International Journal of Engineering Research and General Science Volume 3, Issue 4, Part-2, July-August, 2015
ISSN 2091-2730

INTEGRATED MICROBIAL-VERMIFILTRATION TECHNIQUE FOR AYURVEDIC INDUSTRIAL EFFLUENTS

Divya C Das, Mary Joseph, Deepak Varghese
Department of Biotechnology, MET'S school of engineering,
maryjcherayath@gmail.com,
Phone No: 9567284363

Abstract— Sustainable, effective and cheapest method of effluent treatment is yet to be reality especially for industrial waste water. Many of the existing methods resulted in several environmental problems. So this paper, mainly focuses on the development of treatment method for Ayurvedic liquid effluents by integrating microbial pre-treatment and vermifiltration. Firstly, Ayurvedic effluent was pretreated with a microbial consortium and later fed to a vermifiltration unit. Organic wastes, heavy metals and solids are ingested and absorbed through earthworm's body wall and degraded which was found to remove BOD, COD, total dissolved solids (TDS) and the total suspended solids (TSS) from wastewater. There was no sludge formation in the process and odor free. The final vermifiltered water exhibited a significant reduction in COD by 98.03%, BOD by 98.43%, TSS by 95.8% TDS by 78.66% and oil & grease by 92.58%. The resultant water was clean and disinfected enough to be reused for irrigation.

Keywords: Vermifiltration, Earthworms, Biofilter, Effluent, Odor-free process, Microbial pre-treatment, Irrigation

INTRODUCTION

Human relationship with the environment has been found to change from industrial revolution to the present day. Industrial effluents create a lot of environmental impacts by its uncontrolled discharge which is a great concern in developing countries like India since environmental preservation adds to its economy. Even though industries are needed to fulfil diverse necessities of man, the huge amount of harmful wastes leads to undesirable consequences. Presently, linear waste water disposal systems are followed in ETPs by using the activated sludge method [3]. Environmental regulations insist on zero discharge waste water treatment systems, thereby the refinement of conventional treatment technologies and implementation of new technologies for waste water treatment to meet stringent water quality criteria is made mandatory. [7].

In developing countries, about 80% of the world population use traditional herbal medicines to treat diseases. Herbal medicines and other finished herbal products contain active ingredients from plants or other plant materials. India has a glorious tradition in the field of herbal medicine [8]. Presently, Ayurveda medicines are used to treat different kinds of diseases and are being popular day by day. Herbal pharmaceutical companies generate huge volume of biodegradable wastewater during processing and production. Herbal pharmaceutical wastewater is moderately strong with COD and BOD concentration and can be discharged only after proper treatment [18]. Plant based raw materials are used in preparation of Ayurveda medicines. Condenser waste from evaporators, chemical waste, spent liquors from fermentation operations, sewage, laboratory and floor washing waste contributes to the organic and inorganic matter in Ayurvedic waste water.

Vermifilter, a low- cost sustainable technology has an immense potential for decentralization in rural areas over conventional systems[10]. Vermifiltration, also known by the term lumbrifiltration, is an extension of vermicomposting for solid waste management [4]. Dissolved and suspended solids get trapped in the vermifilter bed. They are processed by complex biodegradation by passive absorption through the body wall of the earthworms and microbes immobilized in the gut area of the earthworms. Aerobic and anaerobic microbes in the soil also promotes the degradation of organic and inorganic matter [11]. The two processes-microbial process and vermi-process were found to work simultaneously in the treatment of domestic as well as industrial wastewater [9]. The resulting effluent becomes highly nutritious and can be reused for irrigation purpose.

In the present paper, pretreatment of the Ayurvedic effluent with enzyme producing strains were studied.

MATERIALS

Eisenia fetida earthworms were collected from the composting units of Kerala Agricultural University (KAU), Mannuthy. Bedding material consisting of straw, cow dung, vegetable scraps was used after suitable sterilization in the upper most layer of vermicompost.

