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# Pathogen removal from municipal wastewater in Constructed Soil Filter

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## ABSTRACT

In this work, a Constructed Soil Filter (CSF) system has been configured for the treatment of wastewater wherein we recreate a soil ecosystem for water purification. Purification capacity and seasonal variability of three such CSF facilities for indicator organisms as well as enteric pathogens monitored over 9–17 months are presented. Indicator organisms include total coliform, fecal coliform, fecal streptococci, heterotrophic plate count, *Enterococcus fecalis*, actinomycetes, and coliphage. Enteric pathogens include *Escherichia coli* 25922, *E. coli* O157:H7, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Staphylococcus aureus*, *Proteus mirabilis*. All three sites show bacterial removal rate constant ( $k_d$ ) in the range of 0.4–1.3 h<sup>-1</sup> which is very high in comparison to the reported values from field as well as lab studies. Log removal for pathogens is typically 2–3 log orders. However, by extended recycling up to 5 log orders removal could be achieved. Among the three sites,  $k_d$  values were in the order of site II > site I > site III, which is also the order of their commissioning, suggesting that these system matures with age. Low hydraulic retention time (0.5–2.0 h), no pretreatment, high removal efficiency, no mechanical aeration, very low energy requirement, and green ambience are the unique features of CSF.

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## 1. Introduction

Human activities affect quality and quantity of existing fresh water resources. Pathogen counts in wastewater continue to be a cause of concern due to limitations of available technologies. Consequently, despite purification, water resources continue to deteriorate. Monitoring studies of water bodies reveal that the main source of pollution is the discharge of sewage (Belmont et al., 2004).

Conventional technologies are machine intensive with limited life, while engineered natural systems show long life and very low energy intensity. The constructed soil filter (CSF) is

such a system and one wherein we recreate a soil ecosystem for water purification and by doing so restore the ecosystems impaired by human activities.

Researchers have reported a bacterial removal rate constant of 0.2–0.5 d<sup>-1</sup> for constructed wetland (Karpiscak et al., 1996; Hench et al., 2003) 0.03–0.05 d<sup>-1</sup> for waste stabilization ponds (Fernandez et al., 1992; Jagals and Lues, 1996; Garcia and Becares, 1997) and 0.1–0.2 d<sup>-1</sup> for algal and macrophytic systems (Karpiscak et al., 1996; Garcia and Becares, 1997).

The rationale for this work is that the oxygen transfer coefficient in the constructed soil filter is in the range of 10<sup>-2</sup> to 10<sup>-3</sup> s<sup>-1</sup>, almost similar to agitated vessels, and hence high

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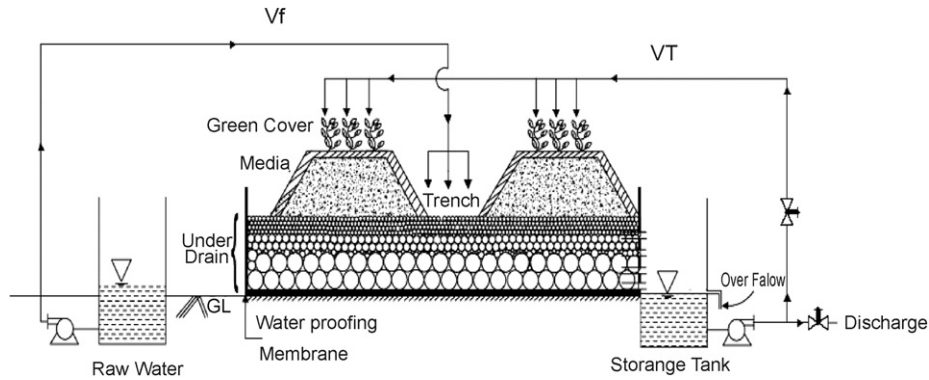


Fig. 1 – Layout of Constructed Soil Filter media.

oxygen levels lead to pathogen destruction. The significance of this paper is that energy consumption is very low due to natural oxygen supply. Consequently the CFS system finds a variety of applications, viz. water and wastewater purification.

Natural systems such as constructed wetlands work in an environment to provide tertiary treatment (Kivaisi, 2001). Similarly, land treatment provides only tertiary treatment (Gross et al., 2007; Sun et al., 1998). So these systems are applicable as a polishing step. In contrast, the CSF system treats raw wastewater directly so as to restore the damaged ecosystem and works in a clog-free recreated soil environment. Thus, CSF combines primary, secondary and tertiary treatment; all in a single evergreen facility.

The objective of this paper is to monitor the field performance of CSF media and its reproducibility accordingly. We show that much higher rate constants for pathogen removal in relation to other systems can be achieved. Field experience with CSF shows that soil system can be engineered as a mature medium for water and wastewater purification. In this paper, we present a study of field performance of three such wastewater purification plants for pathogen removal monitored over a period of 9–17 months.

## 2. Materials and methods

The CFS system for water purification termed as Soil Biotechnology, or SBT, uses a formulated soil environment wherein fundamental processes of nature, viz. respiration, mineral weathering and photosynthesis bring about the bioconversion. The system consists of a medium of suitable mineral constitution, culture containing native microflora, geophagus worm *Pheretima elongata* and bio-indicator plants. Earlier work covers the residence time distribution model for the system (Pattanaik et al., 2004).

