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A comparative study of synchronous treatment of sewage and sludge by two vermifiltrations using an epigeic earthworm *Eisenia fetida*

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ABSTRACT

Reduction and stabilization of sewage sludge during the clarification of municipal wastewater was synchronously shown to be improved significantly in a pilot-scale vermifiltration using an epigeic earthworm *Eisenia fetida*. The present study aimed to select a better filter media suited to vermifiltration performance by the comparisons of sludge yields, the characteristics of the by-products of vermifiltration-vermicast and the abrasions of earthworms between ceramsite and quartz sand. It was observed that the sludge yield of the CVB (Ceramsite Vermibed) ranged from 0.07 to 0.09 kg SS/kgCOD$_{removed}$ at ambient temperature of 4–29 °C, representing 81% and 50% lower than that of the SVB (Quartz Sand Vermibed) and other reduction systems mentioned in this study. In addition, the sludge morphology variations described that the vermicast sludge from the CVB was more completely digested by earthworm than that of the SVB. The abrasions of the body wall of the earthworms in the CVB depicted less injured than that of in the SVB. So the ceramsite as filter media was better suited for the vermifiltration than the quartz sand.

1. Introduction

A large amount of excess actived sludge produced in wastewater purification, if not properly disposed, may pose a potential threat to both the environment and the human health because of heavy metals, organic pollutants and pathogen [1]. Sewage sludge treatment and disposal is, therefore, becoming one of the most serious challenges in WWTPs[1] as a result of legal constraints, rising costs and public sensitivity [2]. It should be realized that biomass production is an important economic factor because the sludge generated is a secondary waste that must be disposed in an environmentally sound and cost-effective manner. Treatment and disposal of sewage sludge from WWTPs accounts for about half, even up to 60%, of the total cost of wastewater treatment [3]. Various approaches relying on either one or a combination of them have been exploited for producing less sludge. Therefore, a number of sludge disintegration technologies for sludge minimization including mechanical methods, electrical methods, chemical methods, physical methods and biological methods (focusing on enzymatic processes such as lyses-cryptic growth, decay, uncoupling metabolism, maintenance metabolism, anaerobic treatment and predation on bacteria) are dealt with [4]. An ideal way to solve sludge-associated problems is to reduce sludge production in the wastewater purification process rather than the post-treatment of the sludge generated [5]. There is therefore a growing interest on sludge reduction by predation on bacteria [1,2,6,7], which is based on the ecological principle that during energy transfer from bacteria to higher trophic level organisms (protozoa and metazoa), energy is lost due to inefficient biomass conversion. Thus the predator may make a large contribution to biomass reduction [8]. The performance of oligochaetes for sludge reduction in biological wastewater treatment has been paid more attentions than that of protozoa for sludge reduction [1,2,6,7,9]. The oligochaetes used for treatment of excess activated sludge can be divided into two groups, firstly, the large aquatic worms such as the Tubificidae, Lumbriculidae and the semi-aquatic or terrestrial Enchytraeidae and, secondly, the small aquatic worms such as Naidids and Aeolosomatids [4]. Although the presence of worms especially small aquatic worms in the aerobic wastewater treatment may lead to substantial sludge reduction, the practical application of worms for sludge reduction is still uncontrollable [1,4].

Earthworms also belong to the order oligochaetes and have the largest sizes. So, vermifiltration, the benefits of earthworms in recycling liquid or solid organic residues, has already been highlighted [10–12]. It is an innovative way to expand the food web using earthworm as the predator and to decrease the sludge produced in water line, and an environmentally sound and economically feasible technology for decentralized wastewater treatment [10,13]. Based on these characteristics, the vermifiltration was designed as a polishing process in order to ensure a long sludge age to realize excess sludge reduction and stabilization during the sewage clarification in this study.

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[1] Wastewater treatment plant.

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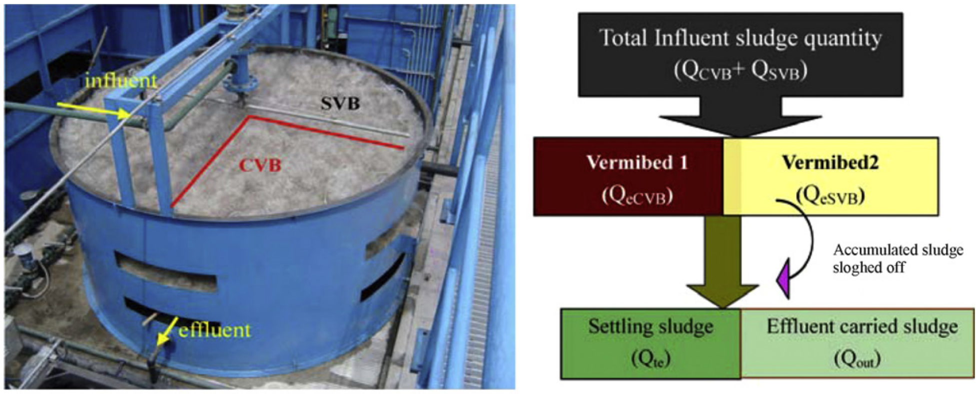

a b

Fig. 1. Field picture of vermifilter and the excess sludge calculation based on mass balance.

