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ORIGINAL PAPER

Effect of organic shock loading on anaerobic performance of pumice-reinforced up-flow anaerobic sludge bed for incineration leachate treatment

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Abstract
The research and application of leachate in the treatment of leachate in UASB reactors are increasing, but UASB also has problems such as poor resistance to organic load impact and unstable operation. In order to improve the anaerobic performance stability of an up-flow anaerobic sludge bed reactor (UASB) subjected to high organic loading shock, pumice was added to the leachate from the incineration. The reinforcement effect of pumice on the anaerobic efficiency of the reactor was studied by increasing the influent load. The results showed that with a gradual increase in the influent organic load [11.6−66.6 kg COD/(m^3•d)] and without the addition of pumice, irreversible acidification took place in the reactor (R1) when the influent load reached 14.51 kg COD/(m^3•d). The average methane output and content were reduced to 39.7 L/d and 66.16%, respectively. In contrast, the reactor (R2) with pumice could still be operated stably when the influent organic load reached 40.04 kg COD/(m^3•d). The COD removal rate reached 91.80%, and the average methane output and content were increased by 42.30% and 15.20%, respectively. The results of analyzing the sludge microbial community structure in the reactor showed that the adding of pumice could selectively enrich the methanogenic bacterium *methanosaeta*, promote the decomposition of volatile fatty acid (VFA), effectively mitigate the acid inhibition effect of VFA on the anaerobic reactor, and enhance the balance between acidogenic bacteria (*Chloroflexi* and *Proteobacteria*) and methanogenic bacterium (*Methanosaeta*) to a great degree. These results prove that the addition of pumice filler can reinforce the resistance of UASB to organic loading.

Keywords Pumice · UASB · Organic shock loading · VFA · *Methanosaeta*

Introduction

Up-flow anaerobic sludge bed reactors (UASB) are commonly used to treat wastewater that contains concentrated organics, and has the advantages of high volumetric loading and low sludge output. During the stable operation of the reactor, the acid and methane production processes are dynamically balanced (Lei 2019; Carrillo-Reyes et al. 2014). However, in real-world situations, the quality and quantity of wastewater fluctuate frequently, and the influent organic loading also varies. Therefore, the reactor is usually in an overload state, which is called organic shock load (Ketheesan and Stuckey 2015). After this shock, due to the difference in the adaptability of acidogenic bacteria and methanogens to the changed environment, the balance between acidogenic and methanogenic bacteria will be destroyed, leading to the accumulation of volatile fatty acids, reduction of methane production efficiency, and lowering of anaerobic digestion performance in the digestion system.

Adding support materials into UASB reactors can improve their resistance to organic shock load. For example, Gao et al. (2018) studied the influence of biochar on the UASB anaerobic process subjected to instantaneous high

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load shock. Under the condition of 80 kg COD/(m^3•d), 12 h and 24 h instantaneous load shock was separately adopted, and the reactor could be stably operated in the presence of biochar with a COD removal rate and methane output of 97.25 ± 0.85% and 0.19 ± 0.01 L CH_4/g COD, respectively. Lei et al. (2019) added granular activated carbon to the UASB reactor treating the waste leachate. The start-up adaptation period was not required for this reactor. After increasing the organic load to 25.0 kg COD/(m^3•d) by gradually shortening the hydraulic retention time, the COD removal rate was still stable and higher than 90%. Wang et al. (2018) employed multi-walled carbon nanotubes as the support that was placed in the UASB reactor to study their effect on the performance of anaerobic granular sludge and microbial community structure. The results showed that the addition of multi-walled carbon nanotubes promoted the transformation of VFA, and the removal rate of total phosphorus was increased by 29.34%, but the addition did not have a significant influence on the removal of COD and nitrogen ($p > 0.05$). Yang et al. (2020) added a nanocomposite of granular activated carbon (GAC) and MnO_2 to the anaerobic system to improve the methane output. The addition of (GAC)-MnO_2 improved the anaerobic digestion performance by enhancing the electron transfer between species and improving enzymatic activity (Yang et al. 2020). Compared to the control group, the COD removal rate and methane output were increased by 77% and 36%, respectively. From the perspective of the microbial community structure, in the presence of (GAC)-MnO_2, methanogens became more abundant, and oxidation processes forming acetic acid were favored, resulting in a decrease in the CO_2 output and increase in the CH_4 output.

