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Application of Vermi-filter-based Effluent Treatment Plant (Pilot scale) for Biomanagement of Liquid Effluents from the Gelatine Industry

Sudhir D. Ghatnekar[1*] • Mahavash F. Kavian[1] • Santosh M. Sharma[1] • Sumukh S. Ghatnekar[2] • Gautam S. Ghatnekar[3] • Angela V. Ghatnekar[3]

[1] Biotechnology Resource Centre, G/1, Adinath, Shaikh Misry Road, Wadala (E), Mumbai - 400 037, India
[2] Flat 12, Agnes Court, Wilmslow Road, Fallowfield, Manchester, M14 6AJ, United Kingdom
[3] 1064, Oak Crest Drive, Charleston SC, 29412, USA

Corresponding author: * **brc_suvash@hotmail.com**

ABSTRACT

The present paper describes the application of three-tier vermiculture biotechnology coupled with vermi-filtration to convert secondary liquid effluents from a gelatine manufacturing unit into 'bio-safe' clean water. The experiments were conducted at a pilot scale. The protocol involved pretreatment of selected effluents using a combination of protease and cellulase. Later, mixed cultures of *Aspergillus flavus* (BRC - 27), *A. niger* (BRC - 28), *Nitrobacter winogradskyi* (BRC - 5), *Spirulina platensis* (BRC - 45), *Oscillatoria princeps* (BRC - 46) and *Chlorella vulgaris* (BRC - 47) were inoculated. The effluents were then passed through a series of 3 vermi-filter (VF) tanks, the uppermost layer of each consisting of bedding material inoculated with selected enzymes, microorganisms and colonies of *Lumbricus rubellus* that served as biofilters followed by a trickling filter system. The final vermi-filtered water exhibited a significant decrease in COD by 90.08 ± 0.176%, and BOD by 89.24 ± 0.544%. In 3 months, the total CFU in the upper bedding layer of specific VF tanks exhibited an exponential increase indicating expected degradation potential of the organic matter in the effluents. The bedding material gradually converted into humified vermicompost on which seedlings of *Canna indica* were planted. The final vermi-filtered discharge was clean and its bio-safety was evaluated by using it for secondary purposes, irrigation and *Spirulina* cultivation.

Keywords: bio-safety, enzyme, *Spirulina*, three-tier vermiculture biotechnology, vermicompost
Abbreviations: BOD, biological oxygen demand; **CFU**, colony-forming unit; **COD**, chemical oxygen demand; **ETP**, effluent treatment plant; **TSS**, total suspended solids; **TT**, treatment tank; **TDS**, total dissolved solid

INTRODUCTION

Developing countries like India have to accelerate economic development while meeting the requirements of environment preservation. Industries fulfill diverse necessities of man. Simultaneously, their activities generate a huge amount of obnoxious wastes. In the recent past, environmental regulations have undergone vast changes. As a result, conventional treatment technologies have been further refined and new technologies for wastewater treatment are being implemented and/or are in the development stage to meet increasingly more stringent water quality criteria (Kumar *et al.* 2008).

Many present wastewater treatment systems are a "disposal-based linear system". Rose (1999) suggested the need to transform traditional linear treatment systems into cyclical treatments to promote the conservation of water and nutrient resources. Amongst the varied biological methods of wastewater treatment adopted, vermiculture biotechnology is gaining wide popularity.

Earthworms have proved to be master bioprocessing agents for the management of organic effluents from diverse sources ranging from domestic sewage to industrial refuse (Ghatnekar *et al.* 2000). Startlingly, they convert effluents that are an undesirable nuisance into coveted plant probiotics in the form of vermicompost. In this context, the present paper describes the application of vermiculture-based wastewater treatment technology with the primary objective of converting liquid effluents into 'bio-safe' clean water.

Since its inception, the Biotechnology Resource Centre (BRC), Mumbai, India has contributed towards uniting the environment and economy by developing innovative, 'bio-safe' waste treatment technologies of global importance (Ghatnekar and Kavian 1992; Ghatnekar *et al.* 2009b). It has successfully commissioned vermiculture-based effluent treatment plant (ETP) in diverse industrial units (Ghatnekar 1994; Ghatnekar *et al*. 1995; Ghatnekar 1999). BRC has developed "three-tier vermiculture biotechnology" that involves the synergistic action of enzymes, microorganisms and earthworms for degradation of complex organic wastes in both solid and liquid forms and converts them into useful plant probiotics and soil conditioners (Ghatnekar *et al.* 2009b).