METHODS

COLLECTION, STORAGE AND PHYSICO CHEMICAL ANALYSIS OF INDUSTRIAL EFFLUENT:

Liquid effluents were collected from Ayurveda Industry. To minimize the potential of volatilization or biodegradation between sampling and analysis, the samples were kept cool as possible without freezing. Samples were cooled with ice or in a refrigeration system set at 4°C. Initial values of BOD, COD, TSS, TDS, Oil and grease, Total nitrogen, Total phosphorous, Sulphate, Chromium, Nickel, Copper present in the liquid effluents were determined.

ISOLATION OF ENZYME PRODUCING STRAINS:

Protease and lipase producing strains were isolated from the effluent by standard plating techniques [13]. A protease (*Bacillus sp*.) and lipase (*Bacillus sp*.) producing was selected from the isolated strains. Antagonism of the two strains were checked by T-streak technique on Nutrient Agar plate. A bacterial consortium was prepared using the selected strains by mixing 5ml of each overnight grown strains in 20ml of nutrient broth [14].

REMOVAL OF OIL CONTENT OF THE EFFLUENT:

Broken brick pieces of varying size were filled inside the column of diameter 6.3 cm. The effluent was passed through the column at a minimum flow rate of 0.025 LPS. The process was repeated for 3 times [1].

PRE-TREATMENT OF THE EFFLUENT:

The experiments were conducted on a lab scale, in the college laboratory. 100ml of Ayurvedic effluent was taken in three 500ml conical flasks each. Each conical flasks was inoculated with protease producing strain (*Bacilllus sp*), lipase producing strain (*Bacilllus sp*), and consortium (Mixture of protease and lipase producing strains) with 1 ml of overnight cultures and incubated at 37˚C at 100rpm for 24 hours. Effect of pre-treatment of effluent with consortium and selected strains on reduction of BOD and COD was studied.

EFFECT OF TIME ON PRE-TREATMENT OF THE EFFLUENT:

100ml of Ayurvedic effluent was taken in three 500ml conical flasks each. Each conical flasks was inoculated with protease producing strain (*Bacilllus sp*), lipase producing strain (*Bacilllus sp*), and consortium (Mixture of protease and lipase producing strains) with 1ml of overnight cultures. Influence of time on pretreatment of effluent was determined by incubating the conical flasks at 37˚C at 100rpm for 24, 48 and 72 hours respectively and reduction of BOD and COD were studied.

PREPARTION OF A LAB SCALE VERMIILTER SETUP:

A lab scale vermifilter setup consist of smooth walled cylindrical plastic tank of 26cm length and 17cm diameter. Lower section resembles a trickling filter consisting of successive layers of pebbles, bricks, gravel, and fine sand laid from bottom to top. The upper bedding material of VF tank was prepared using cow dung and straw as standard bedding materials, and colonies of selected earthworms (Table 1 and 2). For uniform effluent discharge, reservoir with a distribution system was used (fig.1). The treated water was collected at the bottom from the outlet pipe [15].

Serial no.	Parameters estimated	Values
1	Area of the vermi filter system	0.044 m^2
2	Volume of the soil profile in the vermi bed	$3.97*10^{-4}m^3$
3	Porosity of the vermi bed	42.5%
4	Moisture content of the vermi bed	77.40%
5	Temperature of the system	32 ^oC
6	Volumetric flow rate	9.3 L/hr.
7	Hydraulic loading rate (HLR)	0.211 m/hr.
8	Minimum hydraulic retention time (HRT)	2 hrs.

Table 1: Design parameters of vermifilter set up

.

Layers (from bottom to top)	Size range (cm)	Thickness (cm)
Pebbles	1-2	4
Brick	0.05-2	3
Gravel	0.5-1	5
Sand	16-20mm mesh	6
Scraps, cow dung, earthworms	1.5 kg	7

Table 2: Size and thickness of layers

Fig.1: Lab scale setup of vermifilter

VERMIFILTRATION OF RAW EFFLUENT:

The reservoir was filled with 1L of raw effluent without pre-treatment, and was uniformly distributed at a flow rate of 9.3 L/hr to the surface of the vermifilter set up. The entire system was retained for 2hrs. After 2 hrs the treated water was collected at the bottom outlet.The entire set up was allowed to remain to convert the bedding material into humified vermi compost. The treated water collected at the bottom outlet was checked for its BOD, COD, Oil and grease content [16].