Consequently, the major aims of the present study were to explore the potential of reduction and stabilization of the vermicast sludge (a mixture of earthworm casting and excess sludge) produced by vermifiltration and to determine the characteristics of the vermicast sludge including the dewaterability, filterability and morphology. The further aims were to relate the potentials of the reduction and stabilization to the abrasions of the earthworms in two vermibeds.

2. Materials and methods

2.1. Vermifiltration description

The adopted system combined the advantages of the hydrolysis reactor-trickling filter system and incorporated the vermifiltration in order: to improve and support the purification capacity of the hydrolysis reactor-trickling filter system, to stabilize and transform the sludge into useful resource with less operational cost, energy supply, and minimum maintenance expenditure.

The polishing pilot vermifilter (Fig. 1) was located at Shanghai Zhuyuan wastewater treatment plant, China. The effluent collected at the bottom of the trickling filter was pumped and distributed over the top of the vermibed. The vermifilter consisted of a rotary distributor for a uniformly distribution, two staged vermibeds with mesh bottoms and a settler underneath the second staged vermibed (Fig. 1a). The vermibed was circular with a diameter of 3.7 m and a depth of 0.25 m with natural ventilation. The settler with a water-depth of 0.435 m for the vermicast sludge settling, spacing at 0.2 m intervals. The vermifilter was filled with ceramsite and quartz sand with a ratio at 1:3 (v/v, Fig. 1a) divided by an iron net mesh. Therefore, to facilitate discussion, the vermibeds filled with ceramsite and quartz sand were described as the CVB (ceramsite vermibed) and the SVB (quartz sand vermibed), respectively.

The sloughed biofilm sludge generated by trickling filter entered into the vermifiltration was defined as S0, the vermicast sludge produced by the CVB and the SVB were described as S1 and S2, respectively. Sample Se was the vermicast sludge sampled from the end of the run. The characteristics of the ceramsite and the quartz sand are shown in Table 1. A final consideration was that the top of the vermifilter bed was covered with a thin layer of fibrous material to protect earthworms from washed out and against predators.

2.2. *Eisenia fetida*

Eisenia fetida is an epigeic earthworm species which lives in organic wastes and requires high moisture content, adequate amounts of suitable organic material, and dark conditions for proper growth and development [14]. *E. fetida* is being used widespread in existing vermifiltration systems and also proven of its potential for processing of relatively moist organic materials, such as municipal biosolids and animal manure slurries [12,13,15]. This reasoning holds equally well for accumulation studies and in addition *E. fetida* is now readily available from commercial suppliers. The *E. fetida* used in this study were purchased from a Shanghai earthworm culturist.

Table 1
Specifications of quartz sand and ceramsite used.

Items	Quartz sand	Ceramsite
Pore sizes (mm)	1.68–2.05	3–5
Packing density (kg/m^3)	1450	820
Solid density (kg/m^3)	2570	1340
Specific surface (m^2/g)	5	12
Mohs hardness	7.5	6
Porosity (%)	43	52

2.3. Experimental procedure

2.3.1. Experimental conditions

Table 2 showed the operating conditions at the hydraulic loading rates of 2.4, 4.8, 6.0 and 6.72 m^3/(m^2 d), respectively. The chemical characteristics of the vermifiltration influent are described in Table 3. To be clear, the final vermifiltration provides the requisite quality of effluent of the second standard of GB18918-2002 in China [10] under 4 runs operations.

The dewaterability and filterability of the sludge samples therefore were evaluated by SRF[2] and CST[3] in this study. Additionally, the stabilization of the vermicast sludge was determined by VSS/TSS ratio,[4] SVI[5] and VFA.[6] Finally, the abrasions of the earthworm's body wall were related to the efficacy of the two vermifiltrations.

2.3.2. Excess sludge reduction calculation

The expelled vermicast sludge was collected by plastic round container. There was no excess sludge accumulation in the CVB during the 4 operation runs. In contrast, the accumulated sludge washed off from the SVB to the settling chamber using the tap water at the end of each run. Consequently, the sludge reductions based

[2] Specific resistance filtration.
[3] Capillary suction time.
[4] Volatile suspended solids/Total suspended solids ratio.
[5] Sludge volume index.
[6] Volatile fatty acid.

Table 2
Operation conditions of the vermifiltration.

Runs	Date	Influent temperature (°C)	pH	Average illuminance (lx)	DO[a] (mg/L)	Hydraulic loading ($m^3/(m^2$ d))	Organic loading (kgBOD$_5$/(m^3 d))
1	5.1–5.31	17.5–22.8	7.27–7.79	88500		2.4	0.46
2	6.2–6.30	21.5–27.8	7.47–7.9	97400		4.8	0.78
3	7.1–8.10	26.0–28.5	7.45–7.97	102300	4.6–5.33	6.0	0.82
4	8.2–8.25	27.2–28.6	7.59–7.89	102000		6.7	0.93

[a] Dissolved oxygen.