Per Shankar et al. (2005), the single stage CSF process basically involves: (i) preparation of the geophagus earthworms culture *P. elongata* and preparation of bacterial culture; (ii) preparation of the soil media to contain the geophagus earthworms *P. elongata*; (iii) construction of an under-drain first tank and a collection tank herein referred to as second tank; (iv) layering of the media over the first tank; (v) percolation of the organic waste through the layered media; (vi) collection of the treated water in the second tank; (vii) recirculation of the treated water to achieve the desired quality; and (viii) use of

Table 1 – Soil Biotechnology plant details at three sites

|                                       | Site I                 | Site II                | Site III               |
|---------------------------------------|------------------------|------------------------|------------------------|
| Wastewater                            | Domestic + septic tank | Domestic + septic tank | Domestic + septic tank |
| Batch volume, $V_L$ ( $m^3$ )         | 50                     | 30                     | 300                    |
| Pretreatment                          | No                     | No                     | No                     |
| Bed                                   |                        |                        |                        |
| Bed dimensions (m)                    | 25 × 20 × 0.7          | 20 × 12 × 0.7          | 50 × 30 × 0.7          |
| Bed surface area ( $m^2$ )            | 500                    | 240                    | 1500                   |
| Hydraulics                            |                        |                        |                        |
| Flow                                  | Vertical               | Vertical               | Vertical               |
| Mean hydraulic load ( $m^3/(m^2 h)$ ) | 0.040                  | 0.047                  | 0.036                  |
| Raw flow, $v_f$ ( $m^3/h$ )           | 21.6                   | 14.4                   | 39.6                   |
| Recycle flow, $v_r$ ( $m^3/h$ )       | 21.6                   | 10.8                   | 54.0                   |
| Batch time, $t_b$ ( $t_f + t_r$ ) (h) | 6.50 (2.50 + 4.0)      | 4.67 (2.67 + 2.0)      | 11.0 (5.50 + 5.50)     |
| Plant commissioned (year)             | 2002                   | 1995                   | 2003                   |

Site I located at Kanjurmarg, eastern suburb of Mumbai, India; sites II and III located at Chembur, eastern suburb of Mumbai, India.

bio-indicators to monitor the reformed water at various stages of the process.

Site I made in RCC; located in an eastern suburb of Mumbai is spread over an area of 500 m<sup>2</sup>, bed depth of 0.7 m with media consisting of completely weathered Deccan trap basalt. Site I is operated at batch time of 6.5 h and hydraulic loading of 0.04 m<sup>3</sup>/(m<sup>2</sup> h). Site II made in stone masonry; located an eastern suburb of Mumbai is spread over an area of 340 m<sup>2</sup>, bed depth of 0.7 m also using completely weathered Deccan trap basalt. It is operated at a batch time of 4.7 h, and hydraulic loading of 0.047 m<sup>3</sup>/(m<sup>2</sup> h). Site III made in soil embankment is spread over an area of 1500 m<sup>2</sup>, bed depth of 0.7 m with media of weathered Deccan trap basalt. It is operated at a batch time 11 h, and hydraulic loading of 0.036 m<sup>3</sup>/(m<sup>2</sup> h). Sites II and III both are located in the sport complex in an eastern suburb of Mumbai and receive sewage contaminated with septic tank effluent.

Fig. 1 shows schematic of the process together with layout of media. Details of the sites together with media characteristics are given in Table 1. The system consists of raw water tank, bioreactor, and storage tank with associated piping and pumping arrangements. The bioreactor consists of impermeable floor; made typically by using a polymer membrane and containment typically of RCC, stone masonry or soil embankment.

The bioreactor consists of an under drain of stones of various sizes and media details of which are given in Table 1. The medium is laid out in the form of crust and trench arrangements. Suitable plants with tap root system are grown over the crust. The slopes are consolidated via turfing. The objective of culturing geophagus worms is to maintain soil microbial ecology required for water purification. Residence time and oxygen transfer required for the purification are facilitated by using media appropriately as per Shankar et al., 2005.

The process has been run on batch mode. Here the results for batch volume  $V_L$  of wastewater are presented. A batch volume  $V_L$  of wastewater is pumped at a rate  $v_f$  into the system trenches. Water first percolates through the trenches and is collected into the collection tank. It is then pumped on to the media distributed on it with the help of distribution system in order to achieve maximum solid liquid contact while percolating through CSF media. The filtered water then gets collected into the collection tank and is then recirculated ( $v_r$ ) on to the media again. Here the dissolved organics and inorganics are oxidized and the water is purified further. The recirculation ( $v_r$ ) is continued till desired quality is achieved and then the water is discharged. Overall time of operation (wetting cycle) varies from 5 to 11 h. The bed is then left to rest for regeneration for 10–16 h (drying cycle) prior to next cycle of use.

In this work, a list of enteric pathogens and protozoa of considerable interest in public health have been selected for their removal performance via CSF.

### 2.1. Analysis

Sampling frequency was twice a month for sites I–III. Samples of raw wastewater and processed water were collected in sterile 2-L plastic cans and transported to laboratory where they were analyzed within 6 h for microbial parameters and 24 h for

other chemical parameters. Parameters like pH and dissolved oxygen levels were monitored at the site itself.

### 2.2. Physicochemical

pH and dissolved oxygen (DO) were measured using WTW (Germany) Inolab1 pH/Oxi meter. BOD and suspended solids were measured as per standard protocols (APHA, 1998).

### 2.3. Pathogen

Samples were analyzed for various indicator and enteric pathogens by membrane filtration technique (APHA, 1998) using 0.45- $\mu$ m membrane filters (PALL Life Sciences, Mumbai). Appropriately diluted ( $10^{-3}$  to  $10^{-7}$ ) sample (100 mL in volume) volumes, in triplicate, were filtered. Dilution factor varied according to the group of organisms being enumerated and sample source (influent vs. effluent) to ensure 20–250 colonies in the Petri plate. Indicator organisms, viz. fecal coliform (FC), total coliform (TC), heterotrophic plate count (HPC), fecal streptococci (FS), actinomycetes, and coliphage were enumerated using specific media supplied from Hi Media Laboratory Pvt. Ltd., India. Plates were incubated for 24 h at 44.5 °C on M-FC medium for fecal coliform, 24 h at 37 °C on M-Endo agar for total coliform, 24 h at 37 °C on M-HPC agar for heterotrophic plate counts, 48 h at 35 °C on M enterococcus agar for fecal streptococci.