Vermi-filtration is a relatively new technology to process organically polluted water using earthworms as biofilters (Aguilera 2003; Sinha *et al.* 2007; Li *et. al.* 2009). As an extension of vermicompost for solid waste, vermi-filtration was first developed to treat a mixture of solids and liquids from household or animal breeding with high organic pollution (Gardner *et al.* 1997; Bajsa *et al.* 2003; Taylor *et al.* 2003; Li *et al.* 2008). The vermi-filtration-based wastewater treatment plant has been successfully commissioned by BRC at Orient Vegetexpo Ltd., Dindori, Nashik (Ghatnekar *et al.* 2000).

In the present study, a pilot level wastewater treatment system that involves three-tier vermiculture biotechnology coupled with vermi-filtration technology was applied for the treatment of secondary liquid effluents generated by a gelatine manufacturing industry.

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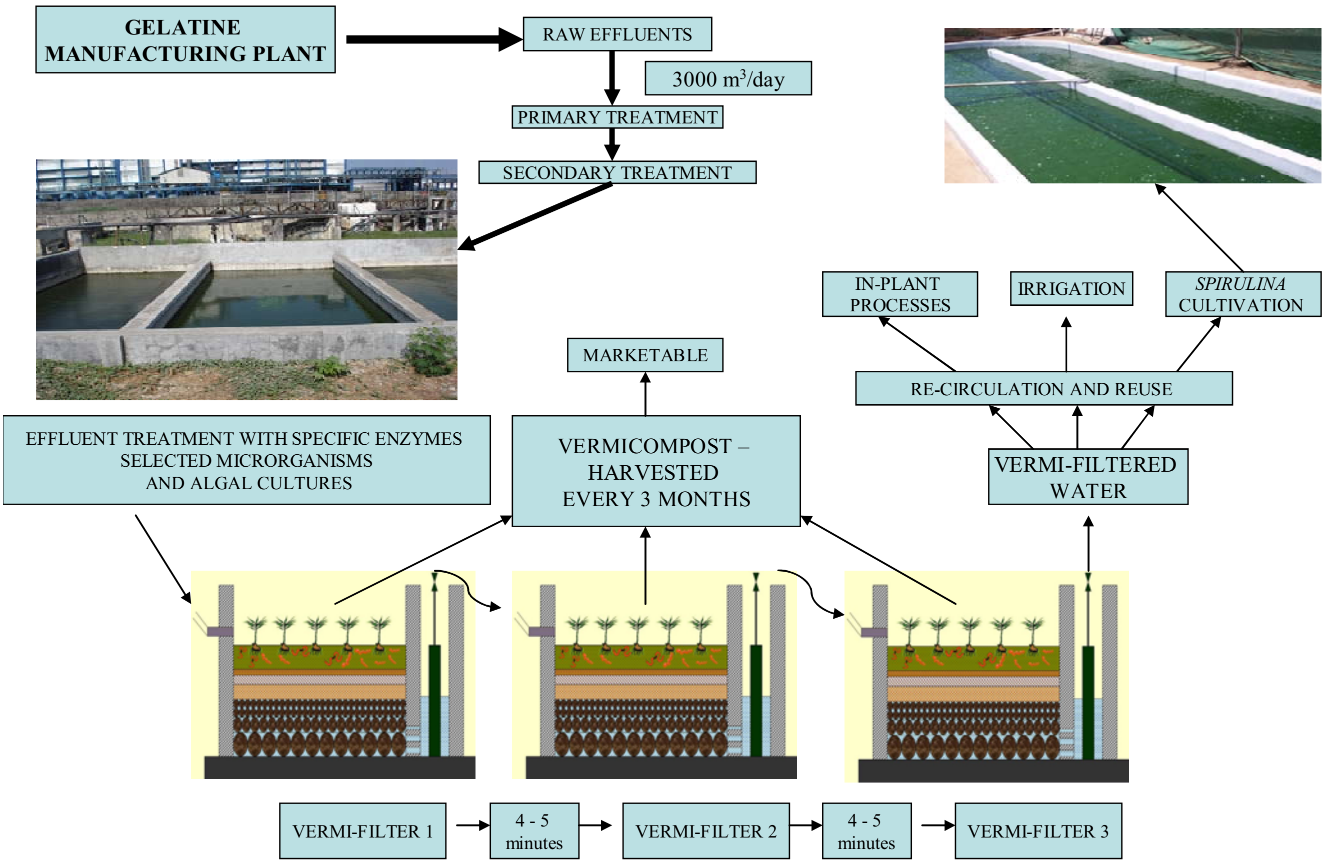

Fig. 1 Treatment tanks used in this study.