VERMIFILTRATION OF PRE-TREATED EFFLUENT:

2L of raw effluent was pretreated with individual enzyme producing strains and consortium at 37˚C for 72 hours with agitation (100 rpm). The reservoir was filled with 1L of pretreated effluent, and was uniformly distributed at a flow rate of 9.3 L/hr to the surface of the vermifilter set up. The entire system was retained for 2hrs. After 2 hrs the treated water was collected at the bottom outlet. The entire set up was allowed to remain to convert the bedding material into humified vermi compost. The trickled water was collected at the bottom outlet and checked for its BOD, COD, Oil and grease content [16].

STUDY OF THE EFFECT OF ENZYME PRODUCING MICROBIAL STRAINS IN THE PRE-TREATED EFFLUENT ON THE EARTHWORMS IN THE VERMIFILTER:

The microbial count in the pre-treated effluent before and after vermifiltration was determined by standard plate count method [13]. The effluent samples before and after vermifiltration were serially diluted and spread plated on Nutrient agar medium. The number of desired colonies of enzyme producing strains were counted. The amount of biomass and number of earthworms before and after vermifiltration were also determined. The above results were analyzed to derive the relationship between the microbial strains and earthworm population. The above procedure was done for protease pre-treated, lipase pre-treated and consortium pre-treated effluents.

RESULTS AND DISCUSSIONS

PHYSICO –CHEMICAL ANALYSIS OF AYURVEDIC EFFLUENT

Ayurvedic effluent was turbid, oily and blackish in color. The characteristics of Ayurvedic effluent is given below in Table no.3

PARAMETERS TESTED	Value (mg/l)
pH	6.23
Total Dissolved Solids	2010
Total Suspended Solids	3020
BOD	3880
COD	7000
Oil &Grease	64.7
Total Phosphorous	55.62
Total Nitrogen	327.55
Sulphate	247.66
Chromium	<0.01
Nickel	<0.01
Copper	<0.01

Table 3: Results of physico-chemical analysis of Ayurvedic effluent

REMOVAL OF OIL AND GREASE FROM THE EFFLUENT

The experiment was carried out using the process of adsorption in which Laterite brick material was used as the adsorbent. The raw effluent was passed at a minimum flow rate of 0.025 LPS through the column containing broken brick pieces. The process was repeated for 3 times. The results proved that Laterite was a powerful adsorbent medium. It can be adopted at the source to remove oil and grease [1].The process resulted in significant reduction of oil and grease from 64.7 mg/l to 7.76±0.2 mg/l with 88±0.25% reduction (Table .4)

Oil and grease Content in raw effluent (mg/l)	Oil and grease content after passage though the column (mg/l)	Percentage Reduction (%)
64.7	7.76±0.2	88±0.25

Table 4: Results of oil removal

PRE-TREATMENT OF THE EFFLUENT:

Ayurvedic effluent was inoculated with protease producing strain (*Bacilllus sp*), lipase producing strain (*Bacilllus sp*), and consortium (mixture of protease and lipase producing strains) incubated at 37˚C at 100rpm in rotary shaker for 72hours. Reduction of BOD and COD by microbial pretreatment was studied in detail and given in table 5 and fig. 2 & 3. Protease and lipase action helps in degradation of proteins and lipids present in the effluent and make it readily available for consumption by microbes and earthworms. Bio management of liquid effluent with enzymes and microbes resulted in significant reduction of organic matter and thereby reducing the BOD load for the vermifiltration process.

EFFECT OF TIME ON PRE-TREATMENT OF EFFLUENT

Ayurvedic effluent was incubated at 37˚C at 100rpm in rotary shaker for 24, 48 and 72 hours respectively with protease producing strain (*Bacilllus sp*), lipase producing strain (*Bacilllus sp*), and consortium (mixture of protease and lipase producing strains). Thus the effect of time on reduction of BOD and COD by pretreatment was studied in detail and has given in table 5 and fig. 2 & 3. In our study, pretreatment with consortium at 72 hours gave the maximum reduction of BOD by 96.1% and COD by 97% which shows increase in contact time with enzymes and microbes decreased the BOD and COD. Agitation at 100 rpm had a significant effect on the growth and activity of the enzyme producing microbes, for the reduction of organic content in the effluent which indicates the oxygen requirement in microbial pretreatment (fig 4& 5).