Table 3
Chemical characteristics of the influent of vermifiltration.

Parameters	COD[a] (mg/L)	BOD$_5$[b] (mg/L)	SS (mg/L)	NH_4-N[c] (mg/L)	TN[d] (mg/L)	TP[e] (mg/L)
Max.	99.8	44	48	29.04	31.8	3.22
Min.	43	20	15	11.03	16.5	0.86
Average	64	32	28	20.0	26.3	1.36

[a] Chemical oxygen demand.
[b] Biochemical oxygen demand.
[c] Ammonia nitrogen.
[d] Total nitrogen.
[e] Total phosphrous.

on sludge mass balance (Fig. 1b) were calculated as the following two equations.

$$R_{CVB} = \frac{Q_{CVB} - Q_{eCVB}}{Q_{CVB}} \quad (1)$$

where R_{CVB}–the sludge reduction percentage of the CVB;
Q_{CVB}–the sludge fed in the CVB;
Q_{eCVB}–the vermicast sludge expelled from the CVB.

$$R_{SVB} = \frac{Q_{SVB} - Q_{eSVB}}{Q_{SVB}} = \frac{Q_{SVB} - (Q_{te} + Q_{out} - Q_{eCVB})}{Q_{SVB}} \quad (2)$$

where R_{SVB}–the sludge reduction percentage of the SVB;
Q_{SVB}–the sludge fed in the SVB;
Q_{eSVB}–the vermicast sludge expelled from the SVB including the accumulated sludge sloughed off at the end of each run;
Q_{te}–the vermicast sludge expelled from the CVB and the SVB;
Q_{out}–the vermicast sludge carried out from the vermifiltration by effluent.

2.4. Analysis procedures

In the pilot scale test, Sludge samples were regularly taken from the vermifiltration at around 9:00 a.m. All analyses including TSS and VSS concentrations, SVI and VFA of the sludge samples were measured according to the Standard Methods [16]. The dewaterability of the vermicast sludge was measured with a CST instrument (Model 319, Triton, UK) equipped with a 10-mm diameter funnel and Whatman No. 17 chromatography-grade paper. The filterability was determined by measuring the SRF using the Buchner funnel method.

A laser particle size analyzer (Hiac 8000A) was also used to measure particle size. SEM[7] (Hitachi S-570, Japan) imaging was performed to obtain micrographs of the sludge samples before and after the CVB and the SVB at the optimum hydraulic loading of 4.8$m^3/(m^2$d). The SEM was operated at 20 kV of acceleration voltage condition.

The earthworm specimen were first fixed by a solution ($V_{40\% formalin}$:$V_{distilled\ water}$ = 1:9), then it was dehydrated in alcohol after washed out by distilled water. Next, it was embedded in paraffin and separated from tissues in 45 °C after sectioned continuously. Finally, it was dried on glass slice and stained by Su Magnolia and 95% alcohol for microobservation.

All sludge samples collected were conducted in triplicates and the data were expressed as the average values.

[7] Scanning electron microscope.

3. Results and discussion

3.1. Sludge reduction by vermifiltration

Table 4 depicts the sludge reduction induced by the CVB and the SVB. The average R_{SVB} and R_{CVB} under the four experimental runs were in the range of 38.2–44.7% and 40.5–48.2%, respectively, at elapsed operation time. It can be noted that a slightly increase of 4.30–7.80% of the sludge reduction occurred in the CVB in comparison with the SVB.

Additionally, the calculated sludge yields of the CVB and the SVB were in the range of 0.07–0.09 kg SS/kgCOD$_{removed}$ and 0.38–0.43 kg SS/kgCOD$_{removed}$ at ambient temperature of 4–29 °C, respectively. These results exhibit a more substantial reduction induced by the interactions of earthworms and microorganisms in the CVB than that in the SVB, suggesting a better favorable dwelling habitat provided for earthworms and microbes by the spherical ceramic pellet of CVB. The explanation will be elucidated in Sections 4 and 5 in detailed.

Concerning on predation on bacteria to minimize sludge reduction in biological wastewater treatment, a variety of researches mainly focus on the predation by protozoa and metozoa in activated sludge system [1,2,6,7], while vermifiltration utilizes a higher and larger oligochaete organism–earthworms in an attached-carrier biofilm reactor to acquire low sludge production. The former reported on the sludge yields of a pilot scale activated sludge

Table 4
Sludge reductions calculated of the vermifilter.