MATERIALS AND METHODS

Materials

1. Industrial effluents

The secondary liquid effluents were procured from the ETP of I.G.C.L., Vapi, Gujarat.

2. Treatment tanks

Treatment tanks (TTs) consisted of a series of smooth-walled cemented, seepage-proof rectangular tanks (9 m × 7 m × 1 m) (**Fig. 1**) that were used for enzymatic and microbial (tertiary) treatment of the selected secondary effluents.

3. Vermi-filter tank

The basic design of the vermi-filter (VF) used in the present study is displayed in **Fig. 1**. It is a smooth walled cemented pit dug in the ground and lined with 30-mm thick stone walls. The floor of the pit consists of rammed clay with water proofing to prevent seepage of water. Over this, successive layers of coarse to fine rubble are laid down. This is followed by a layer of semi-crushed bricks, two layers of gravel and fine sand. This section resembles a trickling filter (Ghatnekar *et al.* 2000). The upper layer of the VF consists of bedding material inoculated with gelatine waste-acclimatized enzymes, selected microorganisms and colonies of *Lumbricus rubellus*.

In each VF tank, the effluents progressively descend through the different strata. The treated water oozes out and accumulates in the side channel through perforations in the lower parts. This vermi-filtered water was extracted using a submersible pump.

4. Standard bedding material

Standard bedding material (consisting of sawdust, cowdung, *Leucaena leucocephala* foliage and bovine urine) formulated by BRC (Kavian *et al.* 1997) was used after suitable sterilization in the uppermost layer of the VF tank.

5. Microbial cultures

Pure cultures of *Aspergillus flavus*, *A. niger* and *Nitrobacter winogradskyi* were obtained from the culture bank of BRC. Thereafter, the effluent-resistant isolates of these species *viz.*, *A. flavus* (BRC-27), *A. niger* (BRC-28) and *N. winogradskyi* (BRC-5) were cultured in flasks and maintained at 30°C and 90 rpm in rotary shakers (Orbitek). Later these isolates were multiplied in semi-automated fermentors (BRC-Bio-Boom).

6. Enzymes

Protease and cellulase for the treatment of selected effluents were obtained from Egenix Biotech Pvt. Ltd., Bangalore.

7. Algal cultures

Axenic cultures of *Spirulina platensis*, *Oscillatoria princeps* and *Chlorella vulgaris* were procured from the culture bank of BRC. Thereafter the effluent-resistant isolates of these species *viz.*, *S. platensis* (BRC-45), *O. princeps* (BRC-46), and *C. vulgaris* (BRC-47) were cultured and acclimatized in plastic tubs (0.45 × 0.32 × 0.12 m).

8. Earthworm cultures

Juveniles, capsules and breeders of *L. rubellus* were obtained from the vermiculture bank of BRC. The earthworms were gradually acclimatized to selected wastes.

9. Canna indica

Seedlings of *Canna indica* were procured from a local nursery at Badlapur.

Pretreatment of selected liquid effluents and vermi-filtration

The experiments were conducted at a pilot scale for a period of 6 months in the field laboratory of BRC located in Badlapur, 50 km away from Mumbai. Major parameters such as pH, BOD, COD, TSS, and TDS of the selected liquid effluents were analyzed before treatment (Trivedi and Goel 1986). The details are presented in **Table 1**.

The liquid effluents were discharged in the TTs to which a combination of protease and cellulase (2: 1) was added. Later, the mixed cultures of waste-acclimatized strains of selected microorganisms and algae were inoculated in the TTs. Initially, the microbial and algal population in TTs required 30 days to attain saturation; thereafter, 70% of the treated effluents were perpetually replaced with a fresh discharge of effluents on a daily basis. Enzymes were added at the same ratio to the effluents discharged in it.