Specific enteric pathogens like *E. coli* O157:H7, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *E. coli* 25922 were also enumerated using special media series, HiCrome™ supplied by Hi Media Laboratory Pvt. Ltd., India. Plates for anaerobic bacteria were prepared in an anaerobic chamber and incubated in anaerobic jar (Hi Media, India Ltd.). Plates were incubated for 24 h at 35–37 °C on Hichrome MS.O157 agar for *E. coli* O157:H7; 24–48 h at 35–37 °C on differential media Hichrome Enterococci agar for *E. faecalis* and *S. aureus*; 24 h at 35–37 °C on differential media Hichrome UTI agar for *P. aeruginosa*, *K. pneumoniae* and *Proteus mirabilis*; 24–48 h at 44 °C under anaerobic conditions on M-CP agar base for *C. perfringens*.

Actinomycete analysis was carried out as per the procedures outlined in standard methods (APHA, 1998). 15 mL of sterile starch casein agar was transferred aseptically to a Petri dish and allowed it to solidify. This formed the bottom layer. To a test tube containing 17-mL liquefied starch casein agar at 45–48 °C, 2 mL of appropriately diluted sample and 1 mL of cycloheximide (1 mg/mL, separately sterilized at 121 °C for 15 min) were added and mixed thoroughly. 5 mL out of this was transferred uniformly over the hardened bottom layer of starch casein agar. All plates were prepared in triplicate and incubated at 28 °C for 6–7 days.

Coliphage analysis was also conducted as per the procedure given in standard methods (APHA, 1998). Frozen host *E. coli* culture was transferred to 44.5 °C water bath. Then 1 mL of host *E. coli* culture, 5-mL sample appropriately diluted and 0.08-mL 2,3,5-triphenyl tetrazolium chloride (TPTZ) were added to each of 5.5 mL of modified Tryptic(ase) soy agar which is previously melted and held at 45 °C before the addition. The contents were mixed thoroughly and poured into separate 100 mm × 15 mm labeled Petri dishes. Plates were incubated at

35 °C in inverted position. Plaques were counted after incubating for 4–6 h.

First-order rate constant were estimated based on the RTD model for small Peclet number and given in Eq. (1) for soil filters (Pattanaik et al., 2004):

$$\ln \left( \frac{C_2}{C_{20}} \right) = - \frac{k_d \tau_r}{(1 + k_d \tau_r)} \frac{t}{\tau_h} \quad (1)$$

where  $C_2$  = contaminant levels in the effluent,  $C_{20}$  = contaminant levels in the influent,  $\tau_r$  = residence time in reactor ( $V_R/v_r$ ; from RTD; 0.21, 0.23, and 0.21 h for sites I–III, respectively),  $\tau_h$  = residence time in holding tank ( $V_T/v_r$ ),  $V_R$  = hold up in reactor,  $V_T$  = hold up in the holding tank,  $v_r$  = recycle rate.

#### 2.4. Media

Soil samples were taken randomly in 2004. Bulk samples were augured from two levels: one at 0–10 cm and other at 20–30 cm. Samples were air dried and mixed in equal proportion to obtain homogenous sample. Soil samples were then sub-sampled. Air-dried soil was then passed through 2-mm sieve and analyzed for most of the physicochemical and microbial studies (except for particle size analysis). Physicochemical parameters includes: moisture content, specific gravity, particle size analysis, organic carbon, hydraulic conductivity, pH, cation exchange capacity, anion exchange capacity, contact angle. Microbial parameters investigated were heterotrophs, actinomycetes and protozoan population to know the microbial diversity in general.

Soil pH was measured with a 10-g soil and 25 mL of 1M KCl suspension, soil paste and 1:5 soil:water suspension with glass electrode. Electrical conductivity ( $EC_{25}$ ) was measured with 1:5 soil:water suspension. Soil moisture content (% MC) was measured by drying 5-g soil at 105–110 °C for 8 h. A particle size analysis of the <2 mm fraction was carried out for each sample according to the procedure of Gee and Bauder (1986). Soil permeability was carried out as per falling-head permeability test method. Percentage oxidizable organic matter was determined by the chromic acid method of Walkley and Black (Black, 1965). Organic carbon content was obtained by multiplying it by 1.33. Cation exchange capacity (CEC) was measured by Na saturation method (Chapman, 1965) using WTW sodium electrode with Eutech Ion meter (Cyberscan 100). Anion exchange capacity (AEC) was measured according to the method of Mehlich (1948). Percentage C, H, and N were

**Table 2 – Microbial characteristics of media at the three sites**

|                                   | CSF media            |                      |                    |
|-----------------------------------|----------------------|----------------------|--------------------|
|                                   | Site I               | Site II              | Site III           |
| Actinomycetes (CFU/g)             | $2.4 \times 10^8$    | $2.4 \times 10^8$    | $2.5 \times 10^8$  |
| Heterotrophic plate count (CFU/g) | $2.8 \times 10^{12}$ | $3.2 \times 10^{12}$ | $3 \times 10^{13}$ |
| Protozoa                          |                      |                      |                    |
| Naked amoebae (cells/g)           | $1.01 \times 10^8$   | $1.01 \times 10^8$   | $1.01 \times 10^8$ |
| Flagellates (cells/g)             | $8.33 \times 10^4$   | $1.0 \times 10^8$    | $4.92 \times 10^4$ |
| Ciliates (cells/g)                | $8.33 \times 10^4$   | $1.43 \times 10^5$   | $8.33 \times 10^4$ |
| Geophagus worm                    |                      |                      |                    |
| <i>Pheretima elongata</i>         | +                    | +                    | +                  |

CFU: colony forming unit; +: present.

determined using CHN analyzer, Thermo Finnegan-FLASHEA 1112 series. Hydrophobicity was measured by contact angle ( $\theta$ ) method to find out the wetting properties of the medium.