Runs	Run1	Run2	Run3	Run4
Hydraulic loading (m^3/m^2 h)	2.4	4.8	6.0	6.7
Operation period (days)	31	30	32	30
Average fed SS concentration (mg/L)	38.9	27.6	25.7	23.7
Average SS concentration of CVB effluent (mg/L)	21.1	14.3	15.1	14.1
Average mixed effluent SS concentration (mg/L)	6.5	5.8	7.6	7.9
Settling sludge concentration (mg/L)	7081	8286	9372	8311
Volume of settling sludge (m^3)	1.61	1.61	1.61	1.61
$Q_{CVB} + Q_{SVB}$ (kg)	28.94	39.74	49.34	47.78
Q_{CVB} (kg)	5.79	7.95	9.87	9.56
Q_{SVB} (kg)	23.15	31.79	39.47	38.22
Q_{eCVB} (kg)	3.14	4.12	5.80	5.69
Q_{out} (kg)	4.84	8.35	14.59	15.93
Q_{te} (kg)	11.40	13.34	15.09	13.38
$Q_{out} + Q_{te}$ (kg)	16.24	21.69	29.68	29.31
Q_{eSVB} (kg)	13.10	17.57	23.88	23.62
R_{CVB} (%)	45.8	48.2	41.2	40.5
R_{SVB} (%)	43.4	44.7	39.5	38.2

Table 5
SRF values of S0, S1 and S2.

Sludge samples	P (Pa)	A (m^2)	μ (Pas)	ω (kg/m^3)	b (s/ml^2)	r (m/kg)
S0	35000	0.0038	0.001	8.7294	0.0317	3.67×10^{12}
S2	35000	0.0038	0.001	12.9779	0.0156	1.21×10^{12}
S1	35000	0.0038	0.001	14.6688	0.0169	1.16×10^{12}

system for domestic wastewater treatment ranged from 0.15 to 0.17 kg SS/kgCOD$_{removed}$ at ambient temperature of 18–23 °C, and that of the submerged MBR was in the range of 0.00–0.15 kg SS/kgCOD$_{removed}$ at the temperature of 20 °C [9,17]. It can be observed that the sludge yield of the CVB in the present study was almost half of the activated sludge system mentioned above and close to the level of MBR system. Moreover, what should be addressed was that the sludge yield of the CVB was also stable at a low temperature of 4 °C, while in activated sludge system, especially the low temperature adversely affects the protozoan and metazoan [1,4]. The better reduction observed in the vermifiltration is ascribed to the joint actions of earthworms and microorganisms and a suited habitat provided for the earthworms by the CVB. In the vermifiltration earthworms are versatile waste eaters and decomposers. They graze on the surplus harmful and ineffective microbes in the wastewater selectively, prevent choking of the medium and maintain a culture of effective biodegraded microbes to function [13]. In a favorabe habitan the activities works better.

3.2. Dewaterability of the vermicast sludge

The SRF and CST values of S0, S1 and S2 were indicated in Table 5 and Fig. 2, respectively. The SRF values of the samples S0, S1 and S2 were calculated as 3.67×10^{12}, 1.16×10^{12}, and 1.21×10^{12} m/kg, respectively, by using the following equation [18]:

$$\mathrm{SRF} = \frac{2bPA^2}{\mu c}$$

where P is the pressure of filtration, N/m^2; A is the filtration area, m^2; μ is the filtrate viscosity, N (s)/m^2; c is the weight of solids/unit volume of filtrate, kg/m^3; b is the slope determined from the t/vol vs. vol plot; vol is the volume of filtrate, m^3; and t is the filtration time, s.

The results showed that the order of SRF of different kind of sludge was: S0 > S2 > S1. Referring to CST, one of the major drawbacks of using the CST test to compare a range of sludge types from different operational treatment works is the large variation in CST due to differences in SS concentration [19], therefore, it cannot be directly used to evaluate the bound water in the sludge [20]. To enable the CST values of the S0, S1 and S2 be compared, a rough correction to cover the deficiency was made to the three sludges as depicted in Fig. 2.

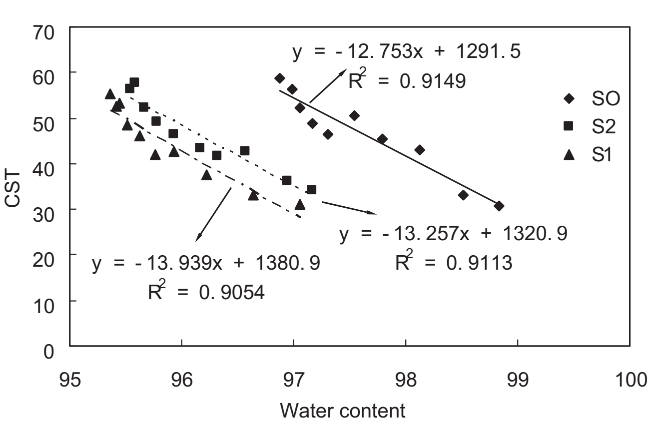

Fig. 2. Comparisons of CST values of S0, S1 and S2.