Time (hrs)	BOD (mg/l)			COD (mg/l)		
	Protease Treatment	Lipase Treatment	Consortium Treatment	Protease Treatment	Lipase Treatment	Consortium Treatment
24	390±2.0	423±4.0	313±3.7	545±2.5	806±2.5	475±3.0
48	324±4.0	393±2.6	264±2.5	426±2.6	751±1.5	386±3.0
72	210±8.5	294±3.0	151±3.5	355±3.0	645±2.5	208±2.0

Table 5: Results of optimization of time for pre-treatment

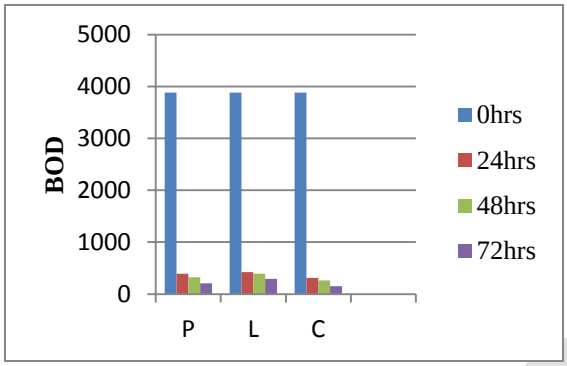

Fig.2: Role of time in BOD removal.

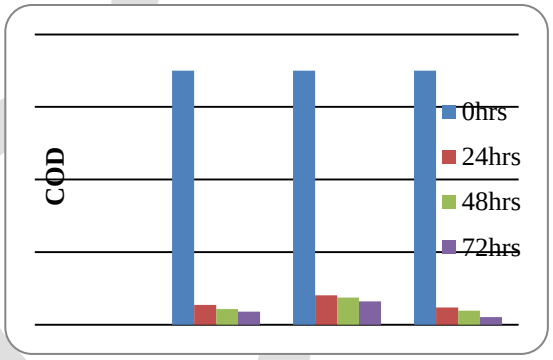

Fig.3: Role of time in COD removal.

EFFECT OF PRETREATMENT ON VERMIFILTRATION

Vermifiltration of raw effluent without pretreatment showed reduction of BOD by 91.95%, COD by 93.23 % and oil & grease by 87.17% (Table 6 & fig 4). 2L of raw effluent was pretreated with individual enzyme producing strains and consortium at 37˚C for 72 hours with agitation at 100 rpm and was uniformly distributed to the surface of the vermifilter set up. The trickled water was collected at the bottom outlet and checked for its BOD, COD, Oil and grease content. The maximum reduction of BOD by 98.43% COD by 98.03% and oil & grease by 92.58% was found in the effluent after vermifiltration process with consortium treatment (Table 6& fig 4). Synergistic action of enzymes, microbes and earthworms lead to considerable reduction of BOD, COD and oil & grease. Studies have shown the gut of endogenic earthworms like *Eisenia fetida* having microbes with cellulolytic activity. The cellulases helps to degrade the cellulose present in the Ayurvedic effluents thereby reducing the BOD and COD [17].

Parameters (mg/l)	Vermi filtered Raw effluent	Vermi filtered Protease pre-treated effluent	Vermi filtered Lipase pre-treated effluent	Vermi filtered consortium pre-treated Effluent
BOD	312.33±4.5	153.33±4.1	167±4.3	61.00±3.2
COD	473.66±4.7	263.00±6.5	374.6±4.5	137.66±2.5
OIL&GREASE	8.4±2.0	8.06±0.2	7.53±0.4	4.8±0.2