Pumice, also known as volcanic rock, is acidic lava with a rich pore structure and porous surface. Pumice has the appearance of honeycomb or slag, and is abundant in China (Ding et al. 2016; Zhang 2010). With research progress in the field, the good adsorption performance and promotion effect of pumice on the immobilization and formation of microbial biofilm have been discovered; this material does not harm the immobilized microorganisms, and does not significantly reduce their biological activity (Quan et al. 2020). Pumice is a natural green filler material that supports the biofilm, and can serve as an artificial support for wastewater treatment (Li et al. 2017). Bin et al. (2020) studied the effects of pumice on the sludge reduction, pollutant removal, and microbial community structure in an anaerobic side stream reactor (ASSR). They found that the removal rates for TN and TP in the reactor improved significantly with the addition of pumice. The Illumina MiSeq sequencing results showed that microbial diversity and richness were the highest in pumice, a large number of microorganisms were fixed on the filler surface in the form of biofilm, and the enrichment of nitrifying bacteria on the filler biofilm improved the nitrogen removal efficiency. Paola et al. (2006) reported that heavy metal ions in the aqueous solution could be effectively removed with pumice, of which the efficiency was higher than those of activated carbon and chitosan. Additionally, the cost was low. Therefore, based on these studies, inexpensive, environmentally friendly pumice that is benign to immobilized microorganisms was selected as the biofilm support in UASB. The leachate from waste incineration power generation was taken as the object to study the influence of pumice on the balance between acid and methane production processes via anaerobic digestion, and the function and mechanism of pumice were expected to be explained from the point of view of microbial community structure.

Materials and methods

Reactor design

Both UASB reactors were made of organic glass (Fig. 1). The reactor column was 730 mm high with a total volume of 9.0 L, inner diameter 80 mm, useful volume 4.5 L. The temperature of the reaction column was maintained at 35 ± 1 ℃ using a water bath jacket. Using a peristaltic pump (1 ~ 100 rpm) to introduce wastewater from the bottom into the reactor, and a duct was provided at the top for gas collection. The gas flowed through the duct and was collected with a tedlar bag. The amount of sludge inoculated in the control (R1) and experimental (R2) groups accounted for 40% of the effective reactor volume, and pumice with particle sizes of 3 ~ 5 mm was added to group R2 to account for 10% of the reactor column volume.

Inoculated sludge and fresh leachate

Incineration leachate was collected from the regulating tank of a waste incineration power plant in Hefei City, and its water quality indicators are shown in Table 1. After dilution, the leachate was used as the wastewater to be tested, and the COD ranges are shown in Table 2. The inoculation sludge, with a moisture content of 83.7% and VSS/TSS of 0.57, was taken from the sludge concentration tank of this plant.

Operation of the UASB reactor

The UASB reactor was operated in a mode of continuous water injection and discharge. The entire operation process was divided into two stages. The sludge was cultivated for 68 days until the operation was stable. High influent organic loading was maintained for 103 days. In this stage, the hydraulic retention time was gradually reduced from 11 to 6 h, while the influent COD concentration was gradually

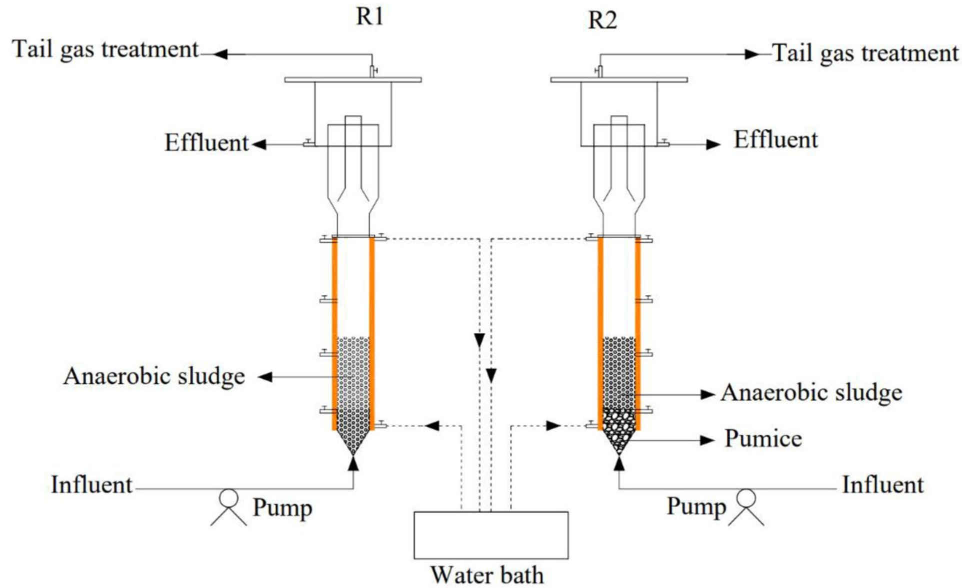


Fig. 1 Schematic illustration of the apparatus

Table 1 Water quality indexes of the leachate from waste incineration power plant

Index	COD (mg/L)	NH_3–H (mg/L)	TN (mg/L)	TP (mg/L)	pH
Values	58,000 ~ 72,000	2000 ~ 3200	3000 ~ 3850	70 ~ 110	4.6 ~ 6.5
Mean values	63,964	2625	3396	89	5.7
Standard deviations	5733	492	311	16	0.9

Table 2 Operating mode of the UASB reactor

Stage	Working condition	Running time (d)	HRT (h)	COD concentration (mg/L)	OLR kg COD/(m^3•d)
High-load-shock stage	Phase I	18	11	10,500−14,000	11.61−14.85
	Phase II	21	8	14,000−15,000	21.05−22.06
	Phase III	64	6	15,000−35,400	27.94−66.63

increased from 10,500 to 35,400 mg/L. The influent organic loading was gradually increased from 11.61 to 66.63 kg COD/(m^3•d).