Fig. 2 shows that there was a significant linear correlation between CST and water content in sludge. The linear equation of S0, S1 and S2 were $y = -12.753x + 1291.5$, $y = -13.939x + 1380.9$ and $y = -13.257x + 1320.9$, respectively. So, the CST of S0, S1 and S2 were calculated as 54.5 s, 28.8 s and 34.9 s, respectively, with the same water content of 97%. The results showed that the order of CST values of the sludge samples was: S0 > S2 > S1, which was in accordance with that of the SRF. The findings suggested that the dewaterability of the sloughed biofilm sludge from biofilter was much poorer than that of the sludge flocs from the vermifilter. The chief cause of the better dewaterability of the vermifilter is that the earthworm is, in fact, a kind of sludge digester: the sludge is softened by the grume excreted in the mouth of the earthworm before it goes to esophagus where it is neutralized by calcium excreted by the inner sides of the esophagus. Then it comes into intestines where it is absorbed and decomposed by enzymes [21]. Finally, the sloughed sludge is discharged in the vermifiltration bed as peat-like materials with a high porosity, aeration, drainage, and water-holding capacity and usually contain more mineral elements-vermicast. Additionally, Volume can be significantly reduced from 1 m^3 of wet sludge (80% moisture) to 0.5 m^3 of vermicast (30% moisture) by vermiconversion process [22]. In the meantime, whether a more favorable habitat provided for earthworms and microorganisms or not is essential for the sludge reduction and stabilization. From the results of CST and SRF, the joint action of the earthworms and microorganisms in the CVB is more effcient than that in the SVB. This can be explained in Sections 4 and 5.

3.3. Vermistabilization of sewage sludge by the vermifiltration

Sludge stabilization processes are the key to reliable performance of any wastewater treatment plant. Earthworm can also assist in, and accelerate the stabilization of organic matter [23]. To evaluate the stabilization of vermifiltration treatment sludge, VSS/SS ratio, SVI and VFA were analyzed.

3.3.1. VSS/SS ratio

VSS/SS ratio indicates the viable microorganisms present in total sludge measured [24] or in other words, reflects percentage of inert matters content in sludge. The greater VSS/SS ratio indicates larger percentages of viable sludge. Under the four experimental runs, as displayed in Fig. 3a, the average VSS/SS ratio of the sloughed biofilm sludge fed was in the range of 58.2–63.3%, after vermifiltration, the VSS/SS ratio of the vermicast sludge of the CVB and SVB ranged from 42.1 to 46.1% and 44.8 to 48.1%, respectively, which meant that the organic matters for these two vermibeds were approximately degraded by 27.6% and 24%, respectively.

3.3.2. VFA

VFA is a key index to evaluate the stabilization of sludge. Low VFA suggests the organics in the sludge are stable. Fig. 3b showed the variations of VFA during the different operation runs. The average VFA of the biofilm sludge before vermifiltration was in the range of 61.3–65.6 mg/L. After vermifiltration the average VFA of the vermicast sludge of the CVB and SVB ranged from 34.1 to 38.2 mg/L and 36.9 to 39.3 mg/L, respectively.

3.3.3. SVI

The average SVI of the biofilm sludge before vermifiltration was in the range of 69.8–76.6 mL/g. After treated by vermifiltration, the SVI of the vermicast sludge of the CVB and SVB were in the range of 33.4–37.8 mL/g and 28.9–32.8 mL/g (Fig. 3c), respectively.

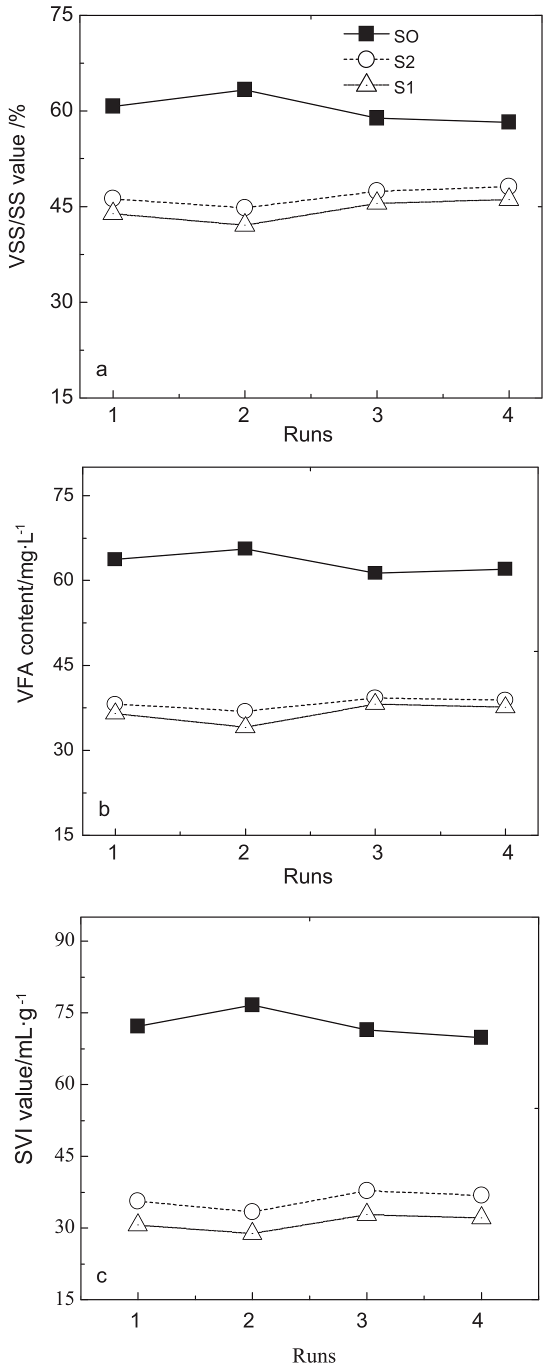

Fig. 3. Comparisons of VSS/SS, VFA and SVI variations of S0, S1 and S2.

