



Review

The use of microbial-earthworm ecofilters for wastewater treatment with special attention to influencing factors in performance: A review



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HIGHLIGHTS

- The application of MEEs for treatment of various wastewater has been reviewed.
- The environment-economic analysis of MEEs was given.
- The influencing factors of pollutants removal in MEEs have been provided and summarized.
- Future research was given on enhancing performance and sustainability of MEEs.

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ABSTRACT

With the unique advantages of lower operational and maintenance cost, the use of microbial-earthworm ecofilters (MEEs) for the wastewater treatment has been increasing rapidly in the recent years. This paper provided an overview of the research activities on the use of MEEs for removing pollutants from various wastewater throughout the world. However, the long-term effective treatment performance and sustainable operation of this system still remain a challenge since the treatment performance would be affected by design parameters, operational conditions, and environmental factors. In order to promote the treatment performance, therefore, this paper also provided and summarized the influencing factors of pollutants removal in MEEs. The design parameters and operational conditions of MEEs include earthworm species and load, filter media type, hydraulic loading rate, nutrient load, packing bed height, chemical factors and temperature. Lastly, this review highlighted the further research on these issues to improve performance and sustainability of MEEs.

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1. Introduction

In industrial society one of the burning issues is the high consumption of water and high demand for cleaning water (Schröder et al., 2007). The wastewater treatment and its

reclamation have become a hard nut to crack especially in the developing countries due to the combined effects of worsening environmentally-unfriendly activity and increasing population (Singh et al., 2015). In fact, numerous technologies for wastewater purification have been widely investigated. Many traditional treatment technologies such as activated sludge treatment, membrane bioreactor and biofilm process have been implied successfully for water pollution control in a lot of countries (Li et al., 2014). However, these wastewater treatment technologies are limited to widespread use, as developing countries cannot afford for the high costs of construction, operation and maintenance (Muga and Mihelcic, 2008). Thus, it is especially necessary to select economically affordable and efficient alternative technologies for wastewater treatment.

Abbreviations: MEEs, microbial-earthworm ecofilters; WWTPs, wastewater treatment plants; TSS, total suspended solids; TDS, total dissolved solids; BOD₅, 5 day biochemical oxygen demand; COD, chemical oxygen demand; TN, total nitrogen; TP, total phosphorus; AOB, ammonia-oxidizing bacteria; HRT, hydraulic retention time; TS, total dissolved and suspended solids; HLR, hydraulic loading rate; LC₅₀, lethal concentration 50.

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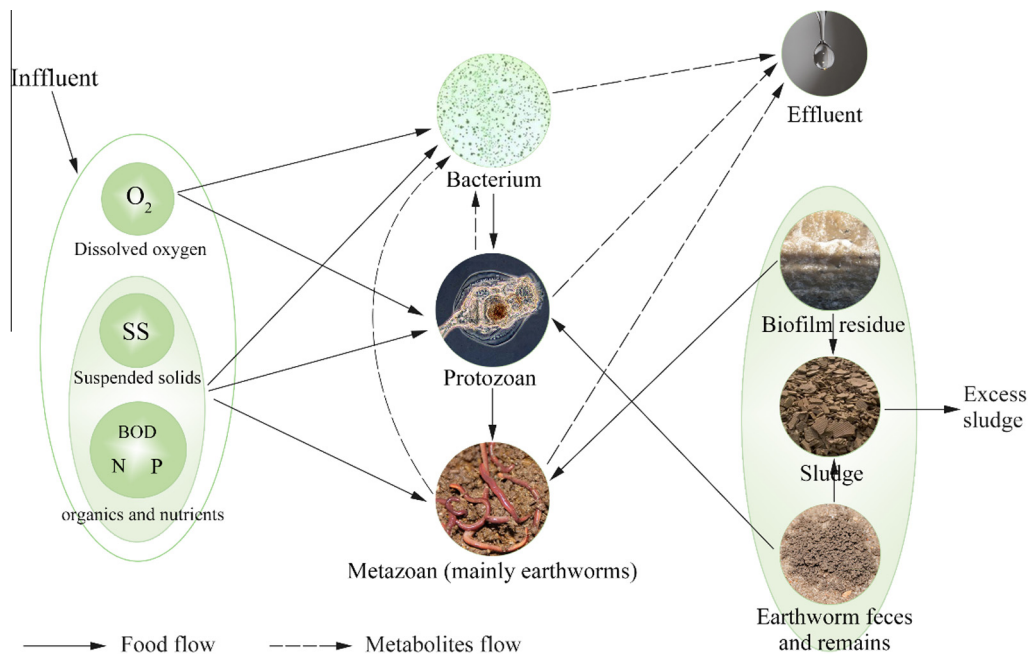


Fig. 1. The diagram for the working mechanisms in MEEs.