Heterotrophic plate count was measured as colony forming unit (CFU)/g after incubation for 48 h with heterotrophic plate count media supplied by Hi Media Laboratory Pvt. Ltd., India. Similarly, actinomycetes were measured as CFU/g of soil grown on starch casein medium (Wellington and Toth, 1994). Protozoan population were enumerated by the most probable number (MPN) method and represented as cells/g of soil (Ingham, 1994).

### 3. Results

Pathogen removal performance reported here covers twice a month monitoring over 17 months for site I and 9 months each for rest two sites. Values reported are arithmetic means of 34 data sets in duplicates for site I, 20 in duplicates for site II, and 18 in duplicates for site III.

Table 2 summarizes the microbial characteristics of the media. It shows that microbial ecology in place is quite rich in terms of actinomycetes, heterotrophic and predator population (protozoa and geophagus worm). These values are far exceeding the values reported in the literature (McCarthy, 1987). Physicochemical characteristics of the media are given as supporting information (SI). Hydraulic conductivity observed in the field was  $10^{-4}$  to  $10^{-5}$  m s<sup>-1</sup>.

**Table 3 – Physicochemical performance of CSF plants**

| Parameter  | Site I         |                | Site II        |                | Site III       |                |
|------------|----------------|----------------|----------------|----------------|----------------|----------------|
|            | Influent (avg) | Effluent (avg) | Influent (avg) | Effluent (avg) | Influent (avg) | Effluent (avg) |
| pH         | 7.0            | 7.4            | 7.0            | 7.1            | 7.3            | 7.6            |
| DO (mg/L)  | 0.7            | 4.8            | 0.8            | 3.4            | 0.8            | 4.0            |
| BOD (mg/L) | 95.3           | 5.9            | 125.2          | 10.4           | 82.5           | 10.6           |
| SS (mg/L)  | 187.9          | 8.3            | 203.5          | 11.2           | 136.6          | 18.2           |

Site I located at Kanjurmarg, eastern suburb of Mumbai, India; sites II and III located at Chembur, eastern suburb of Mumbai, India.

### 3.1. Physicochemical performance

Physicochemical analysis shown are arithmetic means of twice a month monitoring duplicates of 34 samples for site I, duplicates of 20 samples for site II and duplicates of 18 samples for site III. Table 3 summarizes the physicochemical performance of the three CSF plants. Effluent pH of all the plants was found to be close to neutral showing buffering capacity of CSF environment. All effluent shows significant increase in dissolved oxygen levels; up to 3.3–4.8 mg/L. BOD removal rate constants similarly estimated has been found to be 0.38, 1.35, and 0.36 h<sup>-1</sup>, respectively. Significant improvement in the clarity of water was observed due to suspended solids (SS) removal with rate constants of 0.5, 1.6 and 0.35 h<sup>-1</sup>, respectively.

Steen et al. (1999) have provided data on performance of maturation ponds. k<sub>d</sub> values of 0.2–0.5 per day are reported and such systems are reported to deteriorate in performance with time. In contrast, the results of physicochemical performance of CSF indicate that they mature and improve over time.

### 3.2. Pathogen removal performance

Table 4 gives the pathogen levels in the influent as well effluent for all the three sites. Table 4 also gives mean values of K and k<sub>d</sub> values for all three sites, where K is log removal and k<sub>d</sub> is removal rate constants, N<sub>0</sub> and N influent and effluent concentration (CFU/100 mL). All of them show very little variation in the influent pathogen concentration.

Average TC levels in the influent were (2.0–3.5) × 10<sup>8</sup> CFU/100 mL and reduced to (1.5–3.6) × 10<sup>5</sup> CFU/100 mL showing log removal of 2.7–4.5 for all three sites with k<sub>d</sub> of 1.7, 7.2 and 1.5 h<sup>-1</sup>, respectively. In case of FC, average influent concentration was (2–8) × 10<sup>7</sup> CFU/100 mL and reduced to (3.1–8.3) × 10<sup>4</sup> CFU/100 mL for all three sites. Average log removal for all three sites was 2.2–3.4 with k<sub>d</sub> of 1.4, 5.4 and 1.4 h<sup>-1</sup>, respectively and in comparison was observed to be very high as against 1.9–2.7 d<sup>-1</sup> for anaerobic ponds; 0.4–1.9 h<sup>-1</sup> for facultative ponds; 0.4–13.0 d<sup>-1</sup> for maturation ponds; 0.8–7.3 d<sup>-1</sup> for shallow stabilization ponds; and 0.3–1.2 d<sup>-1</sup> for Duckweed ponds (Steen et al., 1999). These k<sub>d</sub> values are also comparable with the reported values of bacterial die-off rate constants for *E. coli* (1.14 d<sup>-1</sup>), fecal streptococci (0.41 d<sup>-1</sup>), *Salmonella* sps. (0.33 d<sup>-1</sup>), and *Shigella* sps. (0.68 d<sup>-1</sup>) in soil (Reddy et al., 1981).

