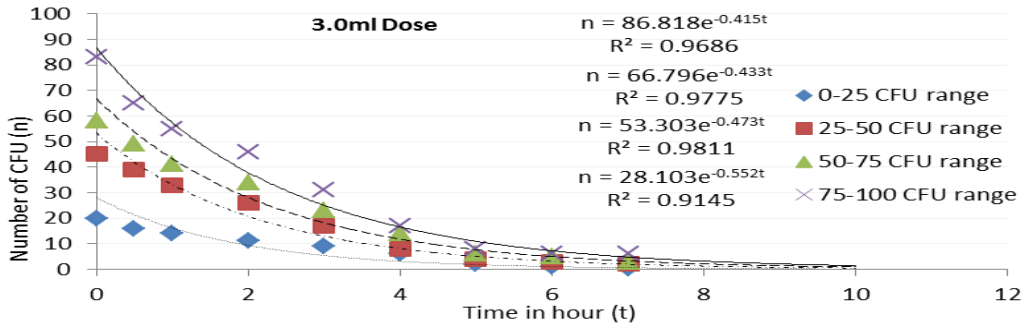
(a)


2.0ml Dose
n = 88.423e-0.182t
R² = 0.9736
n = 65.879e-0.212t
R² = 0.9864
n = 50.381e-0.219t
R² = 0.9798
n = 23.138e-0.191t
R² = 0.9543
0-25 CFU range
25-50 CFU range
50-75 CFU range
75-100 CFU range
Number of CFU (n)
Time in hour (t)

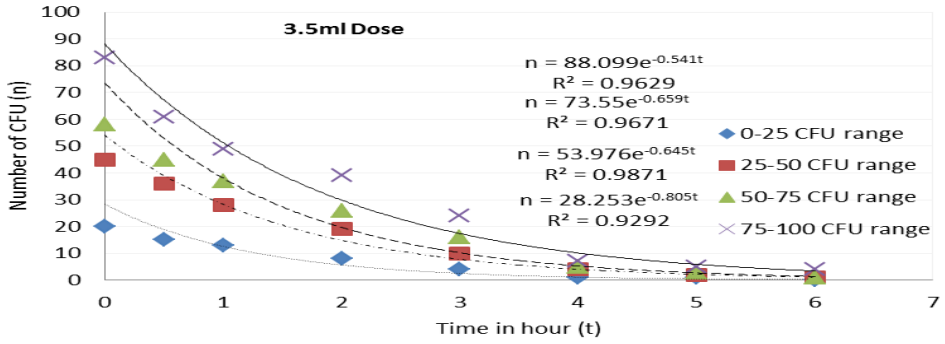
(b)


2.5ml Dose
n = 80.157e-0.243t
R² = 0.9917
n = 62.25e-0.244t
R² = 0.9908
n = 48.474e-0.233t
R² = 0.9859
n = 21.058e-0.226t
R² = 0.9688
0-25 CFU range
25-50 CFU range
50-75 CFU range
75-100 CFU range
Number of CFU (n)
Time in hour (t)

(c)


3.0ml Dose
n = 86.818e-0.415t
R² = 0.9686
n = 66.796e-0.433t
R² = 0.9775
n = 53.303e-0.473t
R² = 0.9811
n = 28.103e-0.552t
R² = 0.9145
0-25 CFU range
25-50 CFU range
50-75 CFU range
75-100 CFU range
Number of CFU (n)
Time in hour (t)

(d)


3.5ml Dose
n = 88.099e-0.541t
R² = 0.9629
n = 73.55e-0.659t
R² = 0.9671
n = 53.976e-0.645t
R² = 0.9871
n = 28.253e-0.805t
R² = 0.9292
0-25 CFU range
25-50 CFU range
50-75 CFU range
75-100 CFU range
Number of CFU (n)
Time in hour (t)