Vermi-filtration

The treated effluents were then passed through three successive VF tanks. The effluent retention time in each VF tank was 4 to 5 min. The filtration process was instant hence effluents could be discharged into the VF system continuously. The parameters under study were analyzed after discharge from each VF tank (**Table 1**).

The degradation potential of the bio-solids contained in effluents was monitored in terms of total CFU and CFU of inoculated species of microorganisms in the upper bedding layer of each VF tank before discharging the effluents and thereafter for a period of 3 months at a 30-day interval (**Table 2**). The standard dilution plate technique was used for analysis (Aneja 2003).

The bedding material of the VF gradually converted into humified vermicompost enriched with probiotics on which seedlings of *Canna indica* were planted. The root systems of these plants also aided partial osmotic filtration. Vermicompost was harvested every 3 months. Three-quarters of this layer was replaced with new bedding material and mixed with the remaining one quarter that served as inoculants of the degradable system (consisting of microorganisms, earthworms and enzymes).

Application of triple vermi-filtered water

The final triple vermi-filtered water pumped out from the 3rd tank was evaluated for its bio-safety by applying it for various secondary uses including washing, toilets, irrigation and *Spirulina* cultivation. The BRC-Spiro medium (Tamhane *et al.* 2005) was used for commercial cultivation of *S. platensis* in the vermi-filtered water.

The growth rate of *S. platensis* cultured in vermi-filtered water *vis-à-vis S. platensis* cultured in potable water was monitored. For this, optical density (O.D.) of the culture was measured with a 215 D visible spectrophotometer (Chemito) at 560 nm at an interval of 10 days for a period of 30 days (**Table 3**). The periodically harvested biomass was dried and preserved for further analysis and trials.

Statistical analyses

All the data collected were analyzed as per the method used by Ghatnekar *et al.* (2009d). The results obtained after the analysis of physico-chemical parameters of the effluents before treatment in relation to vermi-filtered discharge (VF-3) and O.D. of *S. platensis* cultured in control and treated sets were tested for statistical significance using unpaired *t*-tests (Graphpad 2010).

RESULTS AND DISCUSSION

Importance of the gelatine manufacturing industry

Gelatine, a heterogeneous mixture of water-soluble proteins derived from the collagen of animal hide or bone (Narmada Gelatines 2010), is composed of various essential amino acids required for human nutrition. It has a diversity of uses in food science and industry, including wine fining, confectionary, matches and photography (Maree *et al.* 1990); therefore, the gelatine industry is of great importance to society.

Characteristics of gelatine industry effluents

The gelatine manufacturing process primarily involves selective hydrolysis and extraction of collagen from the connective tissues of animals. In India, the conventional raw materials used in gelatine manufacture are bovine bones (Narmada Gelatines 2010)

The major consideration during the manufacture of all forms of gelatine is the generation of large amounts of process waste effluents. These waste effluents contain mineral components and lipid material, which create a high biological oxygen demand (BOD), and can be alkaline or acidic (NOSB TAP Review Compiled by OMRI for the USDA National Organic Program (2002).

Maree *et al.* (1990) reported that the pit effluents of a gelatine manufacturing unit are high in pH, COD and calcium content, which constitute a major source of pollution. Badrinath *et al.* (1991) reported the characterization of ossein (bone) wastewater and its treatment scheme involving the application of chemical and biological unit opera-

Table 1 Analysis of gelatine industry effluents before and after each vermi-filter treatment.

Parameter analyzed	Before treatment	Vermi-filter 1	Vermi-filter 2	Vermi-filter 3 *	% Reduction
pH	6.26 ± 0.04	7.04 ± 0.024	7.08 ± 0.037	7.02 ± 0.02	-11.07 ± 0.307
TS [%]	0.308 ± 0.0109	0.079 ± 0.001	0.0698 ± 0.0011	0.0687 ± 0.00077	77.56 ± 0.638
TDS [%]	0.274 ± 0.0047	0.0642 ± 0.00159	0.0558 ± 0.00178	0.0494 ± 0.00088	81.97 ± 0.181
COD [ppm]	1832.0 ± 13.93	283.6 ± 7.27	209.8 ± 2.73	181.60 ± 4.43	90.08 ± 0.176
BOD [ppm]	445.00 ± 12.45	66.6 ± 1.63	56.8 ± 0.97	47.60 ± 1.12	89.24 ± 0.544

TS, total suspended solids; TDS, total dissolved solids; COD, chemical oxygen demand; BOD, biological oxygen demand

Each value is average of 5 observations ± Standard Error of the mean, '- sign' indicate increase.* The two-tailed P value < 0.0001.