Average HPC levels in the influent were (6.6–18) × 10<sup>8</sup> CFU/100 mL and reduced to (1.4–6.8) × 10<sup>6</sup> CFU/100 mL. Log removal was observed to be 2.2–4.2 with k<sub>d</sub> value of 1.4, 5.8 and 1.2 h<sup>-1</sup>, respectively. Average FS levels in the influent were (2.2–3.8) × 10<sup>6</sup> CFU/100 mL and effluent levels were reduced to (1.9–3.0) × 10<sup>4</sup> CFU/100 mL. Log removal was in the range of 1.7–2.5 with k<sub>d</sub> of 1.0, 3.1 and 1.0 h<sup>-1</sup>, respectively. There was order of difference in the influent levels of actinomycetes, viz. 1.3 × 10<sup>6</sup>, 5.9 × 10<sup>5</sup> and 4.6 × 10<sup>4</sup> CFU/100 mL, respectively. But effluent levels were stabilized to (3.6–6.8) × 10<sup>4</sup> CFU/100 mL. Sites I and II have shown log removal of 1.4–1.6 with k<sub>d</sub> values in the range of 0.6–1.9 h<sup>-1</sup>. Site III have shown lowest log removal of 0.1 ± 0.4 with k<sub>d</sub> value of 0.06 ± 0.1 h<sup>-1</sup>. It could be due to better environment in CSF for their proliferation than removal.

Coliphage levels were measured as (6.3–19) × 10<sup>4</sup> plaque-forming unit (PFU)/100 mL in the influent and reduced to (1.0–12.0) × 10<sup>3</sup> PFU/100 mL showing 0.4–2.8 log order with k<sub>d</sub> of 0.5–3.4 h<sup>-1</sup> for all three sites.

Among the list of enteric pathogens, *E. coli* O157:H7 is of special concern due to its threat to public health. Average *E. coli* O157:H7 levels in the influent were (1.2–3.3) × 10<sup>6</sup> CFU/100 mL and reduced to (1.5–2.4) × 10<sup>4</sup> CFU/100 mL in the effluent. Log removal was in the range of 1.2–2.8 with k<sub>d</sub> of 0.9–3.7 h<sup>-1</sup>. *P. mirabilis* shows maximum k<sub>d</sub> (3.5 ± 0.3 h<sup>-1</sup>) amongst the enteric pathogens with site II. For the rest sites, it was 0.7–2.2 log removal with k<sub>d</sub> of 0.7–0.8 h<sup>-1</sup>. Most of the other enteric pathogen, viz. *E. coli* 25922, *K. pneumoniae*, *P. seruginosa*, *E. fecalis*, *C. perfringens*, *S. aureus* shows influent concentration of 10<sup>5</sup> to 10<sup>6</sup> CFU/100 mL and reduces to 10<sup>3</sup> to 10<sup>4</sup> CFU/100 mL. It shows log removal of 1.0–2.4 for all three sites with k<sub>d</sub> of 0.8–1.5 h<sup>-1</sup> for sites I–III and 1.0–3.8 h<sup>-1</sup> for site II. Hench et al. (2003) has also reported same trend for enteric pathogens.

Total coliform and fecal coliform removal for all three sites are represented graphically in Figs. 2 and 3. It implies that there are no significant variations in seasonal k<sub>d</sub> values. Monsoon precipitation is likely to disturb drying and wetting cycles thereby leading to wash-out effects on pathogen trapped in soil bed media. But no such effect was seen for total coliform and fecal coliform (Figs. 2 and 3).

Summing up, log removal for indicator organisms (TC, FC, HPC and FS) was 2.2–3.7 and for enteric pathogens 0.8–2.4. Site I shows k<sub>d</sub> value of 0.6–1.7 h<sup>-1</sup> for most of the pathogens. Site II shows k<sub>d</sub> values in the order of 1.0–7.0 h<sup>-1</sup>. k<sub>d</sub> values for site III were in the order of 1.0–1.5 h<sup>-1</sup> for most of the indicator organism but show very low k<sub>d</sub> values (0.06–1.0 h<sup>-1</sup>) for enteric pathogens. If we compare the performance for indicator organisms, they were in the order of II > I > III in terms of log removal and k<sub>d</sub> values. This is also the order of age of these plants, implying that the system matures with time; in contrast in the pond system, k<sub>d</sub> decreases due to age factor (Steen et al., 1999).

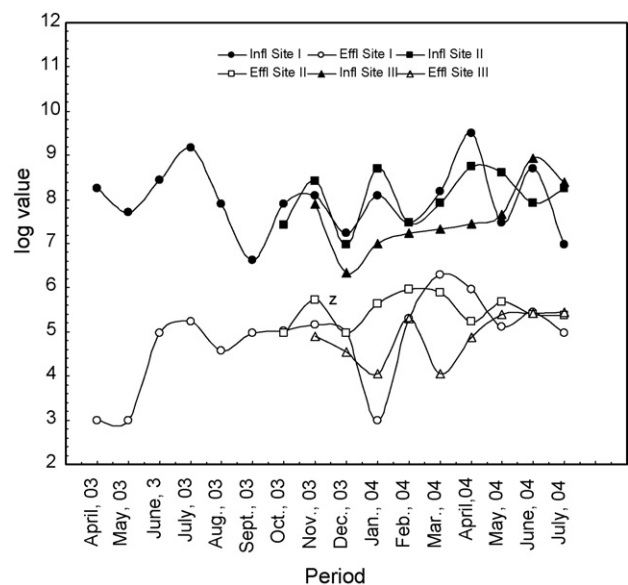


Fig. 2 – Total coliform removal for all three sites.