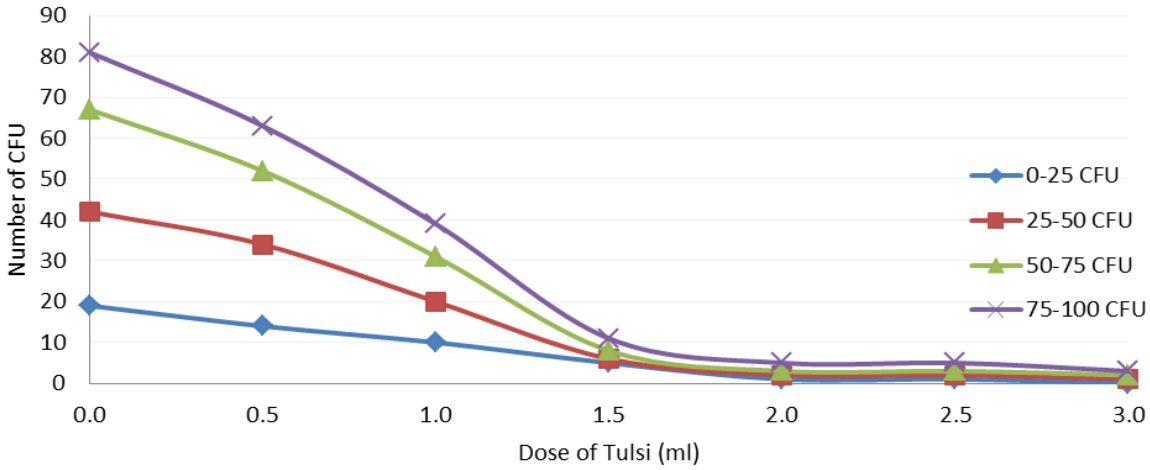
(e)

Figure 4.1 Number of *E.coli* present in water sample after using Tulsi dose (a)1.5 (b)2.0 (c)2.5 (d)3.0 and (e)3.5 ml.

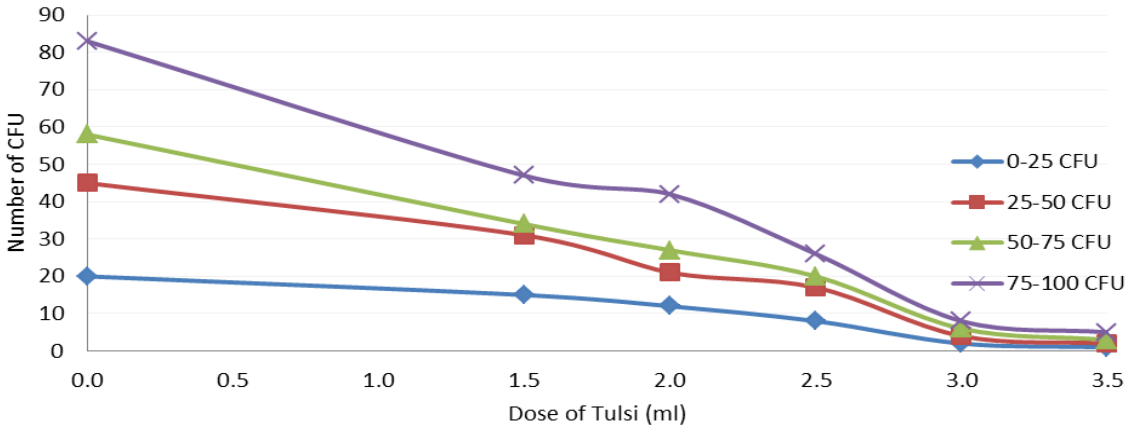
From Figure 4.1, As the contact time increases, the number of coliforms decreases. Time at which 90% removal of *E.coli* is obtained can be taken as contact time. In Figure 4.1(a), the removal of *E.coli* is significant upto 17^{th} hour whereas after that time, number of coliforms is nearly constant. Therefore the contact time was found to be 17 hours for 90% removal of *E.coli* from synthetic water sample for 1.5ml tulsi dose. Similarly, from Figure 4.1 (b), (c), (d) and (e), the contact time was found to be 12, 9, 5 and 4 hours for tulsi dose 2.0, 2.5, 3.0 and 3.5ml respectively. As the dose increases, contact time decreases.

4.1.2 Determination of Dose

After calculation of contact time, dose of tulsi juice was determined by adding different dose of tulsi juice as 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0ml in different concentration of 10ml of Synthetic water sample. Contact time for every dose was 17 hours.. Synthetic water sample of different concentration ranging from 0-25, 25-50, 50-75 and 75-100 CFU were prepared. Similarly, for contact time 5 hours different dose of tulsi leaves juice as 1.5, 2.0, 2.5, 3.0 and 3.5ml were mixed in 10ml of synthetic water sample. For different dose of Tulsi juice, reduction of *E. coli* is shown in Figure4.2(a) and (b).



(a)



(b)

Figure 4.2 *E.coli* reduction vs different dose of Tulsi juice for different CFU concentration for contact time (a) 17 and (b) 5 hours.