One of the alternatives for wastewater treatment in developing countries is microbial-earthworm ecofilters (MEEs) which is a promising economical process for treating point and diffuse sources of wastewater (Tomar and Suthar, 2011). MEEs, a natural engineered system which is based on the symbiotic relationship between earthworms and microorganisms, was first developed by Professor Jose Toha in 1992 at the University of Chile (Aguilera, 2003). The central concept behind MEEs wastewater treatment is that microorganisms perform biochemical degradation of waste material, while earthworms regulate microbial biomass and activity by directly or/and indirectly grazing on microorganisms. These processes are the essential mechanisms for pollutants removal in MEEs (Liu et al., 2012).

The effectiveness of MEEs for wastewater treatment has been demonstrated by a variety of wastewaters, such as domestic sewage, industry wastewater, urban runoff, and livestock wastewater, and at a range of scales (such as small scale, pilot-scale and full-scale) in the recent years (Ghatnekar et al., 2010; Robin et al., 2011; Tomar and Suthar, 2011). MEEs has been shown to provide more improved and consistent wastewater treatment performance than conventional biofilter without earthworm (Sinha et al., 2008). It was also reported that MEEs could be efficient for removing organic matter, nutrients, pathogens, etc. from wastewater and the nitrogen and phosphorus removal rates could reach up to 60.2% and 98.4%, respectively (Arora et al., 2014a; Wang et al., 2011a). In addition to the enhanced wastewater treatment, MEEs has an additional benefit of low excess sludge production (Sinha et al., 2008).

However, as the treatment performance of MEEs can be affected by design parameters, operational conditions, and environmental factors, the long-term effective treatment performance and the sustainable operation still remain a challenge. Thus, the feasibility of MEEs to sustainably eliminate pollutants in wastewater is requiring comprehensive understanding on the influencing factors. Firstly, earthworm species and filter media types are crucial influencing factors for the removal efficiency of MEEs because they are considered as the main biological components of MEEs and can change directly or indirectly the main removal processes of contaminants over time (Sinha et al., 2010). Secondly, the treatment

performance of MEEs is highly dependent on the optimal operating parameters, such as hydraulic loading rate, nutrient load, packing bed height and design of setup, which would lead to variations in removal efficiency of pollutants among different researches (Kumar et al., 2014; Wang et al., 2014; Zhao et al., 2012). Additionally, a variety of pollutant removal processes, such as sedimentation, adsorption, filtration, volatilization, precipitation, earthworm and microbe uptake, are usually directly and/or indirectly influenced by the various internal and external environment factors such as temperatures, pH, ammonia and sodium (Hughes et al., 2009; Yang et al., 2009a,b).

Therefore, the fundamental to the success of long-term effective treatment performance and sustainable operation is the acquaintance of influencing factors and the optimization of design and operational parameters. Meanwhile, the deeply knowledge published in international books and journals on optimizing the treatment efficacy has increased significantly in recent years. Thus, it should be a step in reviewing and discussing the advancement and knowledge on the influencing factors and optimization of MEEs treatment process. The objective of this paper is to provide an overall review on the applications of MEEs for various wastewater treatments and also focus on the development of MEEs considering worm and filter media selecting, operation and design parameters optimizing for the enhancement of wastewater treatment performance. Besides, this paper highlighted the future research considerations for improving the treatment performance of MEEs.

2. Microbial-earthworm ecofilters

2.1. Mechanisms and features

Microbial-earthworm ecofilters is a natural engineered system which is inoculated traditional vermicomposting system into a passive wastewater treatment process by using potentials of earthworms (Athanasopoulos, 1993). In MEEs, microorganisms are responsible for the bio-chemical degradation of waste materials in wastewater, whereas earthworms degrade and homogenize

Table 1
The efficiency and cost comparison between MEEs and conventional WWTPs.

Treatment technology	Removal eff. (%)							References		Cost (US\$/m ³)*		References
	COD	BOD	TSS	TS	TN	TP		Capital cost	Annual (O&M) cost			
MEEs (microbial-earthworm ecofilters)	81.3	98.0	-	-	60.2	98.4		256.4	4.9×10^{-2}	Yang et al. (2011)	Yang et al. (2011)	
WSPs (waste stabilization ponds)	62.0	79.0	67.0	-	-	21.0		854.0	19.0	Comas et al. (2003), Singhirunusom and Stenstrom (2010)	Comas et al. (2003), Singhirunusom and Stenstrom (2010)	
Al (anaerobic lagoon)	Over	-	-	Over	-	-		264.2–	4.0×10^{-5} –	Muga and Mihelcic (2008)	Muga and Mihelcic (2008)	
SBR (sequencing batch reactor)	80.0	-	-	80.0	-	-		1056.7	2.0×10^{-4}	Kalbar et al. (2012a), Molinos-Senante et al. (2012), Singh et al. (2015)	Kalbar et al. (2012a), Molinos-Senante et al. (2012), Singh et al. (2015)	
MBR (membrane bioreactor)	70–90	-	85–	-	55–	25–		2101.4	1.8×10^{-1}	Kalbar et al. (2012b), Molinos-Senante et al. (2012), Singh et al. (2015)	Kalbar et al. (2012b), Molinos-Senante et al. (2012), Singh et al. (2015)	
EA (extended aeration activated sludge)	70–90	99	85–	-	90	70		1463.0	4.4×10^{-1}	Molinos-Senante et al. (2012), Singh et al. (2015)	Molinos-Senante et al. (2012), Singh et al. (2015)	
	70–90	99	85–	-	90	70		1190.4	5.4×10^{-1}	Molinos-Senante et al. (2012), Singh et al. (2015)	Molinos-Senante et al. (2012), Singh et al. (2015)	