Analytical methods

COD, pH, and VFA were determined by the standard methods Wei (2002). The methane content was determined using a portable infrared biogas analyzer (LB-MS4X). The biogas produced during the experiment was collected with a bag (5L), and the gas volume was measured with a 500 mL syringe. These indicators were measured every 3 days.

Acetate kinase was detected using a commercial kit (Shanghai Zhuocai Biotechnology Co., Ltd.). Coenzyme F420 was detected with an enzyme-linked immunosorbent assay kit (Shanghai Zhuocai Biotechnology Co., Ltd.). Detailed instructions regarding usage were provided. Corresponding to each working condition, sludge at the bottom of the reactor was sampled and stored at − 80 ℃ in a refrigerator for testing.

For a better understanding of the evolution of bacterial and methanogenic community structures before and after the shock of high influent organic loading, 20 mL of sludge was sampled from the bottom of reactors R1 and R2 on day 90 and day 156, respectively. The samples were denoted as R1–90, R2–90, R1–156, and R2–156, and sequenced by Baimaike Biotechnology Co., Ltd. The high-throughput sequencing used the bacterial primers of 338F:5′-ACTCCTACGGGAGGCAGCA-3′ and 806R:5′-GGACTACHVGGGTWTCTAAT-3′ as well as the methanogenic primers of MLf5′-GGTGGTGTMGGATTCACACARTAYGCWACAGC and MLr5′-TTCATTGCRTAGTTWGGRTAGTT. During the analysis, operational taxonomic units (OTU) with a similarity of 97% were classified into the same OTU, and the biodiversity of the microbial community was evaluated by Chao and Shannon indexes.

Results and discussion

COD removal efficiencies

Figure 2b shows the COD removal rates of R1 and R2 during the days 68–171. After the successful start-up of UASB, the COD concentrations gradually increased from 950 to 5000 mg/L, and HRT (hydraulic retention time) was 15 h during the whole start-up stage, the removal rates were stable and exceeded 90%. After the 68th day, the influent organic load was increased from 11.61 to 66.63 kg COD/(m^3•d) by adjusting the HRT and COD concentrations(10, 500 ~ 35400 mg/l).

Along with the increase of the influent organic load from 8.94 to 32.33 kg COD/(m^3•d), the COD removal rate of R2 > 90% was maintained stable for 57 days. When the influent organic loading was increased to 33.53 kg COD/(m^3•d), the COD removal rate of R2 declined for the first time. Nevertheless, 12 days later, the COD removal rate of R2 was recovered. On day 137, with an influent organic loading of 40.04 kg COD/(m^3•d), the COD removal rate of R2 declined again. Also, 12 days later, the removal rate rose to 91.80%. When the influent organic load reached a high level of 55.99 kg COD/(m^3•d), the COD removal rate of R2 declined significantly and could not be recovered in the later stage, It may be that the acid buffering capacity of the UASB reactor has reached its limit, and the large accumulation of VFA inhibits the activity of methanogenic bacteria, and the decomposition capacity of organic matter is greatly reduced, resulting in a final value of approximately 65%. When the influent organic loading was increased to 14.51 kg COD/(m^3•d), the COD removal rate of R1 was reduced to < 90%. Moreover, with the increase in influent load, the COD removal rate gradually declined, and the final value was stabilized at approximately 40%, the resistance to organic load impact was increased by nearly 3 times. The data shows that the presence of pumice reinforced the resistance of the reactor to environmental fluctuations. Thereby, the inhibition impact of the high