In figure 4.2(a), For contact time 17 hours, as the dose of tulsi is increased to 1.5ml, there is sharp decrease in number of coliforms, whereas further increase in dose, the number of coliforms is nearly constant. Hence, the dose of Tulsi juice was found to be 1.5ml per 10ml of water sample. In figure 4.2(b), for contact time 5 hours, as the dose is increased upto 3.0ml, there is significant removal of CFU whereas after 3.0ml residual CFU is nearly constant. Hence, the dose of tulsi leaves juice was found to be 3.0ml for 5 hours contact time. As the dose is increased, higher is the removal efficiency. The summary of batch study is shown in Table 4.1

Table 4.1: Summary of batch study

Dose of Tulsi as per 10ml water	Contact time
1.5ml	17 hours
2.0ml	12 hours
2.5ml	9 hours
3.0ml	5 hours
3.5ml	4 hours

The summary of batch study is also shown in Figure 4.3.

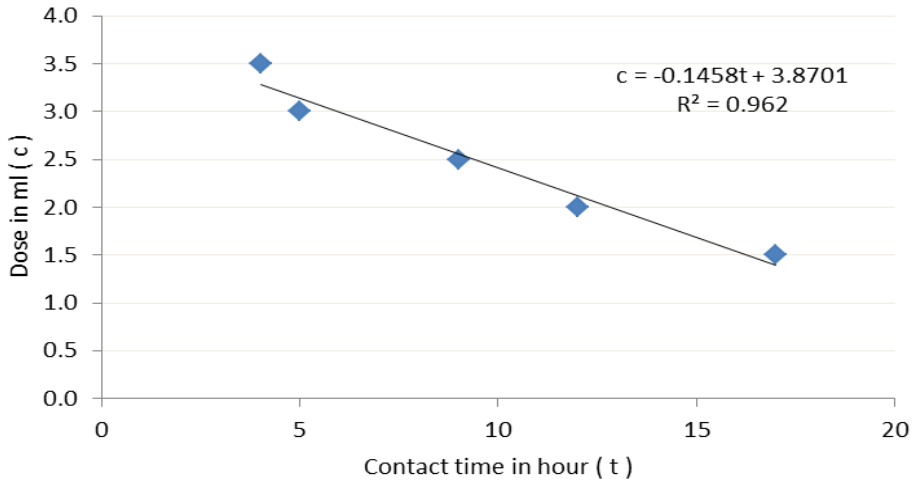


Figure 4.3 Summary of batch study

From Figure 4.3 it is found that as the dose of tulsi leaves juice is increased, the contact time decreases and vice versa.

4.2 Continuous Flow

After various laboratory works, effective dose of tulsi and minimum contact time for maximum removal of *E. coli* were found out and further tests were conducted in continuous flow model.

4.2.1 Flow rates for continuous flow

Continuous flow model was run at flow rates 14.40, 12.00 and 10.28 litre/hour for different ranges of initial CFU i.e. 0-25, 25-50, 50-75, 75-100 CFU/100 ml respectively. Samples were collected from the test ports which are located at 20cm interval. Variation of residual CFU with distance and flow rates are presented in the Figure 4.4 (a),(b),(c) and (d).