* All the monetary units have been converted to US dollars according to the average exchange rate of that year.

the material through muscular actions of their foregut and add mucus to the ingested material, thereby conditioning the filter media and improving its biological activity (Domínguez, 2004). Meanwhile, earthworms can modify microbial community directly or indirectly by three main modes: comminution, burrowing and casting; grazing; dispersal (Brown, 1995). In general, the working principles of MEEs can be illustrated by Fig. 1.

Earthworm predation can effectively condition microbial biomass and protect the filter bed from clogging during long-term running, thereby reducing the needs for maintenance and operation (Wang et al., 2010a,b). Earthworms can improve soil property and aeration so that the soil stabilization and filtration system become effective (Sinha et al., 2008). Therefore, small particle filter materials which have large surface area can be chosen to be applied in MEEs and the treatment performance will be greatly improved. Due to the enhancement of treating ability, land requirements for MEEs decreased (Aira and Domínguez, 2009). Moreover, the existing chain of microbial metabolism has been extended by introducing earthworms and excess sewage sludge produced by MEEs is relatively reduced compared with conventional biofilters (Komarowski, 2001). MEEs is odor-free techniques, and the final water output can be potentially reused for irrigation in parks, gardens and farms, which will facilitate the reuse of reclaimed wastewater upon special permission (Liu et al., 2009).

2.2. Environment-economic analysis of MEEs for wastewater treatment

A successful use of MEEs in developing countries may lie in the fact that this system could fulfill many requirements besides the high treatment efficiency, such as low cost, easy to maintain, and low sludge production. Therefore, it is necessary to pay attention to environment-economic analysis. A series of previous studies indicated that MEEs had an apparent advantage in costs when compared with conventional wastewater treatment plants (WWTPs), as earthworms could handle wastewater without supplement of external energy (Rajiv et al., 2010; Wang et al., 2011b). As can be seen from Table 1, MEEs had less operational cost as it required little energy for pumping of wastewater and no experienced labor. Maintenance expense was also minimal since it did not involve any mechanical devices, except for pumps (Sinha et al., 2008). Moreover, conventional WWTPs faced serious challenge in disposing massive excess active sludge which might cause a potential biohazard to both the environment and the human health if not properly disposed (Li et al., 2014). While MEEs could simultaneously treat sewage and sludge components and transform the sludge into vermi-compost (a nutritive fertilizer of positive economic value) which could be used in agriculture and horticulture (Xing et al., 2011). MEEs was considered as an economically feasible option for vermiculture. For instance, vermiculture is an emerging industry in Australia, and 500 earthworms worth almost \$ 20 in market (Sinha et al., 2008). In addition, the less land requirements for MEEs may be the advantage for their broader application compared with some conventional WWTPs (such as constructed wetland and lagoon), especially in some regions where land resources are scarce and population density is high (Nie et al., 2015).

3. Use of MEEs in wastewater treatment

3.1. Domestic wastewater treatment

The first attempt to the possibility of MEEs application for pollution control was made by Professor Jose Toha in Chile in 1992

Table 2
The use of MEEs for treatment of various types of wastewater reported in the literature after 2007.