Table 2 Microbiological analysis of upper bedding layer of each vermi-filter tank over a period of 3 months.

Microbial status	Days	Total CFU	CFU of inoculated microorganisms		
			A. flavus	***A. niger***	***N. winogradskyi***
Vermi-filter 1	0	2.503 (± 0.053) $\times10^5$	0.79 (± 0.035) ×10	0.98 (± 0.034) ×10	0.91 (± 0.062) ×10
	30	1.65 (± 0.074) $\times10^6$	2.78 (± 0.034) $\times10^{1.3}$	3.34 (± 0.024) $\times10^{1.3}$	2.67 (± 0.039) $\times10^{1.3}$
	60	2.93 (± 0.028) $\times10^6$	1.01 (± 0.043) $\times10^{1.5}$	4.02 (± 0.026) $\times10^{1.7}$	1.51 (± 0.05) $\times10^{1.5}$
	90	5.72 (± 0.074) $\times10^7$	3.94 (± 0.039) $\times10^{1.7}$	5.42 (± 0.037) $\times10^{1.85}$	3.29 (± 0.076) $\times10^{1.5}$
Vermi-filter 2	0	2.06 (± 0.054) $\times10^5$	1.04 (± 0.033) ×10	1.05 (± 0.045) ×10	0.94 (± 0.035) ×10
	30	0.91 (± 0.060) $\times10^6$	3.3 (± 0.065) $\times10^{1.3}$	2.62 (± 0.038) $\times10^{1.5}$	1.63 (± 0.041) $\times10^{1.3}$
	60	2.95 (± 0.063) $\times10^6$	1.95 (± 0.028) $\times10^{1.5}$	3.96 (± 0.029) $\times10^{1.7}$	2.42 (± 0.074) $\times10^{1.5}$
	90	5.096 (± 0.046) $\times10^7$	3.30 (± 0.05) $\times10^{1.7}$	4.51 (± 0.041) $\times10^{1.8}$	3.14 (± 0.023) $\times10^{1.5}$
Vermi-filter 3	0	1.992 (± 0.028) $\times10^5$	1.306 (± 0.014) ×10	1.5 (± 0.009) ×10	1.104 (± 0.018) ×10
	30	2.702 (± 0.023) $\times10^5$	3.328 (± 0.015) ×10	2.5 (± 0.011) ×10	4.718 (± 0.035) ×10
	60	2.406 (± 0.03) $\times10^6$	1.804 (± 0.008) $\times10^{1.5}$	1.49 (± 0.013) $\times10^{1.5}$	0.636 (± 0.012) $\times10^{1.5}$
	90	2.096 (± 0.034) $\times10^7$	1.77 (± 0.031) $\times10^{1.7}$	1.87 (± 0.033) $\times10^{1.8}$	1.74 (± 0.013) $\times10^{1.5}$

Each value is average of 5 observations ± Standard Error of the mean; CFU, colony-forming units tion processes.

During the process of raw gelatin production from cattle bones in the ossein industry, a huge quantity of sinews is discharged as one of the by-products. The bone waste collected from a discharging outlet seems to be fibrous and greases with a pasty matter containing little pieces of bones and hairs. It is composed of a higher quantity of proteins and a small amount of lipids. This is thought to be a major contributor of the odour associated with the ossein industry (Pualchamy *et al.* 2008).

Conventionally these effluents are treated in ETPs using the activated sludge method (Buyukgungor and Gurel 2009; Massachusetts Government 2010). Later, the active sludge is dehydrated to form a sludge cake, which is subsequently disposed. However, the disposal of organically-rich sludge generated from conventional ETPs requires a large dump yard. Furthermore, it may potentially cause environmental pollution by leaching or harbouring pathogens. Discharge of effluents without suitable treatment can cause environmental damage.