Table 6: Results of vermifiltration of pre-treated Ayurvedic effluent

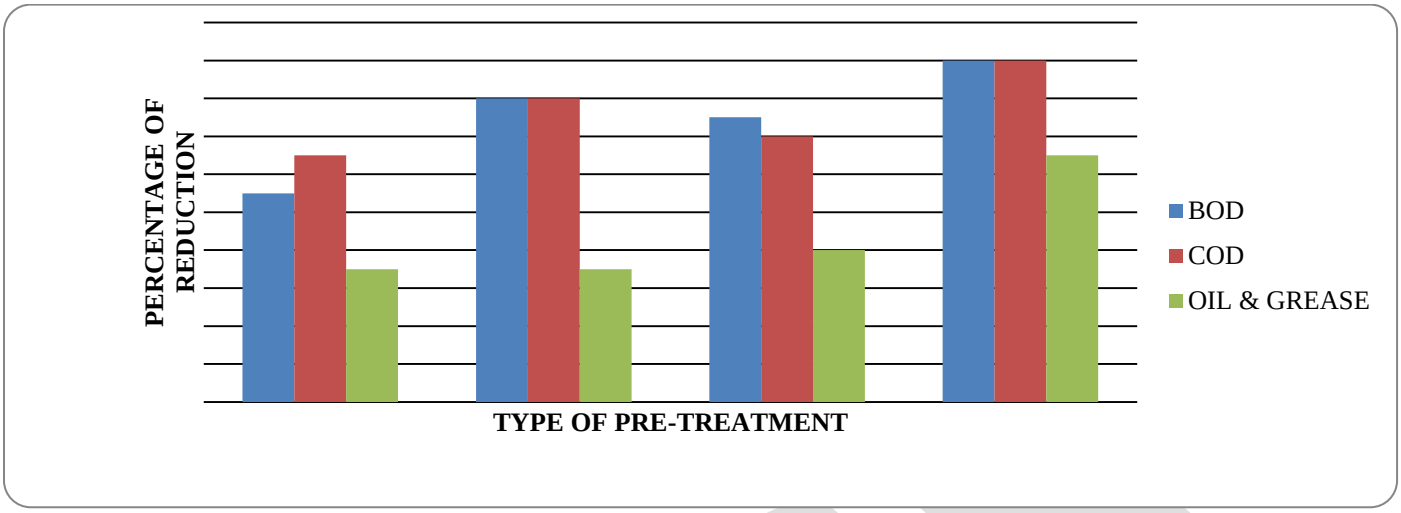

Fig.4: Results for Vermifiltration of pre-treated Ayurvedic effluent

STUDY OF THE EFFECT OF ENZYME PRODUCING MICROBIAL STRAINS ON GROWTH OF EARTHWORMS

Treatment	Number of bacterial colonies in the effluent (CFU) (10^{-9} dilution)	
	Before Vermifiltration	After Vermifiltration
Raw effluent	6.26±0.03	6.83±0.62
Protease pre-treated	10±0.15	12±0.04
Lipase pre-treated	10.48±0.12	11.04±0.02
Consortium pre-treated	14.3±0.05	15.5±0.07

Table 7: No. of bacterial colonies in the effluent (10^{-9} dilution) before and after vermifiltration

Treatment	Biomass of adult earthworms (g)	
	Before Vermifiltration	After Vermifiltration
Protease pretreated	18.35±0.3	23.77±0.05
Lipase pretreated	18.54±0.04	22.48±0.25
Consortium pretreated	18.31 ±0.12	23.64±0.32

Table 8. Biomass of adult earthworms in the pre-treated effluent before and after vermifiltration

Treatment	Biomass of young earthworms (g)	
	Before Vermifiltration	After Vermifiltration
Protease pretreated	9.9±0.25	11.02±0.06
Lipase pretreated	9.85± 0.03	10.70±0.2
Consortium pretreated	10.4±0.09	11.45±0.54

Table 9: Biomass of Young earthworms in the pre-treated effluent before and after vermifiltration

From the above data it was found that for protease, lipase and consortium pre-treatment, the number of bacterial colonies in the pre-treated effluent, remained almost consistent before and after vermifiltration. Number and biomass of earthworms in the vermifilter set up were also found to increase after the vermifiltration process. Increase in the number of earthworms and consistent bacterial count indicated that there was no negative effect on the growth of the desired enzyme producing microbes and earthworms. This proves that the water does not contain harmful compounds and is considerably safe to discharge for irrigational purposes.