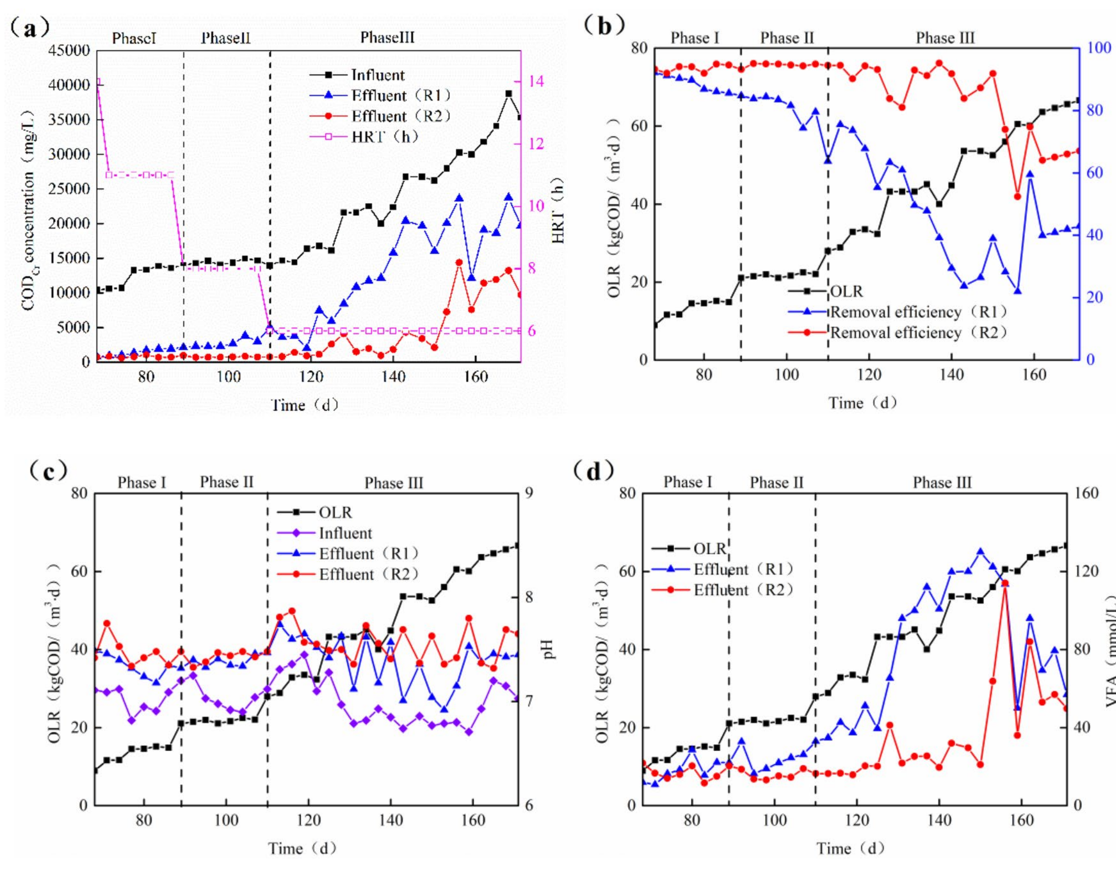


Fig. 2 Changes in the parameters of UASB reactors. **a** Variation of hydraulic retention time with COD concentration of influent and effluent; **b** influent organic load and COD removal efficiencies; **c** pH of influent and effluent water; **d** the VFA production

influent organic load on the anaerobic system could be reduced, and the performance of anaerobic system could recover from the fluctuations more readily.

Changes of pH and VFA

During the actual operation of anaerobic reactors, anaerobic digestion systems are usually inhibited by chemicals such as acids, ammonia, nitrogen, and sulfate (Han et al. 2020; Yuan et al. 2016; Zhao et al. 2020). Among them, the acid inhibition caused by the accumulation of volatile fatty acids is considered to be an important factor that affects the long-term stable operations of anaerobic digestion systems (Zhang et al. 2018). The changes in pH and VFA concentration in the reactors are shown in Fig. 2c and d. During the course of the entire operation, the influent pH of both reactors was maintained between 6.8 and 7.5, and the effluent pH was maintained between 7.0 and 8.0. Figure 3 shows the correlation between the COD removal rate and effluent VFA concentration. The COD removal rates of both reactors were significantly negatively correlated with the effluent VFA concentrations ($r = -0.9$). Under the condition of OLR of 43.23 kg COD/(m^3•d), the cumulative VFA in R1 and R2 increased to 65.4 and 41.2 mmol/L, respectively. Then, the pH declined to 7.12 and 7.36, respectively, and the COD removal rates decreased to 60.93% and 81.02%, respectively. Under the condition of OLR of 55.99 kg COD/(m^3•d), the cumulative VFA in R1 and R2 increased to 122.4 and 63.8 mmol/L, respectively. The pH was decreased to 6.92 and 7.36, respectively, and the COD removal rates declined to 28.27% and 73.95%, respectively, indicating that the shock of the high influent organic load interfered with the balance between acid and methane production, resulting in acid inhibition.

Because methanogens are extremely sensitive to environmental changes, the accumulation of excess VFA will inhibit the activity of the methanogens. It has been documented that the inhibitory effect is principally reflected by the following: (1) When the buffer capacity of the anaerobic digestion system is insufficient to neutralize excess acids, the pH of the anaerobic system declines to the tolerance threshold of methanogens, impeding the methane production process (Wei et al. 2013); (2) Concentrated VFA has a toxic effect on anaerobic systems, thus inhibiting the activity of methanogens (Li et al. 2015). The data acquired show that regardless of the considerable VFA accumulation, the pH in R1 and R2 was still suitable for the growth and metabolism of bacteria and methanogens, which shows that the toxicity of VFA inhibited the COD removal rate; Meanwhile, the presence of pumice could protect the UASB reactor from excessive acidification caused by the shock of high influent organic load, enhance the acid buffer capacity of UASB, and promote the rapid recovery of the balance between acid and methane production processes.