Compared to the average SVI (<80 mL/g) in a pilot conventional activated sludge system [25], these values of the vermicast sludge were very lower, which suggested a better settling property and higher stabilization for the vermicast sludge from vermifiltration was achieved.

In all, the results illustrated in Fig. 3 indicated that the vermicast sludge after vermifiltration treatment had a better stabilization and dewaterability. This benefited from the activities of the earthworms. In vermifiltration, earthworms ingest, grind and digest organic waste with the help of aerobic and anaerobic microflora in their gut, converting it into a much finer, humified, microbially active material [26]. Further, vermicast is a good organic fertilizer and soil conditioner and contains humus with high levels of nutrients such as nitrogen, potassium, calcium, and magnesium. However, what should be addressed that at the end of each run, the SVB were washed by tap water due to the clogging of the accumulated sludge, which meant that the activities of the earthworms in the SVB was less active than those in the CVB.

Table 6
Typical particle sizes of S0, S1, S2 and Se.

Percentage (%)	S0	S2	S1	Se
D10	23.59	30.71	32.36	37.74
D25	45.82	59.00	64.82	77.66
D50	83.07	108.40	126.60	171.60
D75	156.70	196.50	221.40	290.90
D90	367.80	283.10	310.00	408.40

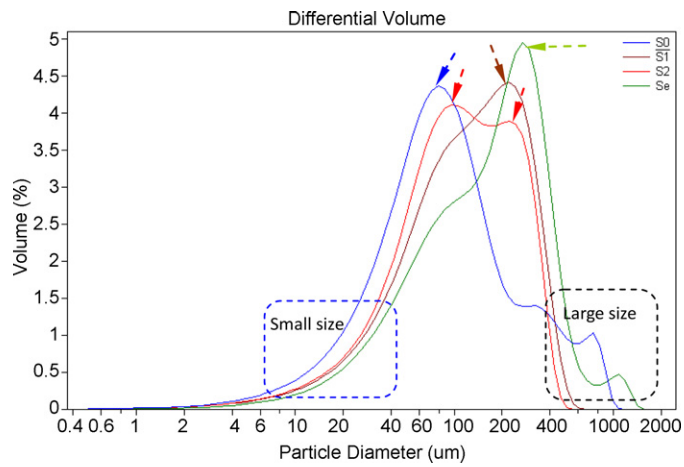

Fig. 4. Comparisons of the sludge particle sizes of S0, S1, S2 and Se.

No clogging was observed during experimental period, which indicated a dynamic balance between the active biomass and inorganic residues in the CVB. Also no inert fractions were accumulated in the bioreactor of the CVB system due to a favorable habitat provided for the earthworms and a more complete digestion for excess sludge fed than that in the SVB.

4. Sludge morphology variations of the vermicast sludge

Sludge morphology was investigated by means of particle size analysis and microscopic observation. The sludge for run 2 was selected as the typical samples. The typical particle sizes of S0, S1, S2 and Se were depicted in Table 6. Fig. 4 depicted the typical particle sizes distribution of different sludge samples before and after vermifiltration. The median particle sizes of S0, S1, S2 and Se were 83.07 μm, 126.6 μm, 108.4 μm and 171.6 μm, respectively, which indicated that the mean sludge particle size increased gradually after treated by vermifiltration due to the strong adsorption and larger specific surface of the vermicast.

SEM examination also revealed the variations of the sludge morphology and the microorganism abundance. The morphology of the sludge samples before and after vermifiltration varied obviously. The SEM photos of sludge samples S0, S1, S2 and Se were shown in Fig. 5. The sample of S0 was shown as a thick slime layer, gel-like slime material, gaps between cells were gradually filled with sticky EPS[8] [27], which were mainly comprised of 65% polysaccharides and other matters, such as protein, nucleic acid and lipids [28]. The morphology of S0 was unclear, almost all of the bacteria were covered with a net-like thin film, and it became very

[8] Extracellular polymeric substance.