IRRIGATIONAL WATER QUALITY CRITERIA OF FINAL DISCHARGED EFFLUENT

The effluent obtained after vermifiltration of the consortium pre-treatment was further analyzed for its biosafety and irrigational water quality criteria (Table 10).

Parameters (mg/l)	Qty in treated effluent (mg/l)	Max. permissible limit in irrigation water (mg/l)
pH	6.2±1.5	6-8.5
BOD	61±3.2	80
COD	137±2.5	150
TSS	130±.007	250
TDS	429±.03	2100
Sulphates	136±.03	1000
Chromium	<0.01	0.1
Copper	<0.01	0.2
Nickel	<0.01	0.2
Total nitrogen	18±.009	30
Total phosphorous	26±.05	50

Table 10: Irrigational water quality criteria of final discharged effluent

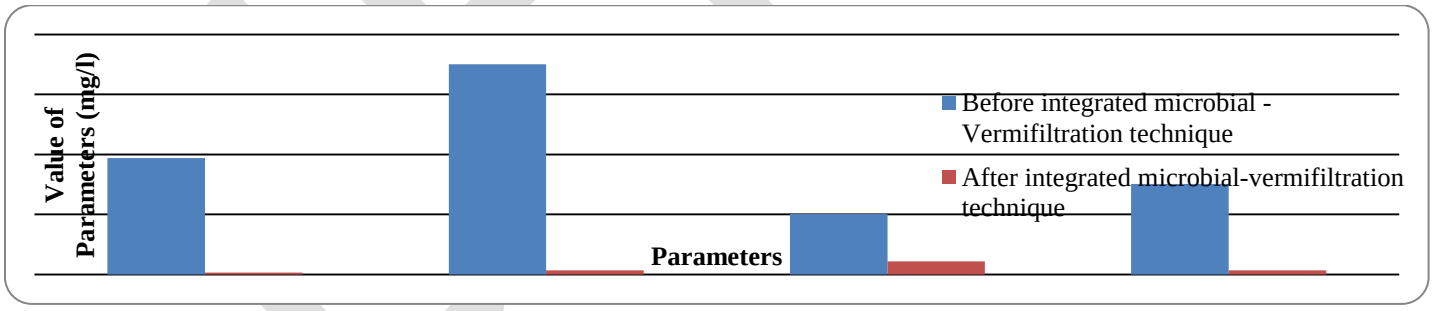

Fig.5: Results of effluent before and after integrated microbial- vermifiltration technique

VARIATION IN PH VALUE OF TREATED WASTE WATER

Results indicate that the pH value of both raw effluent and treated effluent have an average value of 6.2.

REMOVAL OF TOTAL SUSPENDED SOLIDS

Results showed that the microbes and earthworms can significantly remove the suspended solids from the wastewater by over 95%.

REMOVAL OF TOTAL DISSOLVED SOLID

Total suspended solids (TSS) and total dissolved solids (TDS) showed drastic reduction during integrated microbial-vermifiltration process. The total reduction in TDS content was about 75% in vermifiltration unit with pretreatment. Results thus clearly suggested the capability of earthworms to remove solid fractions of wastewater during integrated microbial -vermifiltration processes.

REMOVAL OF BIOCHEMICAL OXYGEN DEMAND (BOD)

BOD is indicates the organic load of wastewater. The BOD load in effluents vermifiltration unit with pretreatment was significantly lower than initial levels and integrated microbial -vermifiltration showed more removal efficiency. Results show that the earthworms can remove BOD load by over 98.43%.

REMOVAL OF CHEMICAL OXYGEN DEMAND (COD)

Results showed that the average COD removed from the wastewater by vermifiltration with pretreatment is over 98.03% indicating degradation of several chemicals by enzymes in the gut of earthworms which is not usually degraded by microbial pretreatment.