Methane output and content

As shown in Fig. 4b, with an OLR value in the range of 8.94–28.87 kg COD/(m^3•d), the methane output from both reactors increased steadily as the influent organic load, increased and the methane output of reactor R2 was higher than that of R1. After increasing the influent organic load further, the difference in methane output from both reactors was gradually increased, and the methane output of R2 was much higher than that of R1. With an OLR value of 43.23 kg COD/(m^3•d), the methane output of R2 was 50.41 L/d, which is higher than the corresponding value for R1 (29.67 L/d); With an OLR value of 55.99 kg COD/(m^3•d), the methane output of R2 was 62.81 L/d, which is higher than the value of R1 (43.81 L/d). Compared to R1, under a high influent organic load, the average methane output of R2 was increased by 42.3%.

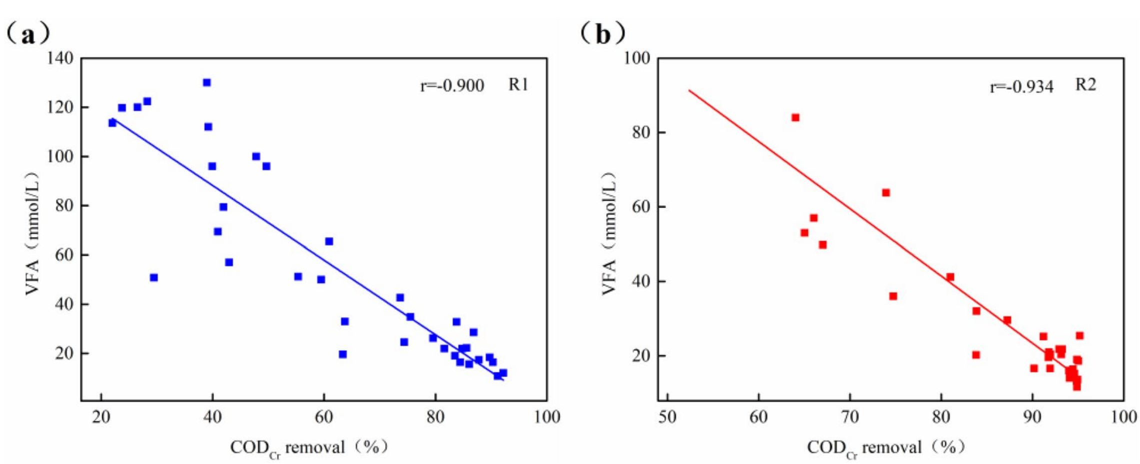


Fig. 3 Pearson correlation analysis results between COD removal rate and effluent VFA concentration. **a** R1; **b** R2

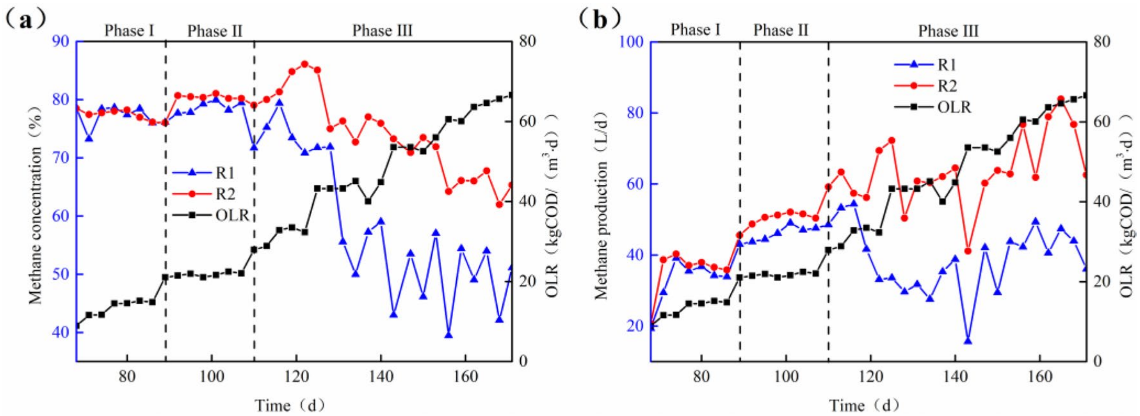


Fig. 4 Biogas production performance of R1 and R2. **a** Methane content; **b** methane output

In the start-up stage, the methane contents in R1 and R2 stabilized at 76.85 ± 1.74% and 77.69 ± 2.23%, respectively; as shown in Fig. 4a, when the OLR values were in the range of 8.94 to 32.86 kg COD/(m^3•d), the methane contents in both reactors were stable and above 70%, and the methane content in R2 was higher than that in R1. When the OLR value reached 43.23 kg COD/(m^3•d), the methane contents in R1 and R2 reached 71.87% and 75.01%, respectively. When the OLR value reached 55.99 kg COD/(m^3•d), the methane contents in R1 and R2 were 57.05% and 71.90%, respectively; Later, the influent organic load was further increased, and the methane content in R2 was maintained in the range of 60–70% while the methane content in R1 was only 50–60%. Compared to R1, the average methane content increased by 15.2% under the high influent organic load. The decline of methane content was caused not only by the toxicity of VFA, but could also be possibly ascribed to the accumulation of H_2 and CO_2 due to excessive acidification. The data shows that in the presence of pumice, the reactor showed better stability and recoverability under the high influent organic shock load, and the inhibitory effect of high influent organic load on the activity of methanogenic bacteria was weakened, promoting the transformation process from VFA to methane.