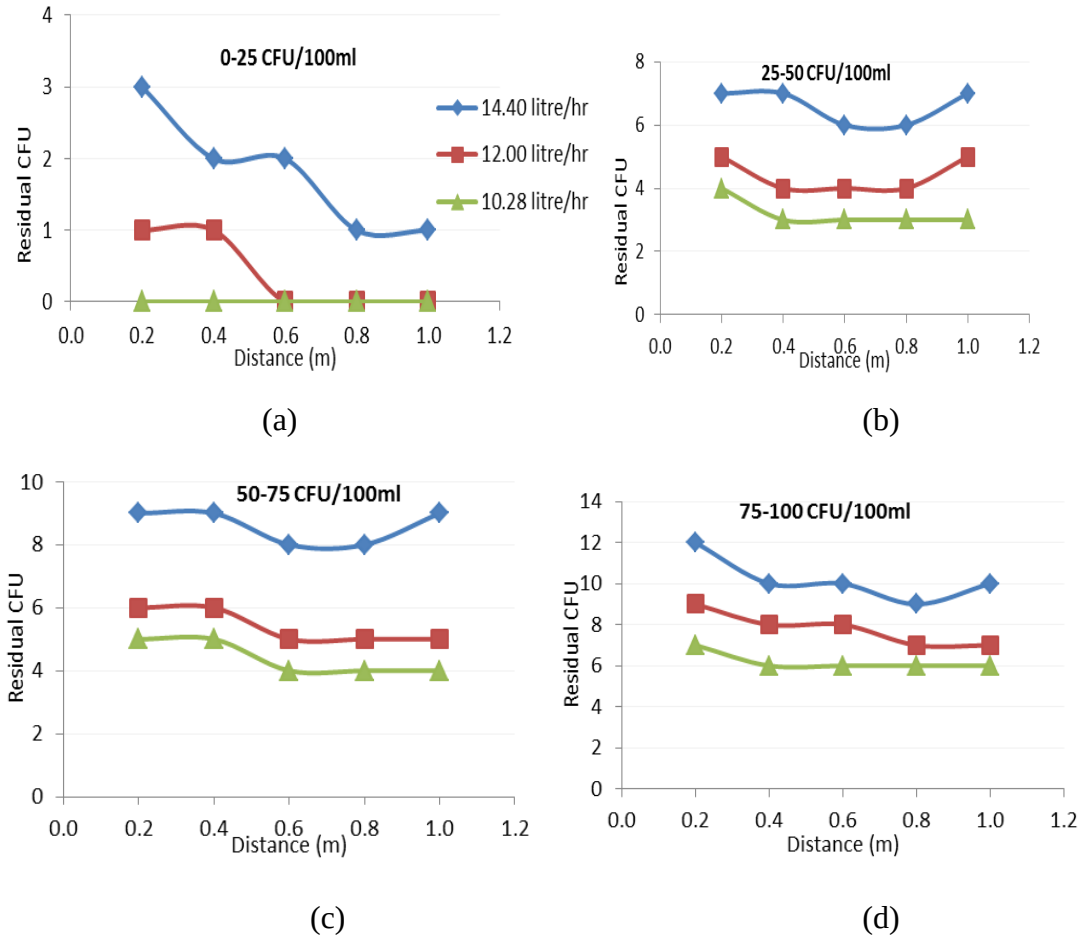
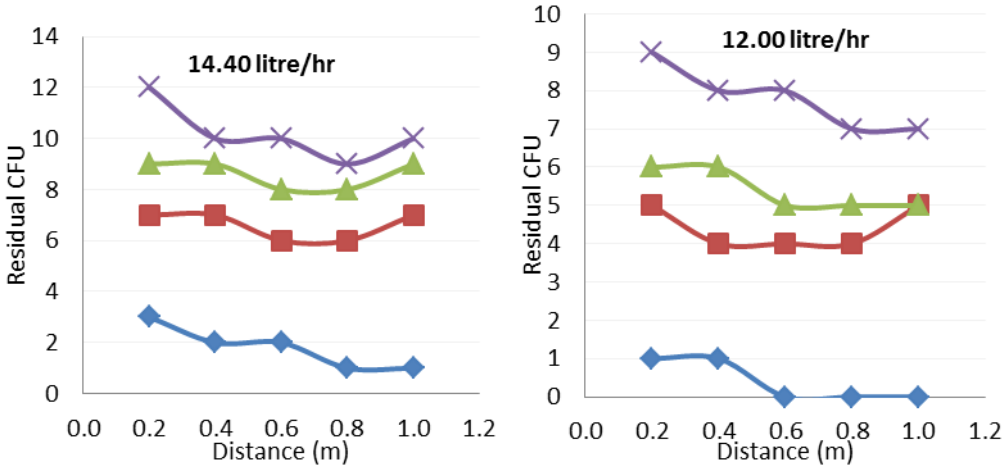


Figure 4.4 Residual CFU at different flow rates for (a)0-25 (b) 25-50 (c) 50-75 (d) 75-100 CFU/100ml range.

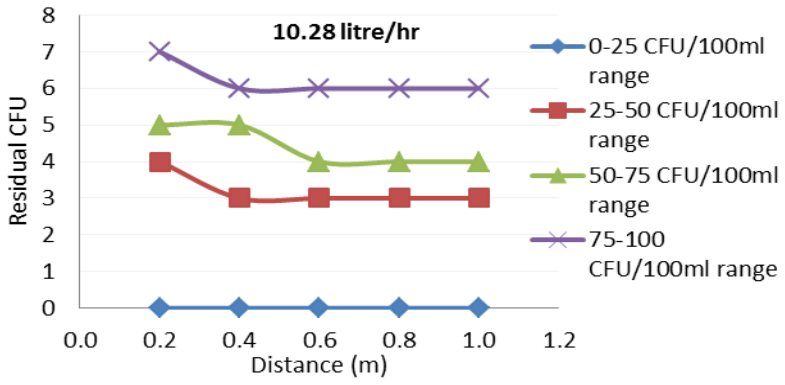
From Figure 4.3 , lower the flow rate, higher is the bacteria removal efficiency of the tulsi dose. Even for same initial CFU concentration there is different final residual CFU concentration for different flow rates. In a flow rate 14.4 litre/hr, the residual CFU is the highest whereas in flow rate 12litre/hr Residual CFU is low and at the lowest flow rate 10.28 litre/hr, the residual CFU is the lowest. Similarly residual CFU is less at distance 0.6m and 0.8m than other test ports.