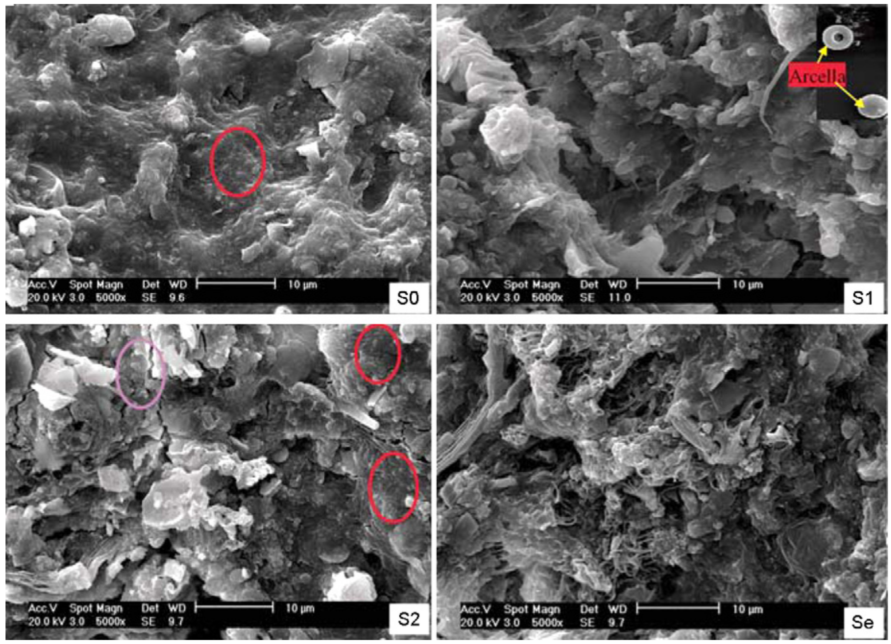

Fig. 5. Scanning electron microscopic images of sludge samples before and after vermifiltration (5000×).

difficult to identify the shape of single bacterium. After treated by vermifiltration the sludge morphology of bacteria of S1 and S2 was clearly observed, and rod- and oval-shaped bacteria were found to be dominant. Additionally, when the vermifiltration operated steadily, the microorganisms became abundant and complex, comprising different amoebae (*Arcella* sp.,), bdelloid rotifers and nematodes. Additionally, many of *Arcella* sp., one of the larger genera of Arcellinida, was detected too, which meant a better nitrification occurrence in the vermifiltration.

A unimodal curve was observed in the floc size distribution of S0, S1 and Se whereas S2 always showed a bimodal distribution (Fig. 4). This indicated that two populations of aggregates are maintained in S2–a dispersed one whose size was around 80–100 μm and a macrofloc population whose mean size was between 200 and 300 μm. The two-peak distribution of S2 (Fig. 4) was clearly demonstrated by microscopic observations shown in Fig. 5. Based on the previous reports [29,30], S0 was high strengh EPS, high SVI and small particle size distribution. This suggest that the smaller aggregates population with a size range of 80–100 μm close to S0 peak in this study may have a denser structure, and thus cause the SVB clogged after a period of operation monthly, which was similar to the membrane fouling as suggested by Lencki and Riedl [31]. The conclusion was correlated well with the results in SRF and CST of S0, S1 and S2. However, other population with the size around 200–300 μm (red arrow) is close to that (brown arrow) of the CVB (Fig. 4). The unimodal of the CVB was very closer to sample Se. All these findings indicated that the vermicast sludge from the CVB was more completely digested than that of the SVB by earthworm. This may be caused by the differences of the earthworms' activites in the SVB and CVB and this will be analyzed in Section 5.

5. Injuries of the earthworm's body wall

Although microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering the biological activity [32,33]. So do in our studied vermifiltration system. At the end of the run 2, the densities of the earthworms were calculated. The maximum density of earthworms was $4.2–17.5 \times 10^3$ worms per square meter and $4.3–18 \times 10^3$ worms per square meter in the SVB and CVB, respectively, which is similar to the density of the relative report [34].

Earthworm's body wall is the direct section to contact with exotic environment. The changes of the exotic environment play a crucial role on the structure and function of the earthworm's body wall. The cuticle layer, epidermal layer and circular muscle layer are closely relative with earthworm's activity and respiratory metabolism. Normally, the cuticle protects the earthworm from dehydration for short periods of time, and provides a tough layer of protection from abrasions. The epidermis can notify the earthworm's central nervous system when danger is near in the form of light, heat, cold, or excessive amounts of water. The circular muscle layer provides the earthworm with its locomotion, contraction, and circular movement. They are crucial to the earthworm's activities; however, they are easy to be injured by the carriers through which the earthworm passes. Consequently, filter media was the key choice and played an extraordinary role in the vermifiltration for the earthworm too. So, microobservation of the cuticle, epidermis and circular muscle layer were performed to evaluate the injuries of earthworm's body wall. The comparison of the injuries in the SVB and CVB were depicted.

The cuticle with no injury (Fig. 6a) is a thin, colorless and transparent acellular layer, which is made up of collagenous fibers of multiple layers. As shown in Fig. 6b, the cuticle of earthworm in cow dung was with complete structure, thin collagenous fibers, clear and orderly cross linkage. However, the cuticle of earthworm in the SVB was obviously injured with thick and coarse collagenous fibers. Compared with the cuticle of earthworm in the SVB, that in the CVB was less injured with a little less thick and coarse collagenous fibers.

Epidermis inside the earthworm's cuticle is composed of sorts of cell monolayer with a large amount of secreting cells. Epidermis (Fig. 6b) in cow dung was more compact than that in the CVB and the SVB. Compared with Epidermis in the SVB, that in the CVB is more compact again, and there were no hollow bubbles.