Enzymatic activity analysis

Acetate kinase is a key enzyme involved in the formation of acetic acid. Its activity reflects the formation of VFA. Acetic acid can be transformed into methane by microorganisms. Coenzyme F420 is one of the characteristic methanogen coenzymes, and can serve as an indicator to monitor the activity of methanogenic archaea (Xue et al. 2020). Figure 5 shows the activities of acetate kinase and coenzyme F420 in the reactors R1 and R2 under different working conditions. Under condition 1 (day 68), the activity of acetate kinase in R2 was 2.83 times that of R1; under condition 2 (day 122),

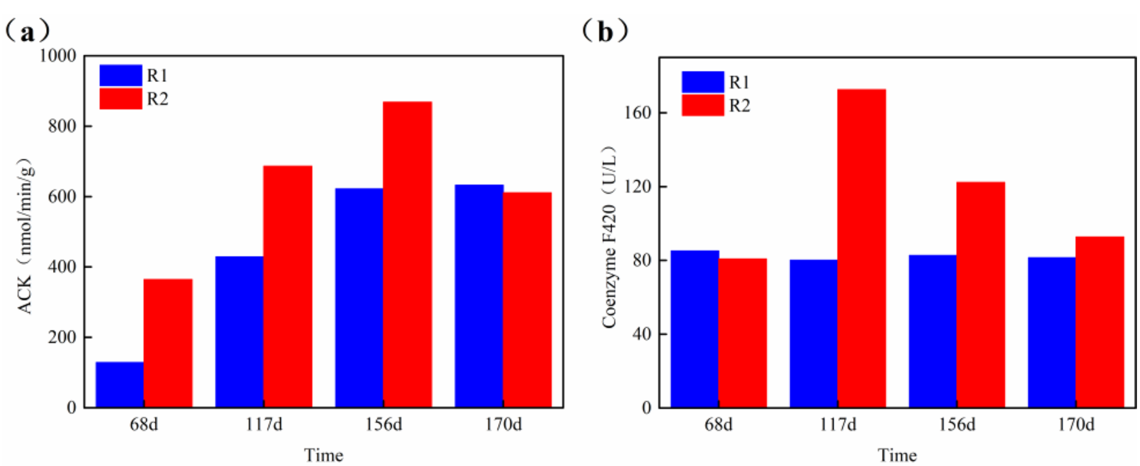


Fig. 5 Enzymatic activity analysis. **a** Acetate kinase; **b** coenzyme F420

the activity of acetate kinase in R2 was 1.6 times that of R1; under condition 3 (day 156), the activity of acetate kinase in R2 reached its maximum of 868.32 nmol/min/g. On day 168, the activity of acetate kinase in R1 and R2 were similar. Meanwhile, the performance of the R2 reactor also became poorer after 156 days. Under working condition 1 (day 68), the activity of coenzyme F420 and methane production performance were similar. Under working conditions 2 (day 122) and 3 (day 156), the activities of coenzyme F420 in R2 were 2.15 and 1.48 times those in R1, respectively. In addition, the methane output of R2 was 2.09 and 1.81 times that of R1, respectively. During the high load shock period, the activity of coenzyme F420 in reactor R1 was principally in the range of 80−85 U/L, resulting in the accumulation of a large quantity of VFA in R1. These results show that the addition of pumice improved methanogen activity in anaerobic sludge, maintained the balance between acid and methane production, and thereby improved the treatment capacity of the anaerobic reactor for incineration leachate. This is consistent with the removal rate for COD.

Microbial community structure

Microbial diversity analysis

Alpha diversity evaluation indexes mainly include the Simpson, Shannon, ACE, Chao, and other indexes. Among them, the ACE and Chao indexes reflect the abundance of microorganisms in a sample, and both indexes are proportional to the abundance. The Shannon index reflects the diversity of microorganisms in the sample, but the diversity is also affected by the abundance and coverage. For the same abundance, the microbial diversity in the sample is proportional to the value of the Shannon index, and coverage reflects the reliability of the sequencing result (Li et al. 2015). Table 3 shows that compared to R1, the numbers of bacterial OTU in R2–90 and R2–156 increased by 2.08% and 1.75%, respectively, and the Shannon indexes increased by 3.44% and 6.06%, respectively; In contrast, the OTU numbers of the methanogens were almost the same, but the Shannon indexes of methanogens in R2–90 and R2–156 were increased by 4.05% and 69.44%, respectively. The high microbial diversity and abundance values rendered the anaerobic digestion system more stable, and played a positive role in the balance between acid and methane production. Pumice is highly porous and has large specific surface area and so, it could provide a suitable surface for the attachment and growth of microorganisms. As a result, the diversity and abundance of bacteria and the diversity of methanogens could be improved, stability of anaerobic digestion system could be maintained, and thereby, the anaerobic efficiency could be improved.