Type of wastewater	Scale	Earthworm species inoculated	Country	Major observations	References
Domestic wastewater	Pilot scale	<i>Eisenia fetida</i> (epigeic worm)	Australia	Removal of BOD ₅ by over 90%, COD by 80–90%, TDS by 90–92%, TSS by 90–95% during the treatment	Sinha et al. (2008)
Swine wastewater	Pilot scale	<i>Eisenia andrei</i> (epigeic worm)	France	Earthworm population have increased 30% in 4 weeks and higher volatilization of water, carbon, and nitrogen were observed	Li et al. (2008)
Rural sewage	Pilot scale	<i>Eisenia andrei</i> (epigeic worm)	China	COD, BOD ₅ and TSS were efficient removed and the treatment efficacy of MEEs was similar as activated sludge process	Li et al. (2009)
Secondary liquid effluents from Gelatine Industry	Pilot scale	<i>Lumbricus rubellus</i> (epigeic worm)	India	Significant removal of COD by 90% and BOD ₅ by 89% during MEEs process	Ghatnekar et al. (2010)
Herbal pharmaceutical wastewater	Pilot scale	<i>Lumbricus rubellus</i> (epigeic worm)	India	Removal of COD by 85.44–94.48%, BOD ₅ by 89.77–96.26% and effluent was color and odour free and no sludge production problem was encountered	Dhadse et al. (2010)
Domestic wastewater	Pilot scale	<i>Eisenia foetida</i> (epigeic worm)	China	Removal rates were COD (47.3–64.7%), BOD ₅ (54.78–66.36%), TSS (57.18–77.90%), TN (7.63–14.90%), and NH ₃ -N (21.01–62.31%). Earthworm population dynamics and enzymatic activities is a good indicator for COD and BOD ₅ removal rates	Xing et al. (2010)
Domestic wastewater	Pilot scale	<i>Eisenia fetida</i> (epigeic worm)	China	COD and NH ₃ -N could be efficiently reduced from the influent in MEEs. There was a positive correlation between the decreasing NH ₃ -N concentration and Shannon biodiversity index for AOB	Xing et al. (2012)
Urban runoff	Small scale	<i>Perionyx sansibaricus</i> (epigeic worm)	India	Removal of TSS by 88.6%, TDS by 99.8%, COD by 90%, NO ₃ ⁻ by 92.7% and PO ₄ ³⁻ by 98.3% in the effluent	Tomar and Suthar (2011)
Toxic wastewaters from the petroleum industry	Small scale	<i>Eisenia fetida</i> (epigeic worm)	Australia	MEEs has been found to remove BOD ₅ by over 90%, COD by 60–80%, TS by 90–95%. Removing toxic chemicals and pathogens also were observed from effluent	Sinha et al. (2012)
Rural domestic sewage	Full scale	<i>Eisenia fetida</i> (epigeic worm)	China	Final effluent met the latest standards of irrigation water quality in China and with low sludge production less than 0.20 kg SS/kg COD _{removed} of MEEs	Liu et al. (2013)
Synthetic wastewater	Pilot scale	<i>Eisenia fetida</i> (epigeic worm)	India	MEEs could efficiently remove BOD, COD, total and fecal coliforms, fecal streptococci and other pathogens. The removal of pathogens was significantly correlated with the antibacterial activity of the isolated microorganisms	Arora et al. (2014a,b)
Rural wastewater treatment	Bench scale, pilot scale and full scale	<i>Eisenia fetida</i> (epigeic worm)	China	The MEEs system can work effectively under a variety of natural and socioeconomic conditions at a reasonable cost	Nie et al. (2015))
Artificial wastewater	Small scale	<i>Eisenia fetida</i> (epigeic worm)	China	Removal of COD from artificial wastewater owe to AOE and ROS in earthworm tissues and daily burrowing length of earthworms. While these parameters was not correlated to NH ₃ -N removal rate	Li et al. (2015)

(Aguilera, 2003). Since then, the experiments on MEEs were carried out increasingly and applied for wastewater treatments successively (Bouché and Qiu, 1998), and the detailed information was shown in Table 2. At the early stage, MEEs was mainly applied to traditional domestic wastewater treatment. Sinha et al. (2008) reported the use of a pilot-scale MEEs to treat domestic wastewater in Australia. The results indicated that MEEs could remove TSS, TDS, BOD₅ and COD more efficiently than another biofilter without worms. For domestic wastewater treatment, Xing et al. (2010) also reported that a pilot-scale MEEs removal efficacy for COD, BOD₅, TSS, TN, and NH₄-N could reach up to 47.3–64.7%, 54.78–66.36%, 57.18–77.90%, 7.63–14.90%, and 21.01–62.31%, respectively. In addition, this research found that earthworm population dynamics and enzymatic activities are two good indicators for COD and BOD₅ removal rates via the correlation analysis. Subsequently, Wang et al. (2011b) tested MEEs for treatment of sewage in laboratory scale with a special attention to analyze the mechanisms of nitrogen transformation. The results showed that MEEs could significantly decrease the level of COD and NH₃-N in the influent. Moreover, there was a positive correlation between the decreasing NH₃-N concentration and the Shannon biodiversity index for ammonia-oxidizing bacteria (AOB). In order to explore the pathogen removal efficiency from domestic wastewater, Arora et al. (2014a,b) tested a pilot-scale MEEs in India. After four months study, the results revealed that MEEs could efficiently remove BOD₅, COD, total and fecal coliforms, fecal streptococci and other pathogens. Furthermore, it reported that the removal of pathogens

