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Research Paper

## TREATMENT OF DOMESTIC WASTE WATER USING NATURAL FLOCCULANTS

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Wastewater generated from wastewater treatment plant contains odors some non pathogens, fecal Coliforms, fungus etc., and chemicals such as phenols, nitrate, chloride etc., in addition to usual constituents such as urine and faeces. The present investigation was hence made in wastewater treatment plant situated in Kallaraimedu of Kodaikanal. Presently the waste water is passing through an underground system to the wastewater plant. In this study analyze the Physio-chemical parameters and biological parameters of septic water before and after treatment with horse and cow dung; and portable water. Isolate and identify the microbes present in horse and cow dung by using biochemical tests.

**Keywords:** Waste water, microbial treatment of waste, Flocculants

### INTRODUCTION

Wastewater can be defined as the flow of use water discharged from homes, businesses, industries, commercial activities and institutions which are directed to treatment plants by a carefully designed and engineered network of pipes. This wastewater is further categorized and defined according to its sources of origin as 1. domestic or sanitary wastewater and 2. Industrial wastewater

Pathogens present in waste water include bacteria, viruses, prions and parasitic worms and Non-pathogenic bacteria. Organic particles such as faeces, hairs, food, vomit, paper fibers, plant material, humus, etc.; Soluble organic material

such as urea, fruit sugars, soluble proteins, drugs, pharmaceuticals, etc.; Inorganic particles such as sand, grit, metal particles, ceramics, etc.; Soluble inorganic material such as ammonia, road-salt, sea-salt, cyanide, hydrogen sulfide, thiocyanates, thiosulfates, etc.; Animals such as protozoa, insects, arthropods, small fish, etc.; Macro-solids such as sanitary napkins, nappies/diapers, condoms, needles, children's toys, dead animals or plants, etc.; Gases such as hydrogen sulfide, carbon dioxide, methane, etc.; Emulsions such as paints, adhesives, mayonnaise, hair colorants, emulsified oils, etc.; Toxins such as pesticides, poisons, herbicides, etc., are the constituents of waste water.

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## SEWAGE TREATMENT

By definition, treatment process means a series of actions or changes. Treatment facilities incorporate numerous processes which in combination achieve the desired water quality objectives. These processes involve the separation, removal and disposal of pollutants present in the wastewater. The treatment of wastewater is accomplished by four basic methods or techniques; physical, mechanical, biological and chemical. Most wastewater is treated in industrial-scale wastewater treatment plants (WWTPs) which may include physical, chemical and biological treatment processes. However, the use of septic tanks and other On-Site Sewage Facilities (OSSF) is widespread in rural areas, serving up to one quarter of the homes in the U.S. The most important mechanical aerobic treatment system is the activated sludge process, based on the maintenance and reconciliation of a complex biomass composed of micro-organisms able to absorb and adsorb the organic matter carried in the wastewater. Anaerobic processes are widely applied in the treatment of industrial wastewaters and biological sludge. Some wastewater may be highly treated and reused as reclaimed water. In some waste waters ecological approaches using reed bed systems such as constructed wetlands may be appropriate. Modern systems include tertiary treatment by micro filtration or synthetic membranes. After membrane filtration, the treated wastewater is indistinguishable from waters of the natural origin of drinking quality. Nitrates can be removed from wastewater by microbial denitrification, for which a small amount of methanol is typically added to provide the bacteria with a source of carbon. Ozone Waste Water Treatment is also growing in popularity, and

requires the use of an ozone generator, which decontaminates the water as Ozone bubbles percolate through the tank.

Treated wastewater can be reused as drinking water, in industry (cooling towers), in artificial recharge of aquifers, in agriculture and in the rehabilitation of natural ecosystems. In order to achieve ecological wastewater treatment, ecological engineering, a closed-loop treatment system is recommended. Many present day systems are a "disposal-based linear system". The traditional linear treatment systems must be transformed into the cyclical treatment to promote the conservation of water and nutrient resources. Using organic waste nutrient cycles, from point-of-generation to point-of-production, closes the resource loop and provides an approach for the management of valuable wastewater resources. Chemical treatment methods enhance the efficiency of other process operations and provide specialized treatment as a result of their addition at various treatment stages. The commonly used physiochemical processes that require chemical action precipitation, chemical oxidation, carbon adsorption, ultra filtration, reverse osmosis, electro dialysis, volatilization and gas stripping. They are either very pollutant specific, or more expensive than dumping and using more water.