4.2.2 Bacteria loads at different flow rates

Water sample containing different concentration of initial CFU were run in the continuous flow model at flow rates 14.40, 12.00 and 10.28 litre/hr. . Residual CFU corresponding to initial load were noted as shown in Figure 4.4 (a), (b) and (c).



(a) (b)



(c)

Figure 4.5 Residual CFU at various distance for different CFU/100ml range for flow rates (a) 14.40 (b) 12.00 and (c) 10.28 litre/hr.

From figure 4.4, for lower initial CFU concentration i.e. 0-25 CFU/100ml range, tulsi leaves juice dose was the most effective. The final residual CFU concentration for flow rates 12.00 and 10.28 litre/hr has decreased upto zero. Samples taken from test ports 0.6m and 0.8m shown best results.

4.3 Cost Analysis

For the economic analysis, synthetic water sample containing *E. coli* was treated with tulsi leaves juice and piyush (0.5% chlorine) available in local market in the batch test at the optimum dose of tulsi leaves juice and piyush respectively. The cost involved in both treatment processes was analyzed. The cost analysis is presented as in Table 4.2

Table 4.2 Cost comparison of tulsi leaves juice and piyush

S.N.	Description	Tulsi leaves juice	Piyush
1	Cost for 100ml	Rs 1.3	Rs 33.33
2	Volume required to reduce 18 CFU/100ml to 0 in 100ml synthetic water sample	25 ml	1 ml
3	Total cost per liter	=Rs1.3/100*25*10 =Rs 3.25/ltr	=Rs 0.33*1*10 =Rs 3.3/ltr

From the Table 4.2, for lower CFU range tulsi leaves juice is even more economic than piyush for water disinfection.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the research conducted the following conclusions can be drawn:

- As the dose is increased, the contact time decreases. For 1.5 ml dose of tulsi leaves, the contact time was found to be 17 hour for *E. coli* removal. When dose is increased to 2.0, 2.5, 3.0 and 3.5 ml of tulsi leaves juice, the contact time reduced to 12, 9, 5 and 4 hours respectively.
- In continuous flow, *E. coli* removal efficiency increases as the flow rate decreases. *E. coli* removal efficiencies found to be 86.67%, 91.11% and 93.33% at the flow rates 14.40, 12.00 and 10.28 litre/hour respectively.
- For lower CFU concentration, the tulsi leaves juice is more effective. For 0-25 CFU/100ml range, *E. coli* removal efficiency was maximum upto 100% whereas for 25-50, 50-75, 75-100 CFU/100ml range, *E. coli* removal efficiency is maximum upto 93.33%, 93.22% and 93.10% respectively.

5.2 Recommendations

The recommendations made from the study are:

- The results from this study showed that the tulsi juice have potential to be used as disinfectant for lower bacterial load and further studies should be conducted in higher bacterial loads
- The contact time and dose for tulsi leaves juice are comparatively very high than that of chlorination. If the antibacterial components of tulsi can be extracted then the contact time and dose could be reduced significantly.
- It is also recommended to study the tulsi leaves juice effect on turbidity of water.

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APPENDICES

A. Equipment and Materials

1. Equipment

   Autoclave
   Incubator
   Hot air oven
   Gas burner
   Inoculating wire and loops

2. Microbiological Media (HiMedia)

   Nutrient Agar
   Nutrient Broth
   MacConkey
   EMB

3. Glass wares

   Beakers
   Measuring cylinder
   Conical flask
   Petridishes
   Glass rods
   Reagent bottles
   Glass slides
   Test-tubes

B. 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 Tulsi juice 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.

C. 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:

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

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

- Vacuum pump or water faucet aspirators,
- Membrane filter assemblies (sterile)
- Sterile petri plates
- Sterile forceps

Procedure

1. With a flamed forceps, transfer a sterile absorbent pad to a sterile petri plate
2. Pour about 2ml of broth to absorbent pad
3. Assemble a membrane filtering unit as follows
    a. Aseptically insert the filter holder base into the neck of a side arm flask

b. With a flamed forceps place sterile membrane filter disc, grid side up, on the filter holding base.
c. Place the filter funnel on the top of the membrane filter disc and secure it to the base with the clamp.