might be attributed to the antibacterial activity of the isolated microorganisms. With more attention to treat rural domestic wastewater, MEEs was also applied in this field. Li et al. (2009) reported the use of MEEs to rural sewage treatment in pilot scale. The results showed that MEEs could continuously process the sewage produced by about 100 farmers. The concentration of COD, BOD₅ and TSS in outflow were rather stable during one year test, despite the fluctuation of hydraulic loading rate and organic input in influent. This system could also remove some nitrogen. However, it was not efficient for phosphorus removal. For treating rural domestic sewage, Liu et al. (2013) also tested a full-scale MEEs in Shanghai, China, during 17-month operation. The results indicated that the final effluent met the latest standards of irrigation water quality in China and the sludge production was less than 0.20 kg sludge solids per kg COD_{removed}. Moreover, the microbial community in MEEs was dominated by phylum *Proteobacteria* and the turnover of biomass carbon was significantly affected by some earthworm-associated bacterial groups including *γ-proteobacteria*, *Bdellovibrio*, *Lysobacter*, and *Myxococcales*. Soon after, Nie et al. (2015) systematically studied MEEs for rural wastewater treatment at three scales: bench-scale, pilot-scale, and full-scale. The results of bench-scale study showed that the largest amount of phosphorous could be removed by Ca/Mg medium. For the pilot-scale study, the high performance in the removal of COD, NH₄⁺-N, and phosphorous was observed. In the full-scale study, the results showed that MEEs could be operated under a reasonably lower cost on the condition of favorable pollutants removal performance

compared with the cyclic activated sludge system and the subsurface soil infiltration system.

3.2. Industrial wastewater treatment and others

In recent years, MEEs application has been significantly expanded to treat industrial wastewaters and some other wastewaters such as herbal pharmaceutical wastewater (Dhadse et al., 2010), petroleum industry wastewater (Sinha et al., 2012), pig slurry (Robin et al., 2011), urban runoff (Tomar and Suthar, 2011), and liquid-state sewage sludge (Xing et al., 2012). For instance, Ghatnekar et al. (2010) studied a pilot-scale MEEs to process wastewater generated from gelatin industry. The results suggested that MEEs could significantly decrease the COD by 90% and BOD₅ by 89%. Dhadse et al. (2010) treated herbal pharmaceutical wastewater by using MEEs in pilot scale. Under 2 days hydraulic retention time (HRT), the results indicated that MEEs could remove 85.44–94.48% of COD and 89.77–96.26% of BOD₅, and the heavy metals removal was also obtained. Furthermore, the effluent was color and odor free and no sludge production problem was encountered. For urban runoff treatment, Tomar and Suthar (2011) explored the performance of MEEs in small scale which was aided with some *Cyprus rotundus* in the top. The results illustrated that MEEs could efficiently reduce the levels of TSS (88.6%), TDS (99.8%), COD (90%), NO₃⁻ (92.7%) and PO₄³⁻ (98.3%), respectively, and the MEEs was more efficient than the biofilter without earthworms in terms of pollutants removal. Besides, Sinha et al. (2012) reported on the use of a small-scale MEEs to treat toxic wastewaters from the petroleum industry in Australia. The MEEs has been found to remove BOD₅ by over 90%, COD by 60–80%, and TS by 90–95%. The removal of toxic chemicals and pathogens were also observed from the effluent.

4. Factors influencing MEEs treatment performance

Since MEEs plays a key role in pollution control, understanding the influencing factors is therefore regarded as fundamental to improve treatment efficacy. In recently, the influencing factors of MEEs have been discussed in the literatures along with various MEEs applications. Particularly, the factors such as worm species and load, filter media type, chemical factors, hydraulic loading rate (HLR) and seasonal temperature may be crucial to establish a viable MEEs and achieve more favorable treatment performance.

4.1. Earthworm species and load

Various earthworm species have various burrowing characteristics. Thus, various earthworm species have different effects on the treatment process. Firstly, the anecic earthworm species prefer making permanent vertical burrows into the deep layers and the treatment efficiency would be reduced because of the decreased contact time between influent and filter matrix (Krishnasamy et al., 2013). The second category are endogeic worms which make extensive non-permanent horizontal burrows. This species may enhance wastewater treatment performance via distributing the influent over a larger area (Hawkins et al., 2008). And thirdly,

epigeic worm species, such as *Eisenia fetida* and *Eisenia andrei*, are a key for decomposition of biomass due to their preference for organic rich substrates compared to the other species (Gajalakshmi et al., 2001). Therefore, epigeic species are most commonly applied to MEEs, which can be seen from Table 2.

The treatment performance of MEEs is influenced by the health, maturity and population density of earthworm. Earthworm load is one of the important factors for efficient running of MEEs (Li et al., 2009). The number of worms per unit area in the vermifilter bed may positively affect the treatment efficiency of MEEs. Sinha et al. (2008) suggested that a relatively high number of earthworms (at least 15,000–20,000 earthworms/m³) in MEEs should be inoculated. Wang et al. (2013a,b) concluded that the removal rate of NH₃-N at 12.5 g of earthworm per liter of soil was higher compared with that at 0 and 4.5. However, some other researches argued that perhaps the worm load in MEEs might not be highly important in treating low strength wastewater as the earthworm density could be adjusted by itself according to the influent (Reinecke and Viljoen, 1990). This view was also demonstrated by Li et al. (2009) who reported that the density of earthworm could be increased simultaneously from 3,000 to 12,000 earthworms/m³.