Table 3 Diversity indexes of bacteria and methanogens in different samples

Type	Sample	Out	Chao 1	Shannon	Coverage
Bacteria	R1–90	673	680.04	7.01	1.00
	R2–90	687	689.50	7.26	1.00
	R1–156	514	548.45	5.78	1.00
	R2–156	523	555.80	6.13	1.00
Methanogens	R1–90	53	53.33	3.08	1.00
	R2–90	60	60.00	3.21	1.00
	R1–156	57	57.60	2.16	1.00
	R2–156	55	55.50	3.66	1.00

Community structure of bacteria

Figure 6 shows the changes in bacterial community structures in different samples at the phylum and genus levels. The dominant bacteria in the entire operation process included *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, and *Synergetes*. It has been reported that these bacteria are the hydrolytic acidification bacteria commonly found in sewage and anaerobic sludge. Compared to R1, the relative abundance of *Proteobacteria* in R2–90 and R2–156 was increased by 1.04 and 1.38 times, and the relative abundance of *Chloroflexi* was increased by 0.22 and 10.99 times, respectively. *Proteobacteria* can degrade complex organics to produce propionic acid (Rakia et al. 2005), and *Chloroflexi* is also active in the degradation of complex organics (Roest et al. 2005). These are also one of the reasons for the high removal rates of organic pollutants. At the genus level, *Macellibacteroides* accounted for 22.00% and merely 2.39% in R1–156 and R2–156, respectively. *Macellibacteroides* belongs to Bacteroides, and it has been proven that its relative abundance is positively correlated with the output of short-chain fatty acids (She et al. 2020). Therefore, VFA was evidently accumulated in R1. The data shows that the presence of pumice affected the compositions and relative abundance of bacterial community structures, promoted the degradation of organic pollutants, and favored the relative balance between bacteria and methanogens.

Community structure of methanogens

Hydrogen and acetate-type methanogens are two main methane formation pathways in anaerobic processes. The latter is generally considered as the most important means of methane production. In these processes, acetate-type methanogens contribute more than 60% of the methane output (Liu et al. 2020). Figure 7 shows the changes in the community structures of methanogens in different samples at the genus level. The primary methanogens included *Methanobacterium,*