4. Attach the rubber hose to a vacuum source(pump or water aspirator) and pour the measured amount of sample.
5. Rinse the inner side of funnel with some amount of distilled water.
6. Disconnect vacuum source, remove the funnel and carefully transfer the filter disc with sterile forceps to the absorbent pad(keep the grid side up).
7. Incubate at 37˚C for 24 hours.
8. Count the number of colonies and calculate the total number of organisms (cfu.)per ml. of given sample

Precaution

- Keep the grid side up.
- Do not invert the plate during incubation
- Do not scratch the filter by handing it with the forceps

D. Batch study to determine contact time

S → Sample

Time(	CFU concentration at 1.5ml dose									at 2.0 ml dose				at 2.5 ml dose				at 3.0 ml dose				at 3.5 ml dose			
hr)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25
0	15	27	30	33	38	55	67	73	81	20	45	58	83	20	45	58	83	20	45	58	83	20	45	58	83
0.5	13	25	28	31	36	50	60	64	67	18	41	52	71	18	40	51	66	16	39	49	65	15	36	45	61
1	12	23	26	29	32	46	54	57	63	17	38	49	61	15	36	46	59	14	33	41	55	13	28	37	49
2	11	23	26	29	31	42	52	50	58	15	34	45	58	13	31	40	48	11	26	34	46	8	19	26	39
3	11	22	25	28	30	39	50	48	55	13	30	39	54	11	27	31	39	9	17	23	31	4	10	16	24
4	10	21	25	28	29	36	50	45	51	12	26	32	49	9	21	25	31	6	8	14	17	1	4	5	7
5	10	20	24	28	28	33	46	45	47	12	21	27	42	8	17	20	26	2	4	6	8	1	2	3	5
6	9	19	24	26	27	30	40	44	41	9	14	21	36	6	13	16	22	1	3	5	6	0	1	1	4
7	8	18	24	23	24	28	38	39	41	7	10	16	29	5	9	12	15	0	2	3	6				
8	8	18	21	22	22	25	34	39	35	6	7	11	23	4	7	9	11								
9	7	17	19	18	21	22	31	37	34	5	6	9	17	2	5	6	8								
10	6	17	18	18	20	22	29	33	34	3	5	7	13	2	5	5	7								
11	5	15	16	16	19	22	28	32	31	2	5	7	10												
12	5	13	13	14	17	20	25	30	27	2	4	5	9												
13	5	11	10	12	15	17	22	15	25	2	3	4	8												
14	3	9	10	12	12	15	18	13	21																
15	3	7	7	9	9	12	14	10	16																
16	2	4	5	8	6	7	10	8	13																
17	2	3	3	4	5	6	6	8	9																
18	2	3	3	4	4	6	6	7	9																
19	2	3	3	4	4	6	6	6	9																
20	1	3	3	4	4	5	6	6	8																
21	1	2	3	4	3	5	5	5	6																
22	1	2	3	3	3	5	5	5	5																
23	1	2	2	3	1	5	5	4	5																
24	1	2	2	3	1	5	4	1	5																

E. Batch study to determine dose

For 17 hours contact time

For Dose Calculation							
	Initial Concn	Final Concn at					
Sn		0.5ml	1ml	1.5ml	2ml	2.5ml	3ml
1	9	6	5	2	0	0	0
2	15	11	8	3	1	0	0
3	19	14	10	5	1	1	0
4	27	21	12	5	2	2	1
5	35	29	15	6	3	3	1
6	42	34	20	6	2	2	1
7	55	42	27	8	2	2	1
8	67	52	31	8	3	3	2
9	73	59	35	10	3	2	1
10	81	63	39	11	5	5	3
11	90	66	47	15	7	4	4

For 5 hours contact time

Sn.	Initial Concn (CFU)	Final Concn at (CFU)				
	0	1.5 ml	2 ml	2.5 ml	3 ml	3.5 ml
1	20	15	12	8	2	1
2	45	31	21	17	4	2
3	58	34	27	20	6	3
4	83	47	42	26	8	5

F. Continuous flow study for different bacteria at various loading and flow rates

a. Calculation of different flowrate

For time t_1 =5 hour

Here length of sedimentation tank is 1.2m.