4.2. Filter media

The filter media can provide a suitable growing medium for earthworms and microbes and also allow successful movement of sewage. Thus, the filter media is a critical design parameter in MEEs (Yang et al., 2001). In addition, filter media may play the most important part in adsorption of various pollutants such as phosphorus (Nie et al., 2015). The selection of an appropriate filter media in MEEs has become an important issue.

The selection of filter media is determined by the hydraulic permeability and the adsorption capacity for pollutants. Poor hydraulic conductivity would result in clogging of systems, and severely decreasing the effectiveness. It has been reported that the porosity of filter media in MEEs had a positive correlation with flow rate, and high flow rate might prevent MEEs from clogging (Jian et al., 2009). In addition, filter media could change the external survival environment of earthworms, and the changes of the external environment played an important part in the structure and function of the earthworm's body wall which is closely relative with the activity and respiratory metabolism of earthworm. For example, some researches indicated that earthworm in ceramsite media presented less injured than in quartz sand media. Therefore, the ceramsite was a more suitable choice as the media in MEEs compared with the quartz sand (Xing et al., 2011). Moreover, low adsorption by filter media could also affect the long-term removal performance of MEEs. The previous studies were mainly carried out on evaluating the effect of different filter media as a vermifilter bed on the performance of MEEs, especially for sustainable phosphorus and pathogen removal from wastewater. For this purpose, the filter media previously utilized for MEEs mainly is easily available and cost effective natural ingredients, such as river bed material, wood coal, glass balls, mud balls, slag-coal cinder ceramsite and quartz sand (Wang et al., 2010a,b; Xing et al., 2011; Kumar et al., 2015).

Table 3
Different natural ingredients selected for MEEs in wastewater treatment (Kumar et al., 2015).

Media	Size (mm)	Porosity (%)	COD eff. (%)	BOD ₅ eff. (%)	TSS eff. (%)
River bed material	6–8	35	72.3	81.2	75
Wood coal	6–8	45	64.6	74.5	64
Glass balls	6–8	40	61.5	72.7	59
Mud balls	6–8	43	59.8	70.9	55

Wang et al. (2010a,b) investigated the treatment performance of converter slag-coal cinder. The results indicated that the average treatment efficiency of COD, BOD₅, NH₄⁺-N and phosphorus removal by the MEEs were 78.0%, 98.4%, 90.3%, and 62.4%, respectively. The converter slag-coal cinder filter played a major role in phosphorus removal. However, this study was restricted to the analysis of nutrients removal like organic contaminants, ammonia nitrogen and phosphorous. The mechanisms for phosphorous removal by converter slag-coal cinder filter should be further explored. In addition, Kumar et al. (2015) evaluated the effect of different natural ingredients, such as river bed material, wood coal, glass balls, and mud balls, as a vermifilter bed on the performance of MEEs. As can be observed from Table 3, the river bed material could maximally reduce the COD, BOD₅ and TSS. What's more, the river bed material showed maximum removal of pathogen such as *total coliform*, *fecal coliform*, *fecal streptococci* and *Escherichia coli*. Besides, Arora et al. (2014b) also found that the river bed material was the most excellent media for removal of the above pathogen.

4.3. Chemical factors

Chemical factors such as pH, ammonia and sodium, may affect the earthworm's survival and the treatment performance of wastewater. Hughes et al. (2007) assessed the possibility of pH biological inhibition and disruption to earthworm in MEEs. This study revealed that the vermicompost and manure media had a relatively high buffering capacity for pH ranging 6.2–9.7 and the earthworm could survive in these pH levels. However, the juveniles was evidently impaired at the higher and lower pH levels, which is probably due to their ability to uptake greater amounts of soluble salts and inability to regulate them. Hughes et al. (2008) assessed the toxicity of ammonia/ammonium to key species (*E. fetida*) in MEEs. The results showed that ammonium with ammonium chloride exhibited relatively low toxicity, which had an LC₅₀ for ammonium of 1.49 g/kg. However, ammonium with ammonium sulfate did not show an influence on mortality at 2 g/kg. As the ammonia hydroxide could change the pH and concentration of ammonia in wastewater, some mortality to the earthworms would occur. However, the effect on system functioning was minimal. Even the effect on nitrifying bacteria was also minimal without linear trend shown with ammonia concentration. In addition, Hughes et al. (2009) found that NaCl was more toxic to the worms than the other common sodium salts found in wastewater. However, the actual risk from NaCl toxicity in MEEs was low and the worms were capable of detoxifying NaCl if the worms were exposed to moderate concentrations of NaCl for a long time. The low solid-water partitioning constant of NaCl led to a very small predicted environmental concentration of NaCl so that the risk from NaCl was low.