Sharma and Rao (1996) reported adverse effects of gelatine factory effluents discharged into the Narmada River. These included a negative impact on seed germination and seedling growth of important crop plants irrigated with river water. The reduction in percentage germination and seedling growth was attributed to high amounts of chloride, calcium, magnesium and other minerals present in the effluents.

In India, Pollution Control Boards in some states are now imposing additional conditions, which read "concerned Industry shall utilize the whole quantity of treated effluent within their premises for plantation and horticulture etc. Effluent shall not be discharged outside the factory premises in any circumstances. Zero discharge conditions shall be maintained" (MPPCB 2009).

Physico-chemical characteristics of effluents selected in the present study

IGCL, Vapi, Gujarat produces food- and pharma-grade gelatine. A gelatine manufacturing plant generates 3000 m^3 of liquid effluents every day, which amount to an average of around 30 tonnes of proteinaceous bio-solid sludge (Ghatnekar *et al.* 2009a, 2009b).

The physico-chemical analysis of selected liquid effluents revealed their acidic nature (pH, 6.26 ± 0.04). They exhibited very high BOD (445 ± 12.45 ppm) and COD (1832.0 ± 13.93 ppm) values. The organic and inorganic content of the effluents contain mostly TS (0.308 ± 0.0109%) and TDS (0.274 ± 0.0047%).

Ghatnekar *et al.* (2009a) had earlier reported that gelatine industry wastes are rich in nitrogen and other minerals like calcium, phosphorus and silica. Moreover, the highly obnoxious odour has been a particular nuisance for the industry and the environment in general. Methanethiol, dimethyl sulfide and dimethyl disulfide are the odour-causing compounds produced from bio-solids (Chen *et al.* 2005). Indeed, these industries are facing a tough time managing the massive waste produced each day.

Pretreatment of selected liquid effluents before vermi-filtration

In the TTs, the complex organic matter in the effluents was degraded by the proteases and cellulases. This process renders it into a nutrient-rich medium for the growth of selected microorganisms and algal species. Microbial populations in the ossein waste from the gelatine industry also promote the production of useful proteases (Jegannathan and Viruthagiri 2009). In addition, the photosynthetic machinery of the algae provided a partial aerobic environment. The pretreated effluents were then subjected to vermi-filtration.

Synergistic action of enzymes, microorganisms and earthworms in vermi-filtration

The role of different types of enzymes, microorganisms, and earthworms for effluent treatments has been previously reported. However, their potential exploitation in effluent degradation in the most optimal way can be attained by their synergistic action in three-tier vermiculture biotechnology (Ghatnekar *et al.* 2009b) that was applied in the vermicast layer of the VF system used in the present study.

Kavian and Ghatnekar (1999) carried out extensive studies on cellulases from *L. rubellus*. Their studies confirmed that enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products. They can also change the characteristics of a given effluent to render it more amenable to treatment or aid in converting effluent material to value-added products.

Kavian and Ghatnekar (1991) also conducted studies on the bio-management of dairy effluents using an *L. rubellus* culture and concluded that sludge cake could support the growth of earthworms without processing. Kavian *et al.* (1996) studied the bio-management of paper mill sludge using vermiculture biotechnology. *L. rubellus* were used to treat approximately 1.5 tonnes of the sludge coming out of the mill daily. The sludge was successfully converted into biofertilizer and plant tonics.

The studies of Hamdi *et al.* (1991) indicated the use of *Aspergillus niger* as an efficient means of protein waste bioconversion while working on waste-water from an olive mill. Kavian and Ghatnekar (1998) demonstrated the utility of fungal species *viz.*, *A. flavus* and *A. niger* in the treatment of pharmaceutical waste. In a separate study, Ghatnekar *et al.* (2009c) reported bio-management of liquid effluents discharged after secondary treatment from the gelatine manufacturing industry using a combination of *A. flavus* and *A. niger*.

In the present study, the liquid effluents discharged in each VF tank were subjected to degradation by selected enzymes, microorganisms and earthworms in vermi-cast layers. These three components synergistically degraded the organic contents of the liquid effluents, thereby initiating a series of alternate aerobic and anaerobic microbial reactions, causing an exponential increase in the population of microorganisms.