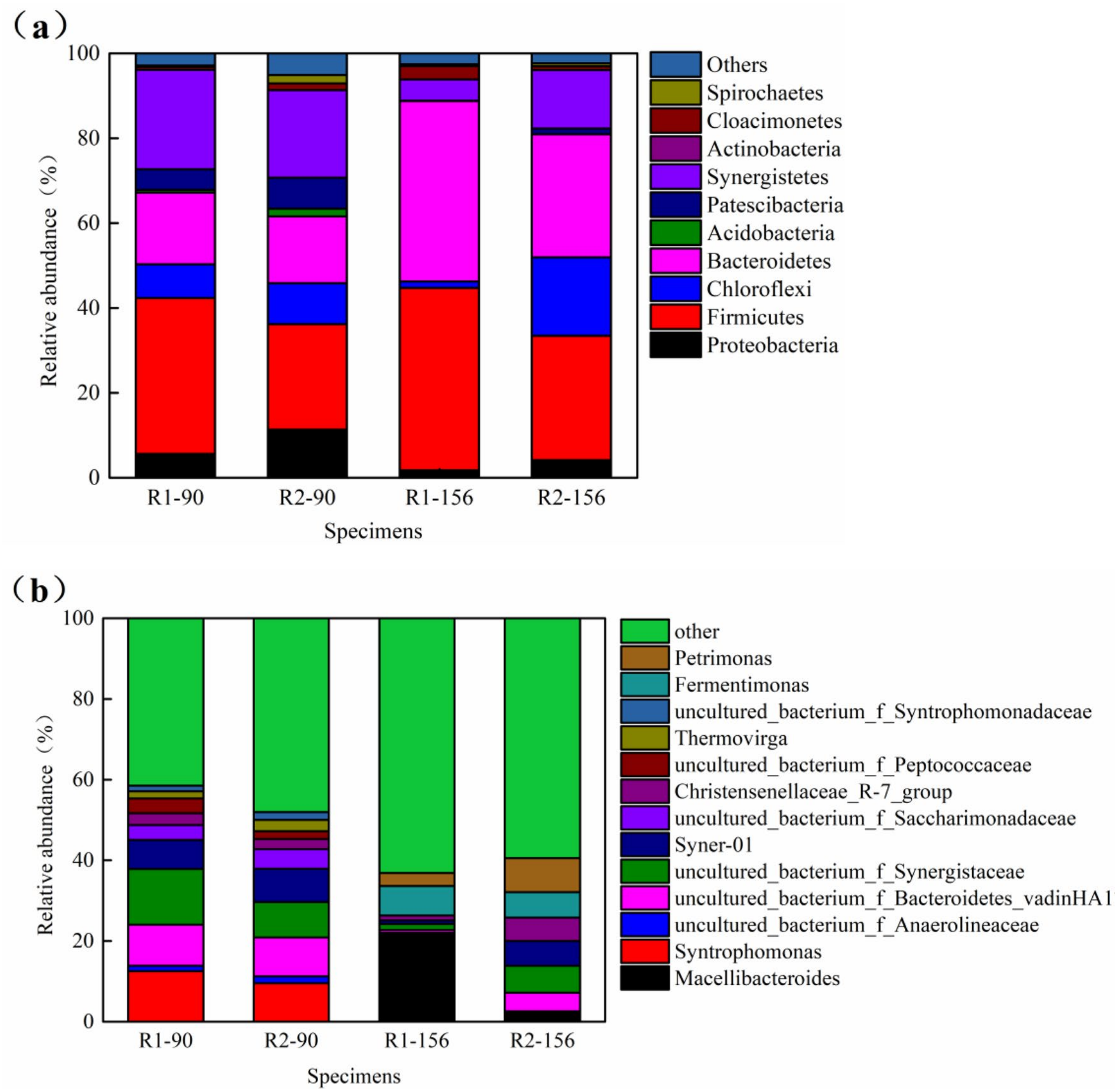
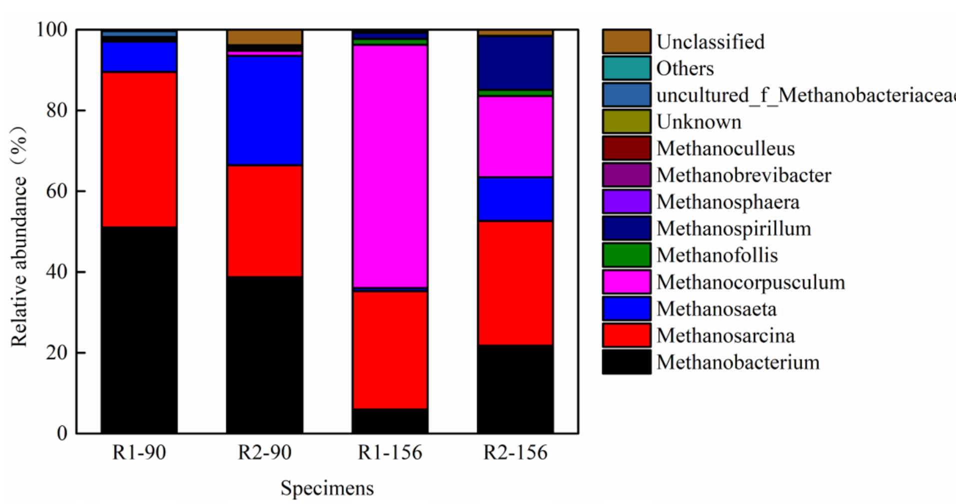


Fig. 6 Compositions of bacterial community structures in R1 and R2. **a** Phylum level; **b** genus level

Fig. 7 Community structure compositions of methanogens of R1 and R2 at the genus level



Methanosarcina, *Methanosaeta*, *Methanospirillum*, and *Methanocorpusculum*. These bacteria can be divided into two categories: hydrogen- type and acetate-type methanogens. Compared to R1, the relative abundance of acetate-type methanogens in R2–90 and R2–156 was increased by 18.68% and 38.37%, respectively, while hydrogen-type methanogens were concentrated in R1. In addition, compared with R1, the relative abundance of *Methanosaeta* in R2–90 and R2–156 was 3.58 and 13.86 times that in R1, respectively. These values show that the presence of pumice changed the dominant methane production pathway, and methanogens were selectively highly enriched, so the transformation efficiency of acid intermediates into methane was improved.

Conclusion

This work focuses on the effect of adding pumice to a UASB on the resistance to shock of high influent organic load and the related mechanisms. It was found that the addition of pumice could significantly improve the microbial diversity and abundance in the UASB reactor, and reinforce the balance between acid-producing bacteria (*Chloroflexi* and *Proteobacteria*) and methane-producing bacterium *Methanosaeta*. As a result, the average methane content of the UASB reactor increased by 15.2%, and the resistance to organic load impact was increased by nearly three times. Especially, under the influence of high load shock, the highly selective enrichment of *Methanosaeta* effectively enhanced the acid buffer capacity and anaerobic efficiency stability of the UASB reactor.

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Author contributions Authors are encouraged to include a statement that specifies the contribution of every author to the research and preparation of the manuscript. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by YZ, QH, ZJ, TL, GX, YZ and HW. The first draft of the manuscript was written by QH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent to participate Not applicable.

Consent to publish Not applicable.

Compliance with ethical standards Not applicable.

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