**Table 4 – Pathogen removal status of sites I–III**

| Organism                  | Site I            |                   |     |       | Site II           |                   |     |       | Site III          |                   |     |       |
|---------------------------|-------------------|-------------------|-----|-------|-------------------|-------------------|-----|-------|-------------------|-------------------|-----|-------|
|                           | Influent          | Effluent          | K   | $k_d$ | Influent          | Effluent          | K   | $k_d$ | Influent          | Effluent          | K   | $k_d$ |
| Total coliform            |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $3.5 \times 10^8$ | $2.4 \times 10^5$ | 3.6 | 1.7   | $2.0 \times 10^8$ | $4.0 \times 10^5$ | 2.8 | 7.2   | $2.1 \times 10^8$ | $1.5 \times 10^5$ | 2.8 | 1.5   |
| S                         | $3.2 \times 10^8$ | $2.3 \times 10^5$ | 0.9 | 0.3   | $6.9 \times 10^7$ | $1.9 \times 10^5$ | 0.1 | 0.4   | $3.1 \times 10^8$ | $1.2 \times 10^5$ | 0.5 | 0.1   |
| Fecal coliform            |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $2.0 \times 10^7$ | $3.1 \times 10^4$ | 2.6 | 1.4   | $1.9 \times 10^7$ | $8.3 \times 10^4$ | 2.2 | 5.4   | $8.3 \times 10^7$ | $6.5 \times 10^4$ | 2.8 | 1.4   |
| S                         | $1.9 \times 10^7$ | $2.4 \times 10^4$ | 0.4 | 0.1   | $1.3 \times 10^7$ | $1.8 \times 10^4$ | 0.5 | 0.3   | $1.3 \times 10^7$ | $5.0 \times 10^4$ | 0.6 | 0.1   |
| Heterotrophic plate count |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.4 \times 10^9$ | $2.0 \times 10^6$ | 3.3 | 1.4   | $1.8 \times 10^9$ | $6.8 \times 10^6$ | 2.6 | 5.8   | $6.7 \times 10^8$ | $1.4 \times 10^6$ | 2.8 | 1.2   |
| S                         | $9.8 \times 10^8$ | $2.0 \times 10^6$ | 0.9 | 0.3   | $8.3 \times 10^8$ | $5.8 \times 10^6$ | 0.4 | 0.2   | $4.1 \times 10^8$ | $1.2 \times 10^6$ | 0.4 | 0.1   |
| Fecal streptococci        |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $3.3 \times 10^6$ | $2.3 \times 10^4$ | 2.3 | 1.0   | $2.2 \times 10^6$ | $3.0 \times 10^4$ | 1.9 | 3.1   | $3.8 \times 10^6$ | $1.9 \times 10^4$ | 2.3 | 1.0   |
| S                         | $2.7 \times 10^6$ | $2.3 \times 10^4$ | 0.2 | 0.1   | $5.0 \times 10^5$ | $1.3 \times 10^4$ | 0.2 | 0.1   | $2.6 \times 10^6$ | $1.2 \times 10^4$ | 0.1 | 0.1   |
| Escherichia coli 25922    |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.2 \times 10^6$ | $1.5 \times 10^4$ | 2.3 | 1.0   | $1.5 \times 10^6$ | $2.1 \times 10^4$ | 1.7 | 3.3   | $3.3 \times 10^6$ | $2.4 \times 10^4$ | 2.2 | 1.0   |
| S                         | $9.5 \times 10^5$ | $1.4 \times 10^4$ | 0.5 | 0.2   | $1.3 \times 10^6$ | $1.5 \times 10^4$ | 0.4 | 0.2   | $2.9 \times 10^6$ | $1.6 \times 10^4$ | 0.4 | 0.1   |
| E. coli O157:H7           |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.1 \times 10^6$ | $1.2 \times 10^4$ | 1.9 | 0.9   | $1.5 \times 10^6$ | $1.6 \times 10^4$ | 1.9 | 3.7   | $3.5 \times 10^7$ | $1.9 \times 10^4$ | 2.8 | 1.5   |
| S                         | $7.0 \times 10^5$ | $4.7 \times 10^3$ | 0.2 | 0.1   | $7.8 \times 10^5$ | $4.7 \times 10^3$ | 0.2 | 0.3   | $3.4 \times 10^7$ | $1.7 \times 10^4$ | 0.8 | 0.2   |
| Klebsiella pneumoniae     |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.5 \times 10^6$ | $1.6 \times 10^4$ | 2.2 | 0.9   | $3.2 \times 10^6$ | $5.5 \times 10^4$ | 1.8 | 3.0   | $4.3 \times 10^6$ | $2.9 \times 10^4$ | 2.2 | 1.0   |