v= 1.2/5=0.24m/hr

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

Discharge, q= a*v = 0.06*0.24 = 0.0144 m^3/hr = 14.40 litre/hr

Similarly, for time t_2=6 hour and t_3=7 hour

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

b. Determining suitable Flowrate

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

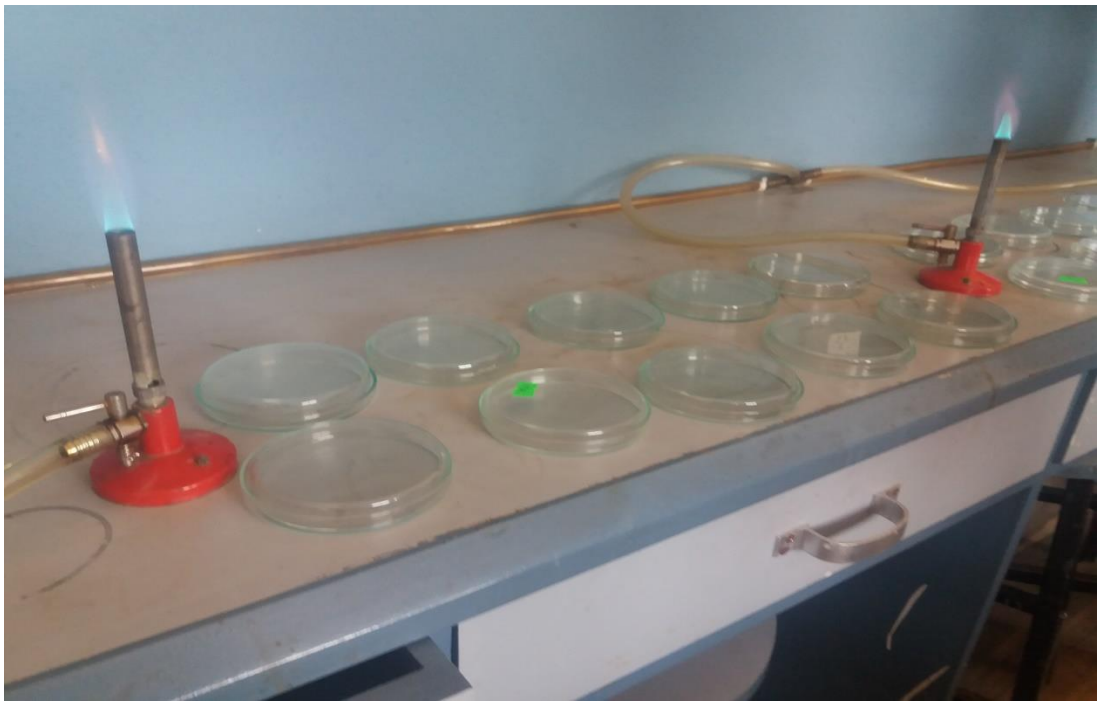
Sn	Initial CFU	Flow Rate	Contact time(hr)	Final CFU count from test ports				
				0.2	0.4	0.6	0.8	1.0
1	18	14.40	5	3	2	2	1	1
2	18	12.00	6	1	1	0	0	0
3	18	10.28	7	0	0	0	0	0

Sn	Initial CFU	Flow Rate	Contact time(hr)	Final CFU count from test ports				
				0.2	0.4	0.6	0.8	1.0
1	45	14.40	5	7	7	6	6	7
2	45	12.00	6	5	4	4	4	5
3	45	10.28	7	4	3	3	3	3