The action of bacteria and other micro-organisms are biological methods of treatment, which play a vital role in the removal of pollutants which cannot be effectively achieved by other means.

## SEPTIC TANK TREATMENT BY BACTERIA

Although there are many methods used, there are numerous concerns raised regarding the

presence of constituents including heavy metals, pathogens and other toxic substances. This requires the selection of the correct disposal method focusing on efficient and environmentally safe disposal. New technologies are being produced to assist in the treatment and disposal of sewage sludge, conforming to strict environmental regulations. One of these new technologies being proposed is the use of Effective Microorganisms (EM).

Bacteria are living organisms, part of the earth's biological system. In a septic system bacteria such as *Bacillus subtilis* aid in digesting waste matter inspects the system approximately every three years. The sludge is pumped when necessary to maintain a proper operating system to provide a healthy environment for bacteria to achieve optimum population levels and proper operation within the septic tank.

The septic tank processes convert the organic nitrogen compounds into ammonium; however no conversion of ammonium to nitrate is expected to occur in the septic tank. Since in the septic tank phosphorus concentration is high wastewater coliform bacteria are not removed because reduction of ammonium to nitrate does not occur.

## **MATERIALS AND METHODS**

### **Flocculant Collection and Analysis of Flocculant**

Flocculant was collected from Kodaikanal area between Jan-Feb. 2011. Horse dung was used as Flocculant1 and cow dung was used as Flocculant2.

### **Isolation and Identification of Microbes Present in Horse and Cow Dung**

Microbes present in the flocculants were isolated

and identified by using selective and differential media. MR-VP medium (Methyl Red Voges Proskauer), nutrient agar, nutrient broth, tryptic soy agar, starch agar, Mannitol salt agar, Simmon Citrate agar, lactose Broth were used. The pour plate technique was used for bacterial enumeration and determines the bacterial count in a milliliter or gram of a specimen. After incubation, colonies appearing on the agar are counted. Each colony represents one colony forming unit (CFU). The CFU/ml or CFU/g is then calculated. Gram staining was used to distinguish between gram positive and gram negative bacteria.

## **EXPERIMENTAL DESIGN**

### **Sample Preparation**

Two litres of septic water samples were collected from water treatment plant, Kallaraimedu, Kodaikanal and dispensed into 4 conical flasks. The volume of sample in each conical flask was 500ml. The first conical flask with the septic water sample was not treated Septic water untreated (SWUT). 5g of Horse dung was weighed and added into a conical flask containing 500ml of the sample. This was named as septic water treated Flocculant1 (SWTS 1). 5g of Cow dung was weighed and added into a conical flask containing 500ml of the sample. This was named as septic water treated Flocculant2 (SWTS 2). The known Bactizyme was used as an internal positive control it was weighed 5g and added to the sample (SWTS 3). The 500ml of distilled water with no dung was kept as Negative control (SWNC). Potable water was used as Standard (SWSS).

### **Analysis of Septic Water**

The collected samples were subjected to

systemic analysis of the below stated physiochemical parameters, biological testing and microbial characteristics noted as Day 0. The analysis was continued for 20 consecutive days. The samples were tested for the following physiochemical parameters pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Solids (TS), Chloride, Nitrate. The septic water treated samples (using Flocculant 1 and Flocculant 2) were serially diluted and microbial analysis was done for bacteria - Total viable count (TVC) Presumptive test, confirmed test, completed test. Faecal coliform (FC) was tested and the number of colonies on the EMB agar plates were counted and recorded as CFU/ml. Staphylococcus was also tested (Cauppuccino, 1992). Total fungal count (TFC) was performed as per the procedure outlined in the Microbiology Laboratory Manual (Cappuccino, 1992). The fungal colonies were counted and recorded as CFU/ml.

## RESULTS AND DISCUSSION

### pH

The sample from SWUT remained acidic. However the change of pH in septic tank was observed to be very rapid in treatment time from day 0 to 20 days. The water slowly moved towards neutral pH. Low pH in elevated temperature, polymerization and condensation reactions take place resulting in the reduction of soluble and insoluble materials. Reduction of pH is due to chlorine because it degrades the volatile fatty acids (Baloch and Akunna., 2003 and Mohammed *et al.*, 2008).

### Total Suspended Solids

The total Suspended Solids in the samples of the treatment system decreased from 0.50g to 0.03g septic tank from Day0 to Day 20.