| S                         | $9.3 \times 10^5$ | $1.6 \times 10^4$ | 0.3 | 0.1   | $1.8 \times 10^6$ | $4.1 \times 10^4$ | 0.4 | 0.2   | $1.2 \times 10^6$ | $1.8 \times 10^4$ | 0.4 | 0.1   |
| Pseudomonas seruginosa    |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $2.8 \times 10^6$ | $6.4 \times 10^4$ | 1.9 | 0.8   | $7.6 \times 10^6$ | $1.0 \times 10^5$ | 1.8 | 3.8   | $6.6 \times 10^6$ | $2.8 \times 10^5$ | 1.8 | 0.72  |
| S                         | $2.5 \times 10^6$ | $6.3 \times 10^4$ | 0.7 | 0.3   | $5.4 \times 10^6$ | $6.7 \times 10^4$ | 0.8 | 0.4   | $2.0 \times 10^6$ | $2.4 \times 10^5$ | 0.6 | 0.1   |
| Enterococcus faecalis     |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.3 \times 10^6$ | $1.0 \times 10^4$ | 2.2 | 1.0   | $3.0 \times 10^5$ | $1.8 \times 10^4$ | 1.2 | 1.7   | $2.0 \times 10^6$ | $7.3 \times 10^3$ | 2.4 | 1.1   |
| S                         | $1.3 \times 10^6$ | $1.0 \times 10^4$ | 0.5 | 0.2   | $1.6 \times 10^4$ | $5.5 \times 10^3$ | 0.2 | 0.1   | $1.7 \times 10^6$ | $7.6 \times 10^3$ | 0.1 | 0.1   |
| Clostridium perfringens   |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $4.2 \times 10^6$ | $1.2 \times 10^4$ | 2.1 | 1.2   | $1.8 \times 10^6$ | $4.2 \times 10^4$ | 1.7 | 2.5   | $2.3 \times 10^5$ | $8.3 \times 10^3$ | 0.8 | 0.6   |
| S                         | $4.1 \times 10^6$ | $7.0 \times 10^3$ | 0.7 | 0.3   | $1.7 \times 10^6$ | $5.8 \times 10^4$ | 0.3 | 0.2   | $2.0 \times 10^5$ | $5.4 \times 10^3$ | 0.2 | 0.2   |
| Staphylococcus aureus     |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $6.6 \times 10^5$ | $5.3 \times 10^3$ | 1.5 | 0.8   | $5.6 \times 10^5$ | $1.7 \times 10^4$ | 1.2 | 1.0   | $2.0 \times 10^5$ | $3.7 \times 10^3$ | 1.0 | 0.7   |
| S                         | $6.0 \times 10^5$ | $4.4 \times 10^6$ | 1.1 | 0.4   | $7.4 \times 10^5$ | $9.5 \times 10^3$ | 0.8 | 0.4   | $1.6 \times 10^5$ | $3.6 \times 10^3$ | 0.9 | 0.2   |
| Actinomycetes             |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.3 \times 10^6$ | $6.8 \times 10^4$ | 1.4 | 0.6   | $5.9 \times 10^5$ | $4.6 \times 10^4$ | 1.6 | 1.9   | $4.6 \times 10^4$ | $3.6 \times 10^4$ | 0.1 | 0.1   |
| S                         | $1.2 \times 10^6$ | $6.6 \times 10^4$ | 0.7 | 0.3   | $6.6 \times 10^5$ | $7.1 \times 10^4$ | 0.9 | 0.5   | $3.9 \times 10^4$ | $2.6 \times 10^4$ | 0.4 | 0.1   |
| Coliphage                 |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $1.9 \times 10^5$ | $1.2 \times 10^4$ | 1.6 | 0.6   | $6.3 \times 10^4$ | $1.0 \times 10^3$ | 1.1 | 3.4   | $1.7 \times 10^5$ | $1.2 \times 10^4$ | 1.3 | 0.5   |
| S                         | $1.2 \times 10^5$ | $1.1 \times 10^4$ | 1.1 | 0.4   | $5.7 \times 10^4$ | $5.6 \times 10^2$ | 0.6 | 0.8   | $1.4 \times 10^5$ | $1.1 \times 10^4$ | 0.9 | 0.2   |
| Proteus mirabilis         |                   |                   |     |       |                   |                   |     |       |                   |                   |     |       |
| M                         | $5.4 \times 10^5$ | $7.7 \times 10^2$ | 2.3 | 0.7   | $2.2 \times 10^5$ | $2.7 \times 10^3$ | 0.7 | 3.5   | $5.8 \times 10^4$ | $7.7 \times 10^2$ | 1.2 | 0.8   |
| S                         | $1.0 \times 10^6$ | $6.7 \times 10^2$ | 0.8 | 0.3   | $3.4 \times 10^5$ | $4.4 \times 10^3$ | 0.5 | 0.5   | $4.5 \times 10^4$ | $7.2 \times 10^2$ | 1.0 | 0.2   |