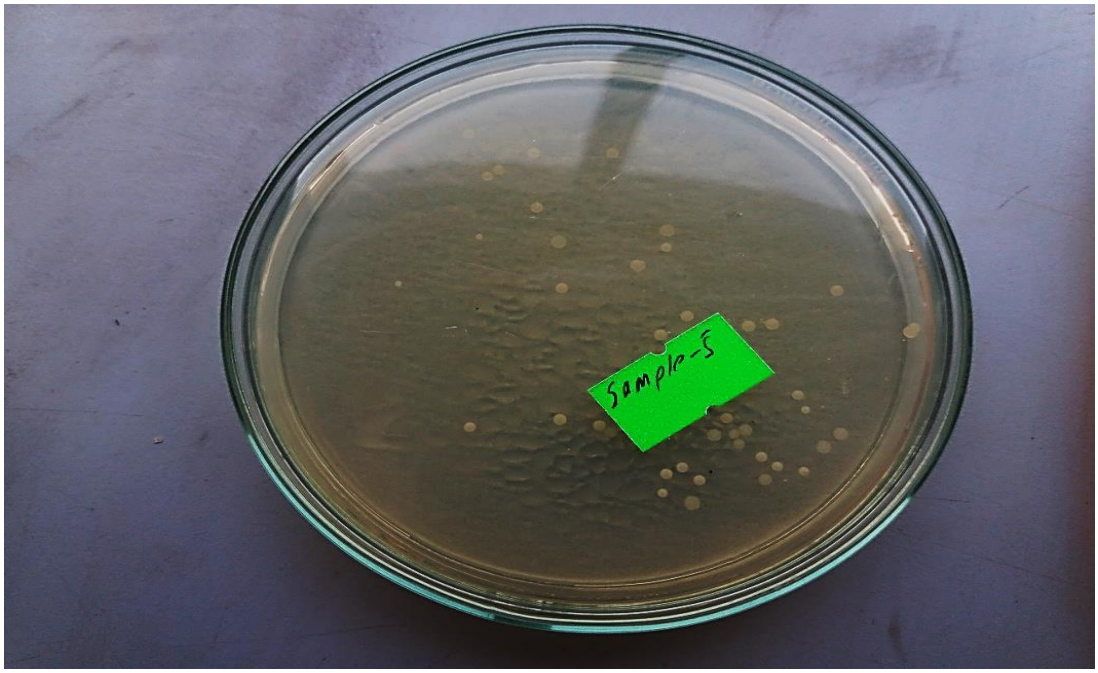
Sn	Initial CFU	Flow Rate	Contact time(hr)	Final CFU count from test ports				
				0.2	0.4	0.6	0.8	1.0
1	59	14.40	5	9	9	8	8	9
2	59	12.00	6	6	6	5	5	5
3	59	10.28	7	5	5	4	4	4

Sn	Initial CFU	Flow Rate(L/hr)	Contact time(hr)	Final CFU count from test ports				
				0.2	0.4	0.6	0.8	1.0
1	87	14.40	5	12	10	10	9	10
2	87	12.00	6	9	8	8	7	7
3	87	10.28	7	7	6	6	6	6

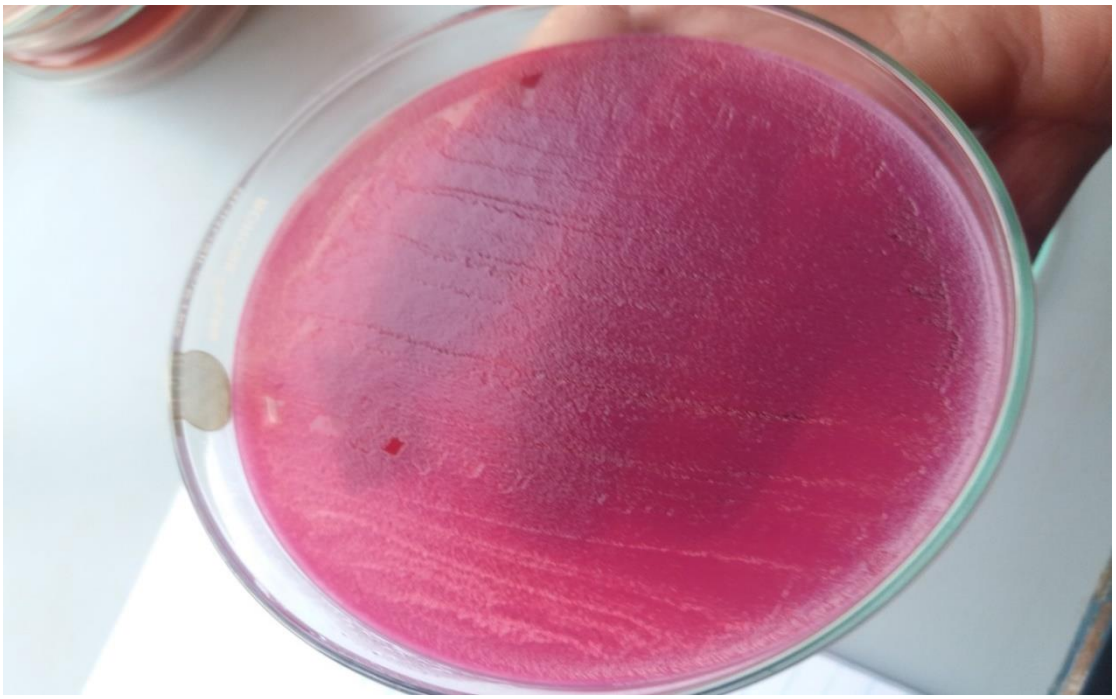
PHOTOGRAPHS



P1: Preparation of NA plate



P2: Number of CFU in water sample in NA plate



P3: *E. coli* culture in EMB agar plate

P4: Membrane Filter test

P5: Experimental setup for continuous flow study

P6:Determination of water characteristics using conductivity meter and pH meter