### Total Dissolved Solids

The total Dissolved Solids of the samples of the treatment system was maintained at from SWUT from 0.01g. The same levels were maintained in standard, controls in all the day of observation. TDS was contributed predominantly by chlorides, Sulphates, phosphates, Mg, Ca, Na, K etc. High level of salts could adversely affect the biological treatment process. Some of them are reported to remain unchanged during the treatment process (Vishnu *et al.*, 2008).

### Chloride

Chloride content gradually decreased from Day 0 to Day 20. In Day 0 untreated septic sample chloride content is 295.13mg/l and in day 20 it is 200.22mg/l. This is due to the inoculation of the Flocculants which act on compounds present in the sample. The increasing value of chloride might be due to the precipitation of chlorides of Calcium and Aluminium phosphate on hydrous aluminium oxide and the presence of more suspended solids (Ngtez *et al.*, 1999; Rashed and Soltan, 2002).

### Nitrate

Nitrate content gradually decreased from Day 0 to Day 20. Due to low hydraulic retention time (HRT) in the septic tank, ammonium oxidizing bacteria could not get sufficient time to produce effects. Active by passing of the waste water in septic tank resulted in high concentrations of  $\text{NH}_3$  and  $\text{HNO}_2$  (Manipura and Burgess, 2008).

Nitrogen is considered as one of the most important nutrient causing water pollution and removal of compounds having nitrogen is considered as the major activity in waste water treatment (Koren *et al.*, 2000 and Kasia *et al.*, 2005). Nitrogen was reduced much when treated

with SWTS 1, SWTS 2 and SWTS3. Decrease or increase in nitrogen content is due to bacteria which requires carbon source. These microbes use NO<sub>3</sub>-N as the terminal electron acceptor in the absence of oxygen (Zhao *et al.*, 1999). The organic nitrogen of waste water in the present investigation was observed to be increasing and gradually decreases due to the conversion of organic bound nitrogen in to an ammonium compound.

### Microbiological Parameters for Potential Faecal Pathogens

#### Faecal Coliform

The Higher Faecal Coliform rate was noticed in Day 0 and it was gradually decreased from Day1 to Day 20.

#### Staphylococcus

On day 0, 2-3 colonies were noticed and after treatment effectively from Day 1 to Day 20 no staphylococcal colonies were obtained. There is no fermentation in lactose broth and no color change from Day 0 to Day.

#### Fungal Count

The total Viable Count of bacterial colonies present in the sample. The viable count of fungi shows reduction in treatment from Day 1 to Day 20. The fungal colonies were identified based on conidial structure - *Candida* Sp, *Aspergillus*, *Mucor*.

### Identification and isolation of microbes from flocculants

Flocculant collected was used for isolation of microbes. The cultures F 1A, F 1B, F 1C and F 1F are horse dung isolates and F 2A, F 2B, F 2C and F 2F are cow dung isolates were identified by colony morphology, growth characteristics and biochemical tests.

### Identification

The group of cultures were grown in differential and specialized media for the growth of specific microbe. The cultures identified as

Flocculant collected was used for isolation of microbes. The culture F 1A (*Pseudomonas* sp), F 1B (*Bacillus subtilis*), F 1C (*Enterococcus* sp), F 1F (*Aspergillus* sp), F 2A (*Bacillus subtilis*), F 2B (*Streptococcus* sp), F 2C (*Lactobacillus* sp), and F 2F (*Candida* sp) are non pathogenic naturally occurring microbes present in horse dung and cow dung and are antibiotic resistant that destroy the majority of human pathogenic organisms in waste water through biological competition (Gerba *et al.*, 1980; Ward *et al.*, 1984 and Placha *et al.*, 2008). The data obtained from the existing treatment system of waste water indicate that the treated wastewater match with positive control and standard.

The main species involved in effective waste water treatment include Lactic acid bacteria – *Lactobacillus plantarum*, *L. casei*, *Streptococcus lactis*; Photosynthetic bacteria – *Rhodospseudomonas palustris*, *Rhodobacter spaeroides*; Yeasts – *Saccharomyces cerevisiae*, *Candida utilis* Actinomycetes – *Streptomyces albus*, *S. griseus*; Fermenting fungi – *Aspergillus oryzae*, *Mucor hiemalis* (Diver, 2001). The basis for using these EM species of microorganisms is that they contain various organic acids due to the presence of lactic acid bacteria, which secrete organic acids, enzymes, antioxidants, and metallic Chelates (Higa and Chinen, 1998). This study has identified microbes in Flocculants 1 are *Pseudomonas* sp., *Bacillus subtilis*, *Enterococcus* sp., *Aspergillus niger*, and in Flocculant 2 are *Bacillus subtilis*, *streptococcus* sp., *Lactobacillus* sp., *Candida* sp.