K =  $\log(N_0/N)$ ;  $k_d$  ( $\text{h}^{-1}$ ), std = standard deviation,  $N_0$  and  $N$ : influent and effluent concentration (CFU/100 mL);  $\tau$  = residence time in reactor (0.21, 0.23 and 0.21 h for sites I–III, respectively); M: mean; S: standard deviation.

Fecal and total coliform level as per WHO guidelines for irrigation reuse, i.e.  $\leq 10^3$  CFU/100 mL (WHO, 1989), was achieved with extended recycling of 6–7 h for site I as shown in Fig. 4. This shows that by appropriate choice of retention time (or batch time) pathogen levels as desired can be achieved. With  $k_d$  values typically of  $1.0 \text{ h}^{-1}$  for fecal coliform, 8 log order reduction will require a retention time of 18 h. It also implies that with typical  $k_d$  of  $1.0 \text{ h}^{-1}$ , 8 log reduction in FC can be

achieved with retention time of 18 h. A photograph of one of the facilities is shown in supporting information (SI).

#### 4. Discussion

Fate of bacteria, i.e. bacterial removal and reduction, when applied onto soil, is governed by two distinct processes—retention (filtration and/or adsorption) and die-off (Ellis and

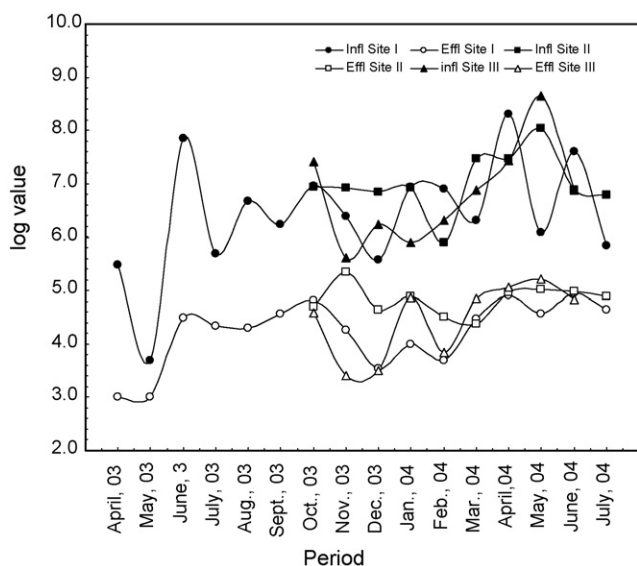


Fig. 3 – Fecal coliform removals for all three sites.

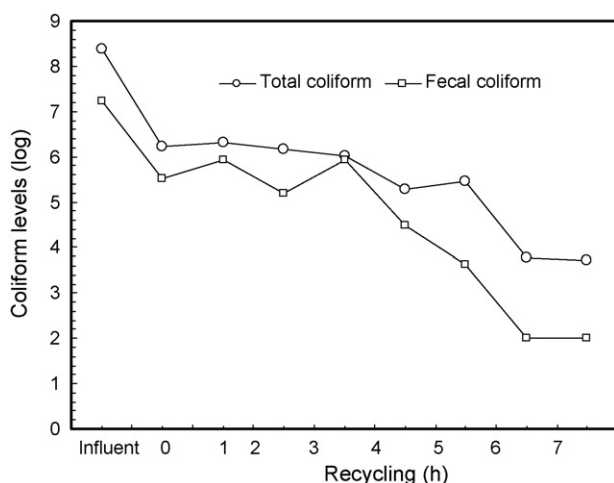


Fig. 4 – Effect of extended recycling on indicator organism removal.

McCalla, 1976; Reddy et al., 1981; Crane and Moore, 1984). There are two aspects to pathogen removal in CSF: (i) property of media to retain pathogens during filtration, i.e. bacterial adhesion, (ii) unsuitable physicochemical environment for pathogen survival in CSF and (iii) predation of these pathogens to regenerate bed for further adhesion.

Extreme acidic and alkaline pH has a direct effect on bacterial survival. A pH range of 9.0–9.5 is considered as lethal levels for pathogen survival (Pearson et al., 1987). However, CSF reports effluent pH of the order of 7.2–7.6, yet very good  $k_d$  values. Elevated temperature with drying conditions as in CSF effectively increases die-off rates (Crane and Moore, 1984). Increased moisture content also favors bacterial survival (Reddy et al., 1981). Alternate drying and wetting cycle subjects the system to alternate saturated and unsaturated flow regime which results into moisture variation in the system. These extreme fluctuations in moisture profile affect pathogen removal rates in CSF. BOD removal from wastewater

makes septic bacteria more susceptible to die-off than their natural counterparts in soil. Williams et al. (1994) have given an empirical equation for fecal coliform removal as a function of BOD for constructed wetlands as

$$\text{Fecal coliform } \{ \log(\text{CFU}/100 \text{ mL}) \} = (0.074 \times \text{BOD}_5) + 1.95 \quad (2)$$

Observed relationship between BOD and fecal coliform shows good agreement between them.

Many workers have correlated increased dissolved oxygen with bacterial die-off (Fernandez et al., 1992; Pearson et al., 1987) in aquatic environment. Fernandez et al. (1992) have claimed 8–10-folds increased bacterial die-off rate constant ( $k_d$ ) for total coliform, fecal coliform, fecal streptococci and *Clostridium* in response to short aeration treatment (2h per day) in aquatic environment. Observed relationship between effluents dissolved oxygen levels (3.37–4.8 mg/L) in CSF and  $k_d$  values (0.63–1.17  $\text{h}^{-1}$ ) for FC shows good consistency. Earlier work on CSF indicates that for organic loading of  $<0.15 \text{ kg/m}^2$  per day and hydraulic loading of  $<0.05 \text{ m}^3/(\text{m}^2 \text{ h})$ , reduction potentials of  $>600 \text{ mV}$  are typical in CSF (Pattanaik, 2001) and so aerobic oxidation is the dominant reaction at work. The highly toxic potential and near neutral pH together with ecology of medium brings about significant reduction in the pathogens.

Soil is a complex heterogenous system. It is more diverse and dense with respect to its microflora. One gram of soil holds  $10^{12}$  to  $10^{16}$  cells and  $10^5$  to  $10^6$  different types of species (Curtis and Sloan, 2005). There is a resistance by indigenous soil microflora to new addition (Ellis and McCalla, 1976). Their competition for space and nutrients limits the pathogen survival in soil. Enteric pathogen also gets subjected to various toxic and antibiotic secretions. Certain bacteriophage and free-living soil organism are known to parasitize *E. coli* cells (Reddy et al., 1981).

Presence of geophagus worms (also known as earthworms) is known to reduce organic matter content of soil and thus makes the environment more unsuitable for pathogens. They are known to maintain active prey-predator chain in soil ecology. CSF medium is rich in its microbial ecology (Table 2) and hosts very hostile environment for wastewater microflora and hence the observed reduction.

## 5. Conclusion

It is shown that CSF works in a soil environment and removes chemical contaminants as well as pathogen in a single facility open to atmosphere. In India, there are no pathogen level based guideline for sewage discharge into receiving water bodies. In this work, it is shown that by extended recycling, fecal counts less than  $10^3 \text{ CFU}/100 \text{ mL}$  as per the WHO guideline for wastewater irrigation (WHO, 1989) can be achieved.

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