CONCLUSION

Integrated microbial- Vermifiltration technique for the treatment of ayurveda liquid effluent was developed. It is a decentralized and cost effective method which can be applied to treat both domestic and industrial waste water treatment. A huge reduction in various effluent parameters like BOD, COD, TSS, TDS, Oil & grease, was observed. Presence of heavy metals in Ayurvedic effluent is almost zero. Vermifiltration of the effluent pre-treated using the bacterial consortium at optimum conditions showed maximum reduction in the above mentioned parameters. Treated effluent well suited the irrigational water quality criteria.

REFERENCES:

[1] Ajith Hebbar H, Jayantha K. S (2013), Oil and Grease Removal from Wastewater Using Laterite as an Adsorbent Material. Volume 3, Issue 5, 2250-2459.

[2] Bouché MB, Qiu JP (1998). Concrete contributions of earthworms in the environmental study. Doc. Pédozool. Intégrol. 3: 225-252.

[3] Buyukgungor H, Gurel L (2009). The role of biotechnology on the treatment of wastes. African Journal of Biotechnology 8 (25), 7253 – 7262.

[4] Ghatnekar, S. D., Kavian, M. F., Sharma, S. M., Ghatnekar, S. S., Ghatnekar, G. S., & Ghatnekar, A. V. (2010). Application of Vermi filter-based Effluent Treatment Plant (Pilot scale) for Biomanagement of Liquid Effluents from the Gelatine Industry. Dynamic Soil, Dynamic Plant Vol 4 Special Issue 1, 83-88.

[5] Indian J.Environmental Protection,vol 27,No.6, June 2007.

[6] Kharwade A.M. and Khedikar I. P. (2011).Laboratory scale studies on domestic grey water through vermifilter and non-vermifilter. Journal of Engineering Research and Studies, 2 (4): 35-39

[7] Kumar L, Rajan R, Sabumon PC (2008). Development of an ecologically sustainable waste water treatment system. Water Science and Technology 58(1), 7-12.

[8] Nandy, T.; Kaul, S. N.; Szpyrcowicz, L. (1998). Treatment of herbal pharmaceutical wastewater with energy recovery. International Journal of Environmental Studies. Vol 54, 83-105.

[9] Sinha R. K., Bharambe G. and Chaudhari U (2008). Sewage treatment by vermifiltration with synchronous treatment of sludge by earthworms: a low cost sustainable technology over conventional system with potential for decentralization. The Environmentalist, 28, 409-420.

[10] Taylor M, Clarke WP, Greenfield PF (2003). The treatment of domestic wastewater using small-scale vermicompost filter beds. Ecol. Eng. Vol 21: 197-203.

[11] Tomar, P., &Suthar, S. (2011). Urban wastewater treatment using vermi- biofiltration system. Desalination Vol 28 no.2, 95-103.

[12] Wang, S., Yang, J., Lou, S., & Yang, J. (2010). Wastewater treatment performance of a vermifilter enhancement by a converter sla-coal cinder filter. Ecological Engineering Vol 36, 489-494.

[13] Benson, H. J. Microbiological Applications 8th Edition. New York: McGraw Hill, 2002. Fig. 21-1. Pg. 87. Bacterial Population Counts. ICBN# 0-07-231889-9.

[14] Payel Sarkar, Andrew Roysten Rai and Shilpi Ghosh (2013), Degradation of Aromatic Petroleum Hydrocarbons (Btex) By A Solvent Tolerant Bacterial Consortium. Vol 7, ISSN 1982 – 3932.

[15] A.Merly Xavier, A.Logeswari, S.Mano, Dr.M.Thirumarimurugan, Dr.T.Kannadasan (2013), Vermiculture For The Treatment Of Diary Effluents. Vol 1, ISSN : 2320 – 5083.

[16] Standard Methods For the Examination of Water and Wastewater (2005).

[17] Fujii K, Ikeda K, Yoshida S (2012) Isolation and characterization of aerobic microorganisms with cellulolytic activity in the gut of endogeic earthworms. Int Microbiol. Sep; 15(3):121-30.

[18] Atul P. Vanerkar, , Abhay B. Fulke, Satish K. Lokhande, Manisha D. Giripunje, and Shanta Satyanarayan (2015), Recycling and treatment of herbal pharmaceutical wastewater using *Scenedesmus quadricuada*, Current Science, Vol. 108, No. 5