Organic materials within wastewater originate from plants, animals or synthetic organic compounds, and enter wastewater via a number of routes including human wastes, detergents, and industrial sources (Taylor *et al.*, 1997). In the current wastewater treatment process (either municipal or domestic on-site) microorganisms play a significant role in the treatment of domestic sewage. Many different organisms live within the wastewater itself, assisting in the breakdown of certain organic pollutants (Taylor *et al.*, 1997). The creation of an antioxidant environment by microbes assists in the enhancement of the solid-liquid separation, which is the foundation for cleaning water (Higa & Chinen 1998).

Research revealed that *Aspergillus niger*, *Trichoderma harzianum*, *Bacillus cereus* and *Bacillus subtilis* isolated from cow dung can reduce the growth of *Sclerotium rolfsii*, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Helminthosporium maydis* and *Rhizoctonia solani* with inhibitory zones of up to 58%. Furthermore, *B. subtilis* isolated from cow dung can enhance plant growth, sulfur oxidation, phosphorus solubilisation and was found to produce industrial enzymes such as amylase and cellulase (Swain and Ray, 2006).

## CONCLUSION

SWTS 1 and SWTS 2 were found to be Potential Flocculant for treating waste water. Both F 1 and F 2 were found to have *Bacillus subtilis*. Other microbes found were *Pseudomonas sp.*, *Enterococcus sp.*, *Aspergillus niger.*, *Streptococcus sp.*, *Lactobacillus sp.* and *Candida sp.*. This study concluded that the microbes present in SWTS 1 and SWTS 2 are known to treat waste water. SWTS 1 have the best activity when compared to SWTS 2. Hence we conclude from

tables and figures shown above that effect of SWTS 1 and SWTS 2 have the best activity. Further studies need to reduce the treatment time. The effectiveness of individual microbes identified in this study in waste water treatment should be evaluated.

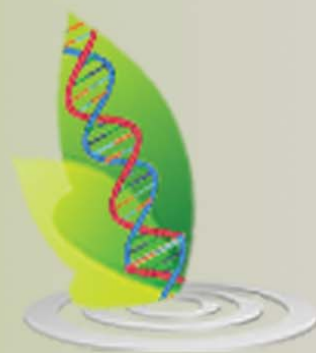
## REFERENCES

1. A Subset of Naturally Isolated Bacillus Strains Show Extreme [www.ncbi.nlm.nih.gov/pubmed/20626457](http://www.ncbi.nlm.nih.gov/pubmed/20626457)
2. An Introduction to bacterial Identification Page 1: General Principles this Page Amplifies Material Discussed in our UW-Madison Microbiology 102 laboratory courses.
3. Bsci424—Pathogenic Microbiology - Fall 2000 <http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/CatalaseTest.htm>
4. Cappuccino G J (1992), *Microbiology- A Laboratory Manual*, 3<sup>rd</sup> Edition Benjamin, Cumming Publishing Company Inc., Newyork.
5. Carl Lamanna (1940), "The taxonomy of the genus bacillus I. Modes of spore germination", *Journal of Bacteriology*, pp. 340-360.
6. G C Delzer and S W McKenzie (2003), "Five-Day Biochemical Oxygen Demand", U.S. Geological Survey, TWRI Book 9 ChapterA7.2, pp. 1-21
7. How to test water purity/e-how.com [www.ehow.com/how\\_378\\_test\\_waterpurity...](http://www.ehow.com/how_378_test_waterpurity...)
8. Identification flow charts Bergey's Manual of Determinative Bacteriology All of the Unknowns will fall into the Following Groups in Bergey's Manual of Determinative