Abundance of capillaries filled with the circular muscle layer, among the circular muscle layer more or less gaps exist. Collagen micro-fibers were distributed largely in the gap. The circular muscles in cow dung (Fig. 6c) were more compact with clarity textures than that in the CVB. However, those in the SVB were looser with

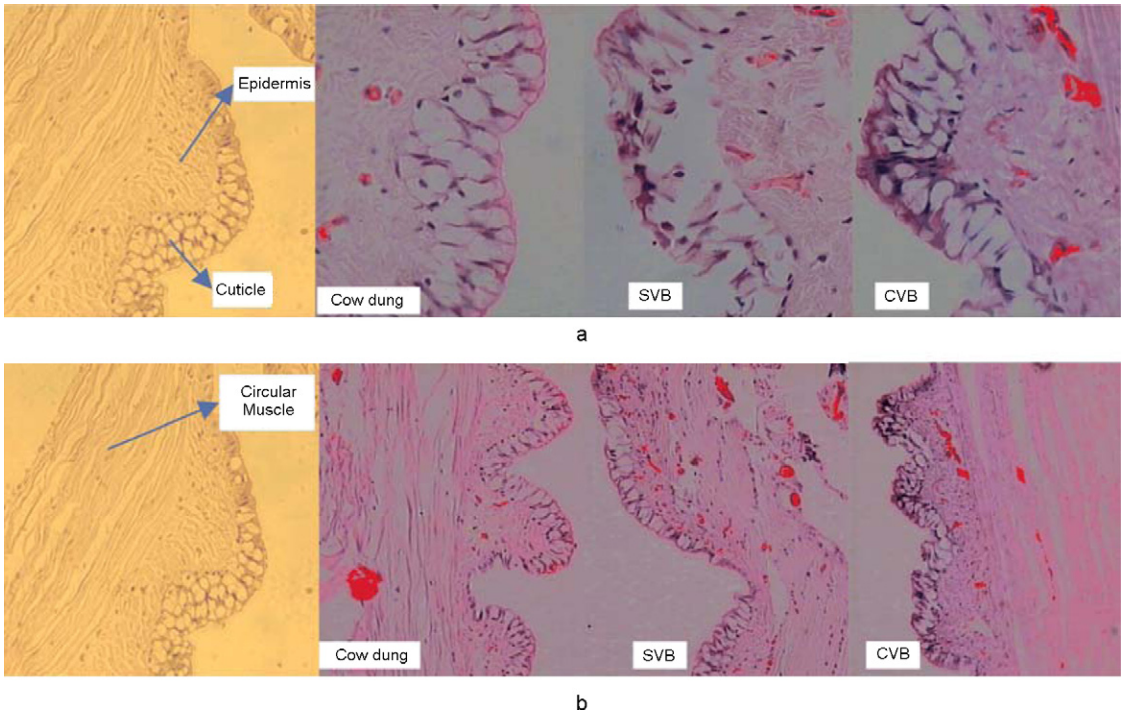

Fig. 6. Microstructure of histological section of the earthworm's section (10 × 20, slice thickness: 7 μm).

disorder textures than that in the CVB. In addition, fractures of the circular muscles were easy to be found in the SVB.

From the experimental results, it was obvious that the abrasions in the CVB were less slight than that in the SVB, which meant that the ceramsite was more suitable for the survival of earthworm than the quartz sand. This may be associated with their physio-chemical characteristics. Quartz sand with irregular solid square edge angle shape has smaller particle sizes (1.68–2.05 mm), larger bulk density and larger true specific gravity than that of ceramsite, which will do a server damage on the earthworm's body wall when earthworm passes. On the contrary, the ceramsite with round solid ball shape, good air permeability and excellent dispersibility is easy for the earthworm's burrowing which is favorable for the ventilation of the vermibed.

On the other hand, the ceramsite is lower thermal expansion rate and thermal conductivity, which provides a protection from the influences of the extreme high temperature and low temperature. Compared with the quartz sand, therefore, the ceramsite could regulate the vermibed temperature and played a temperature-keeping role when the extreme high or low temperature reached.

Finally, in the CVB the distribution of the earthworm was even, though in the SVB the earthworm dwelled in between 0 and 15 cm in depth despite of the total depth of 30 cm of the vermifiltration. The even distribution can aerate the CVB vermibed completely. Additionally, the surface of the ceramsite assumes multi-pore, multi-edges and corners, rough, easy-linked membrane, and certain mechanical strength, is one kind of ideal microorganism carrier. The rather high porosity of the material also allowed diffusion of gases, especially ingress of oxygen and release of carbon dioxide, which can accelerate the degradation of organics by microbes. So the ceramsite is selected as the medium for the vermifiltration.

6. Conclusions

Synchronous sewage sludge treatment during the clarification of municipal wastewater was obtained by vermifiltration at the hydraulic loading of 4.8 $m^3/(m^2\ d)$. The ceramsite was selected as the filter media for vermifiltration due to its low sludge yield and good stabilization of the vermicast sludge. The vermicast sludge produced by the CVB was more completely digested by the earthworms compared to that of the SVB. Additionally, the abrasions of the body wall of earthworms suffered in the CVB were less slight than that in the SVB, which meant that the ceramsite can provide a suitable endemic environment for the earthworms.

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