4.4. Temperature

Temperature is a crucial factor for the growth and metabolic activity of microorganisms and diversity of microbial community (Nedwell, 1999). Earthworm is a poikilotherm and the body temperature is significantly associated to outside temperature, which would make earthworms mortality under higher or lower temperature (Edwards and Lofty, 1980). Yin et al. (2011) reported that the effect of the filter bed temperature on the removal of organics was in accordance with the binomial distribution and the removal of NH₄⁺-N was in accordance with the Sigmoidal equation. However, there was no obvious influence on the removal of TN and TP. Besides, the range of optimal temperature for earthworm survival was 16–25 °C. Subsequently, Wang et al. (2013a,b) studied the performance of MEEs fed synthetic wastewater over the course of one year. The results showed that MEEs could efficiently eliminated COD and NH₃-N from wastewater during summer and autumn.

When temperatures and ammonia-oxidizing bacteria activities were the highest in autumn, the NH₃-N degradation rate constant ($K_{\text{NH}_3 - \text{N}}$) generally peaked. Arora and Kazmi (2015) also explored the effects of seasonal temperature on the treatment efficiency with a special attention to pathogen removal in MEEs. The results showed that COD and BOD₅ reduction, indicator organisms and pathogen removal, earthworm population, bacterial and actinomycetes number were significantly affected by the variation in ambient temperature, but TSS removal and fungi number were not affected. During the spring and autumn period in which the mean temperature was 25–27 °C, higher removal of BOD₅ and COD was accomplished. In this optimum temperature range, the activity, growth and reproduction of earthworm species (*E. fetida*) were the most active. However, the pathogen removal efficacy of MEEs increased with the increase in temperature, which implied that the pathogen removal efficiency of MEEs was remarkable impacted by temperature.

4.5. Operating and design features

4.5.1. Hydraulic loading rate

Hydraulic loading rate (HLR) is defined as the rate at which influent enters the MEEs. HLR of MEEs can be presented as:

$$\text{HLR} = \frac{V}{A \times t}$$

where V (m³) is the volumetric flow of the wastewater. A (m²) is the area of profile, while t (h) is the time taken by the wastewater to flow through profile. HLR could be determined by several factors such as structure, effluent quality, bulk density, profile aeration and method of effluent application (Siegrist et al., 2000). At a high HLR, hydraulic retention time (HRT) of the system could be reduced. Thus, the treatment efficiency would be affected (Sinha et al., 2008). The mainly reason was that wastewater required a certain contact time with the biofilm which grew and was attached on filter media to allow for the adsorption, transformation, and reduction of contaminants (Hughes et al., 2006). Fang et al. (2010) investigated the effect of HLR on pollutants removal by MEEs. The results showed that the HLR exhibited varying influences on NH₃-N, TN, NO₃-N and TP removal. However, COD and NO₂-N exhibited no significant difference at various HLR. Kumar et al. (2014) also illustrated the effect of hydraulic loading rates on the wastewater treatment in MEEs. The wastewater was treated at four different hydraulic loading rates of 1.5, 2.0, 2.5 and 3.0 m³/m d, respectively, and the optimum results were observed in the rate of 2.5 m³/m d. At this optimum HTR, the removal efficiency of BOD₅, TSS and TDS were 96%, 90% and 82%, respectively.

4.5.2. Nutrient load

The C/N ratio of influent plays an important role in wastewater decontamination (Xia et al., 2008). Microbes must change their C/N/P stoichiometry as a function of growth rate. There was a positive correlation between growth rates and N/C in many heterotrophic organisms, including bacteria (Elser et al., 2003; Makino et al., 2003). The efficient biological wastewater treatment depended on the knowledge of the organisms involved and how they responded to different nutrient load conditions. Moreover, the low C/N ratio in the influent might result in low efficiency of nitrogen and phosphate removal (Zhao et al., 2010). Zhao et al. (2012) reported the performances of MEEs in treating synthetic domestic sewage under different C/N ratios of 3:1, 6:1 and 9:1, respectively. The MEEs achieved the highest nutrient removal efficiency when the influent C/N ratio was controlled at 6:1.

Besides, the organic load is another type of nutrient load in MEEs, and should be regulated to realize satisfactory treatment performance. The optimal design of organic load plays an impor-

tant role in optimizing earthworm growth and activity and improving treatment performance of MEEs. Li et al. (2014) reported that the organic load decreased with the decrease in depth, which led to the decrease in earthworm population growth and activity. The approach which made the earthworms in the under layer gain more available organic food was to input different proportions of influent into respective depths.