In 3 months, the total CFUs in bedding materials of successive VF tanks 1, 2 and 3 increased to 5.72 (± 0.074) × 10^7, 5.096 (± 0.046) × 10^7 and 2.096 (± 0.034) × 10^7, compared to their respective CFUs, 2.503 (± 0.053) × 10^5, 2.06 (± 0.054) × 10^5 and 1.992 (± 0.028) × 10^5 before vermi-filtration. The CFUs of inoculated microorganisms also increased substantially. This increase in CFUs of the selected microorganisms was highest in VF 1 [*A. niger*, from 0.98 (± 0.034) × 10 to 5.42 (± 0.037) × $10^{1.85}$, *A. flavus*, from 0.79 (± 0.035) × 10 to 3.94 (± 0.039) × $10^{1.7}$ and *N. winogradskyi*, from 0.91 (± 0.062) × 10 to 3.29 (± 0.076) × $10^{1.5}$ (**Table 2**)]. Among all the inoculated microorganisms, *A. niger* exhibited the highest CFU count, followed by *A. flavus* and *N. winogradskyi* in all VF tanks.

An increase in CFUs of inoculated microbial species displayed an expected degradation potential of the organic content in selected liquid effluents.

Conversion of vermi-cast layer into vermicompost

In the present study, the bedding material in the uppermost layer of each VF tank was harvested in a 3-month period. It had converted into a humified vermicompost enriched with probiotics.

In a previous study, Li *et al.* (2008) applied vermi-filtration technology to treat diluted manure from a piggery, using earthworms and producing vermicompost. Bajsa *et al.* (2003) reported considerable reduction in pathogens in the end product of vermicomposting and vermi-filtration processes to a level that could then be safely applied to land.

Importance of earthworms in vermi-filtration

Organic matter degraded by microorganisms was further digested by colonies of earthworms living in bedding material. Various actinomycetes inhabiting the earthworms' guts also triggered degradation of solid contents. Zhao *et al.* (2010) reported an intensified bacterial diversity in the VF due to the presence of earthworms (*Eisenia foetida*), especially in response to nutrients in their casts.

Earthworms and microorganisms cooperate in VF to ingest and biodegrade organic wastes and other contaminants in wastewater. This extends the food chain in normal bioprocesses and thus greatly improves sewage treatment efficiency (Li *et al.* 2009).

Earthworms increase the hydraulic conductivity and natural aeration by granulating clay particles (Ghatnekar *et al.* 2000). They also grind silt and sand particles, increasing the total specific surface area, which enhances the ability to 'adsorb' organics and inorganic from wastewater. Intensification of soil processes and aeration by earthworms enable the stabilization of soil and the filtration system to become effective and smaller in size (Ghatnekar 1994; Ghatnekar and Kavian 1995; Ghatnekar *et al.* 1995; Sinha *et al.* 2008).

Zhao *et al.* (2010) observed that the presence of earthworms in VF led to significant stabilization of sludge by enhancing the reduction of volatile suspended solids. Specifically, earthworms in the VF were capable of transforming insoluble organic materials to a soluble form and then selectively digesting the sludge particles to a finer state, which facilitated further degradation of organic materials by the microorganisms in the reactor.

Hughes *et al.* (2008) reported that ammonia had very low toxicity on the survival of earthworms in the VF. In this study there were no observable adverse effects of the selected effluents on the earthworm population.

Enhanced functioning of vermi-filters

The composition and proportion of filter material is the core of vermi-filtration technology (Li *et al.* 2009). Different kinds of filter material have been reported, such as domestic organic waste (Bajsa *et al.* 2003; Taylor *et al.* 2003), gravel, sand, soil (Sinha *et al.* 2008), wood chips, bark, peat, and straw (Li *et al.* 2008). The upper bedding material used in the current study facilitated the acclimatization of earthworms and microorganisms in selected effluents, and enhanced the degradation process (Kavian *et al.* 1997) while the lower strata, similar to a trickling filter system, further cleansed the water (Ghatnekar *et al.* 2000).

In recent times, various attempts have been made to improvise VF systems. Wang *et al.* (2010) reported the enhancement in rural domestic wastewater treatment performance of vermi-filters by a converter slag–coal cinder filter. While vermi-filtration was effective for insoluble organic matter and suspended solid removal, the converter slag–coal cinder filter played an important role in the removal of phosphorus.

In this study, 3 successive vermi-filters were used to enhance the filtration process.