- Bacteriology (The pink book on the shelf in the laboratory).
9. J A F Op den kamp, I Redai, and I L M Van deenen (1969), "Phospholipid Composition of *Bacillus subtilis*", *Journal of Bacteriology*, Vol. 99, No. 1, pp. 298-303.
  10. Francis Borgio J, Jesvin Bency B, Ramesh S and M Amuthan (2009), "Exopolysaccharide production by *Bacillus subtilis* NCIM 2063, *Pseudomonas aeruginosa* NCIM 2862 and *Streptococcus mutans* MTCC 1943 using batch culture in the Different media", *African Journal of Biotechnology*, Vol. 9, No. 20, pp. 5454-5457.
  11. Junko Ebina, Tsuyoshi Tsutsui and Toyozo Shirai (1983), "Simultaneous Determination of Total Nitrogen and Total Phosphorus in Water Using Peroxodisulfate Oxidation", *Water Research*, Vol. 17, No. 12, pp. 1721-1726.
  12. Kannan N (2002), "Laboratory Manual In General Microbiology", Panima Publishing Corporation, New Delhi, 2<sup>nd</sup> Edition.
  13. Manivasakam N, "Chemical And Microbiological Analysis of Mineral Water and Packaged Drinking Water", Sakthi Book Services, Coimbatore.
  14. Michalski R and I Kurzyca (2006), "Determination of Nitrogen Species (Nitrate, Nitrite and Ammonia Ions) in Environmental Samples by Ion Chromatography", *Polish Journal of Environmental Studies*, Vol. 15, No. 1, pp. 5-18
  15. Nitrite/Nitrate, calorimetric method Photometric endpoint determination Roche Applied Science 68298 Mannheim Germany For life science research only. Not for use in diagnostic procedures. Cat. No. 11 746 081 001 Version August 2005
  16. Op den Kamp JA, Kauerz MT, van Deenen LL (1972), "Action of phospholipase A2 and phospholipase C on *Bacillus subtilis* protoplasts", *J Bacteriol*, Vol. 112, No. 3, pp. 1090-1098.
  17. Oxidation/Fermentation Of Glucose Test by Standards Unit, Department for Evaluations, Standards and Training 2.1 09.12.10 Page no: 1 of 10 BSOP TP 27i2.1 This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency [www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk) Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)
  18. Punitha S, Balamurugan S, Kuberan T and Suresh Kumar R (2010), "Isolation and Characterization of Agriculturally important microbes from Panchakavya and their enzymatic activity", *Journal of Bioscience. Resources.*, Vol. 1, No. 3, pp. 194-201
  19. Robert Kranz, Kathleen Weston-Hafer, Eric Richards, April Bednarski, Elgin C R and Wilhelm Cruz (2006), Identifying Unknown Bacteria Using Biochemical and Molecular Methods from Howard Hughes Medical Institute (HHMI), Washington University in Saint Louis
  20. Standard Methods for the Examination of Water and Wastewater. 20th Ed. American Public Health Association, American Water Works Association, Water Environment Federation, <http://www.norweco.com/html/lab/Inthelab.htm>
  21. Swain M R, Ray R C and Nautiyal C S (2008), "Biocontrol Efficacy of *Bacillus Subtilis* Strains Isolated From Cow Dung Against Postharvest Yam (*Discorea*



- rotundata* L.) Pathogens”, *Current Microbiology*, Vol. 57, pp. 407-411
22. T J Murray A (2011), “Comparative Study of Colon Bacilli Isolated From Horse, Cow, And Man”, *Journal of Infectious Diseases*, Vol. 13, pp. 161-174
23. The Identification of Bacteria-Spencer Reames [http://www.accessexcellence.com/AE//AEC/AEF/1994/reames\\_identification.php](http://www.accessexcellence.com/AE//AEC/AEF/1994/reames_identification.php)
24. The Obligate Anaerobic Microflora Of Farm Yard Manure [journals.cambridge.org/articles\\_5002](http://journals.cambridge.org/articles_5002)
25. Typical results for biochemical tests [http://inst.bact.wisc.edu/inst/index.php?module=Book&func=displayarticle&art\\_id=124](http://inst.bact.wisc.edu/inst/index.php?module=Book&func=displayarticle&art_id=124)
26. Van Iterson W and den Kamp J A (1969), “Bacteria-shaped gymnoplasts (protoplasts) of *Bacillus subtilis*”, *J. Bacteriol.*, Vol. 99, No. 1, pp. 304-315.
27. Wahyudi A, Cahyanto MN, Soejono M and Bachruddin Z (2010), “Potency of Lignocellulose Degrading Bacteria Isolated From Buffalo And Horse Gastrointestinal Tract And Elephant Dung For Feed Fiber Degradation” *J. Indonesian Trop. Anim. Agric.*, Vol. 35, No. 1.
28. Yaowei Kang Patent application title: *Bacillus subtilis* Strain IPC8 Class: AA01N6300FI USPC Class: 504117 Patent application number: 20090318292



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