4.5.3. Packing bed height

Variation in the earthworm packing bed height may directly change the distribution of COD concentration in MEEs. For $\text{NH}_3\text{-N}$ and TN elimination, adjusting packing bed height results in different aerobic-anoxic microenvironments (Akhavan et al., 2013). In addition, both growth and reproduction of earthworm are related to system humidity, filter media ventilation and the degree of earthworm metabolism. Adjusting the packing bed height might change the key factors mentioned above which affect the earthworm population characteristics (Molina et al., 2013). Thus, packing bed height is a crucial factor for nutrient removal in MEEs (Zhao et al., 2009). Wang et al. (2014) investigated the effects of MEEs height on pollutants removal from synthetic domestic wastewater. The results showed that variation in MEEs height had a significant effect on COD and TP removal rates, earthworm population, and actinomycetes numbers, but had no effect on $\text{NH}_3\text{-N}$ and TN removal rates, bacterial and fungi numbers. In addition, good nutrient removal efficiency, vigorous earthworm activity and high microbial numbers were achieved when the height was 60 cm.

4.5.4. Enhancing design

In order to improve the quality of the treated wastewater from MEEs, some enhanced MEEs have been developed. Wang et al. (2011a,b) designed a three-stage MEEs to enhance the nutrients removal from rural domestic wastewater treatment. The average removal efficiencies of COD, $\text{NH}_3\text{-N}$, TN, and TP were 81.3%, 98%, 60.2% and 98.4%, respectively. This indicated that the three-stage MEEs had higher COD, $\text{NH}_3\text{-N}$, and TP removal efficiencies compared with traditional MEEs. It might be benefit from the advancement of oxygen demand concentration in the effluents by designing three section. Tomar and Suthar (2011) carried out experiments in a novel MEEs which comprise of earthworm and construction wetland system to treat urban runoff. The results indicated that the novel MEEs could cause significant reduce in level of TSS (88.6%), TDS (99.8%), COD (90%), NO_3^- (92.7%) and PO_4^{3-} (98.3%). In addition, there were about 38.8, 20.8, 80.6, 50.8 and 144.6% removal efficiency of TSS, TDS, NO_3^- , PO_4^{3-} and COD, respectively, in the novel MEEs. This removal efficiency was higher than another reactor without earthworms. In France, a hybrid MEEs was built behind a 30 pregnant sow's piggery (Morand et al., 2011). The wastewater treatment system consisted of screen, MEEs, macrophytes pond and constructed wetland. The concentrations of nitrogen, microorganisms and endocrine disruptors were drastically decreased. Moreover, the phosphorus and potassium were eliminated through the byproducts harvesting.

5. Future research direction

MEEs, as a new type of ecological wastewater treatment technology, is becoming a research hotspot in the field of wastewater treatment, especially in developing countries. The current review indicated that many factors contributed to the limitation of contaminant removal in MEEs and the advancement in design and operation would realize effective treatment and sustainable operation. However, given the increasingly stringent water quality criteria for wastewater treatment and wastewater utilization, MEEs

still has some limitations and it is necessary to further research and develop. In summary:

- (1) The review on earthworms and filter media selection showed that the pollutants removal from wastewater in MEEs was vitally dependent on earthworms and filter media. In the future, more attention should be paid in proper earthworm species selection, such as high uptake of wastewater solids, and tolerance of high intensity of contamination, cold climate, extreme pH and sodium toxicity. Meanwhile, we should investigate whether the earthworms could be used as bio-indicators in MEEs. In addition, different filter materials would have different impacts on pollutants treatment performance. Some non-conventional filter media (i.e., industrial byproduct, artificial products, and agricultural wastes, etc.) which has high adsorption capacity and can facilitate the removal processes, should be developed and applied to MEEs.
- (2) The review on operating and design parameters indicated that the environmental, hydraulic and operating conditions were rather critical for realizing optimal treatment performance. Thus, optimizing these conditions requires extensive survey in future researches. In particular, the investigation of organic load (F/M) should be enhanced because the organic load connects closely with earthworms and microbes which are both crucial to decompose organic matter or nutrients transformation. Moreover, research of the key route and mechanism which correspond to higher pollutants removal should also be taken into account.
- (3) While the research and actual application in MEEs have been going on development, future studies urgently need new technologies and methods applied in MEEs for the enhancement of wastewater treatment performance. These technologies and methods may include: combination of various filter media, integration of MEEs with traditional sewage treatment technologies, designing multi-stage MEEs, addition of various plants, etc.

6. Conclusion

Overall, this paper revealed that the MEEs wastewater treatment technology was mostly used in developing country and various wastewaters have been successful treated by MEEs mainly at pilot scale. However, the literature review also illustrated that the treatment performance of MEEs would be affected by design parameters, operational conditions, and environmental factors. In order to realize long-term effective treatment and sustainable operation and meet increasingly stringent water quality standards, future studies should concentrate on the comprehensive assessment of earthworms and filter media under real life conditions, optimization of operating and design parameters, exploration of novel enhancement technologies.

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