Parameters monitored during treatment of selected liquid effluents

After successive filtration in 3 VF tanks, TS and TDS reduced by 77.56 (± 0.638) and 81.97 (± 0.181)%, respectively. In addition, COD and BOD values exhibited a significant reduction by 90.08 (± 0.176) and 89.24 (± 0.544)%, respectively. The acidic pH of the effluents was also raised to neutrality (7.02 ± 0.02) at the end of the 3rd vermi-filtration. Details of changes in characteristics of liquid effluents after each vermi-filtration are presented in **Table 1**. The recorded data were analyzed using an unpaired *t*-test. The values exhibited statistical significance at a 95% confidence interval.

Previous studies have also demonstrated neutralization of waste water pH by earthworms, as well as elimination of any foul odour during VF processing (Hughes *et al.* 2007; Sinha *et al.* 2008).

Table 3 Growth rate of *S. platensis* in vermi-filtered discharge (Treated) in relation to potable water (Control) recorded in terms of Optical Density (O.D.) of the culture.

Number of days	O.D. of culture at 560 nm			
	Control	% I	Treated	% I
0 *	0.49 ± 0.0080	-	0.55 ± 0.011	-
10 * *	0.64 ± 0.011	23.43	0.69 ± 0.003	20.28
20 # #	0.79 ± 0.006	37.97	0.82 ± 0.003	32.92
30 # #	0.91 ± 0.006	46.15	0.88 ± 0.003	37.5

Each value is average of 5 observations ± Standard Error of the mean, **% I** – percentage increase in absorbance in relation to zero day reading.
* The two-tailed P value = 0.0021, * * The two-tailed P value = 0.0023, # The two-tailed P value = 0.0136, # # The two-tailed P value = 0.0052

Application of vermi-filtered water

The final vermi-filtered water obtained from tank 3 was clean and 'bio-safe', making it suitable for secondary usage in irrigation (Ghatnekar *et al.* 2000; Ghatnekar and Kavian 2003; Sinha *et al.* 2007), and for commercial cultivation of *S. platensis*. The growth rate of *S. platensis* cultured in vermi-filtered water (treated) exhibited a 37.5% increase in absorbance (O.D.) in relation to the day zero reading. It was comparable to the control system (*S. platensis* cultured in potable water) that depicted a 46.15% increase in O.D. (**Table 3**).

Application of vermi-filtration process

Since the 1990s, studies on vermi-filtration technology from small to pilot scales have been conducted. Almost all of the earlier experimental vermi-filtration processes showed a perfect efficacy on sewage treatment, with high removal rates of COD, BOD and TSS, as well as some ability to remove N and P (Sinha *et al.* 2008). Some researchers further developed vermi-filtration to treat municipal sewage with relatively low organic loads (Bouché and Soto 2004; Xing *et al.* 2005; Sinha *et al.* 2008).

Ghatnekar *et al.* (2000) reported the use of a vermi-filtration system for treatment of waste water from a vegetable dehydration unit at a rate of 100 million m^3 per day. The treated pure water was then used for irrigation of vegetable plots where onion (*Allium cepa*), cabbage (*Brassica oleracea*) and chilli (*Capsicum annuum*) were cultivated. Ghatnekar and Kavian (2003) suggested the utilization of a 'VF-Biotreatment Plant' to treat wastewater from a sewage plant of a small town for irrigating agriculture.

Sinha *et al.* (2007) applied vermi-filtration technology to treat primary liquid waste products from the dairy industry. They reported a reduction in BOD (5 days) by over 98%, COD by 80-90%, TDS by 90-92% and TSS by 90-95%.

In the present study, the efficacy of the vermi-filtration system for the treatment of secondary liquid effluents from the gelatine manufacturing industry was evaluated.

CONCLUDING REMARKS

The results of this present study indicate that the combination of three-tier vermiculture biotechnology and vermi-filtration technology is suitable for treatment of secondary liquid effluents generated by the gelatine manufacturing industry. The organic content in selected liquid effluents has the expected degradation potential and is harmless to the earthworm population in the vermi-cast layer. The vermicompost obtained in the process may find use as plant probiotics and soil conditioners. The vermi-filtered water is clean and can be recycled for inplant production processes, various secondary usages and irrigation. The technology used in the present study has the potential to address waste management and water crisis simultaneously.

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