The Effect of Trace Element Addition on the Performance Efficiency of An Anaerobic Moving Bed Biofilm Reactor Treating Wine Vinasse

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The effect of trace element addition on the anaerobic digestion of wine vinasse in an anaerobic moving bed biofilm reactor was investigated in terms of applied organic loading rate, soluble COD removal efficiency, effluent propionate content and biogas production rate during the whole experiment. The addition of the first series of trace element combination with Fe (50 mg/l), Ni (10 mg/l), Co (10 mg/l) from day 178 to 181 did significantly optimize the reactor performance although the propionate levels were still high with ranges from 1.01 to 3.34 mg/l. Associated to this addition, the well-buffered pH system of the reactor was established, which made the reactor operated under the acidic influent. The addition of the second series with individual Fe from day 210 to 213 and the addition of the third series of trace element combination with Fe and Ni from day 214 to 217 did not affect the performance of the reactor distinctly. The addition of the fourth series of trace element combination with Fe (50 mg/l), Ni (10 mg/l), Co (10-20 mg/l), Cu (2 mg/l), Zn (20 mg/l), and Mo (0.8 mg/l) from day 218 to 225 can markedly reduce the propionate levels from about 2.0 g/l to 0.5 g/l, resulting in the performance of the reactor to be enhanced greatly. At the end of the experiment, soluble COD removal efficiency of the reactor was up to about 86% with an organic loading rate of 18.43 g COD/ l d, effluent propionate level was below 1.0 g/l, and biogas production rate was 8.6 l/l d. The addition of trace elements to the influent of the reactor resulted in a steady accumulation of Ni, Co and Mo in the suspended and attached biomass. The experiment result showed that using trace element combination to stimulate the performance of the reactor was quite available with the short-term dosing strategy, providing a promising method for sustainable anaerobic digestion of high strength wastewater.

Key words: Trace element, Anaerobic degradation; Wine vinasse; Biomass; Anaerobic moving bed biofilm reactor.

The application of anaerobic digestion processes for wastewater depollution and energy recovery requires not only organic matter, but also essential major and trace elements for the growth and activity of the methanogenic consortium in anaerobic systems. Several specific trace metals, including Fe, Co, Ni, W, Cu, Zn and Mo, are essential for the enzyme and cofactors involved in the biochemistry of fermentation and methane production. Limiting the trace elements required by the enzyme and cofactors can disturb the total anaerobic digestion process, and the poor performance of an anaerobic system can be amended or improved by supplementing single or combinations of trace elements through a faster substrate turnover and lower levels of volatile fatty acids (VFA), especially propionate[1-16].

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In recent years, much considerable attention has been paid on the effect of different trace element addition on the performance of anaerobic process or the conversion of VFA[3, 10-11]. The dissolved trace elements from aqueous media adsorbed to the biomass matrix can act as a stock of trace elements to maintain the metabolic requirements during anaerobic system operation, without any further trace element addition. So the investigation of trace elements will allow a more economic and effective control of the process.

The objective of the present study was to define the effect of single or combined trace elements on the performance of laboratory-scale anaerobic moving biofilm reactor (MBBR) to treat wine vinasse, and investigate the content variations during the different operation days of the reactor.

MATERIALS AND METHODS

Reactor

Experiments were performed using a 32.9 litres anaerobic MRRB (height: 89 cm; internal diameter 23.8 cm) made of Plexglas and filled with the frustum cylindrical polyethylene support (density 0.92 g/cm^3, diameter 35-29 mm and height 29 mm) as biofilm carrier (Bioflow 30). The filling support was accounted for 66 % total reactor volume, and the available specific surface area of the support was about 211m^2/m^3. The reactor was kept in a temperature-controlled water bath which was set at 39°C.

The reactor was sequentially mixed by a submerged Suprema centrifugal pump fixed at its bottom. The mixing regime was only for 2-8 times per hour, each mixing times lasted only for 1.25 minutes, which can make almost all supports in the reactor moved one and more cycles.

Influent vessel was stirred mechanically, and the reactor was fed with Masterflex L/S peristaltic pump. Hydraulic retention time (HRT) was adjusted by controlling the flow rate of the feeding.

Vinasse composition and inoculum sludge

Two kind of raw wine vinasses were used in the study. The low strength vinasse was only used for feeding the reactor from day 1 to 49, with total COD of 16.68 g/l, soluble COD of 15.52 g/l, pH of 5.75, suspended biomass (SS) of 2.06 g/l, and volatile suspended biomass (VSS) of 1.13 g/l. The high strength vinasse was used for feeding the reactor from day 50 onward because the low strength vinasse was used up, with total COD of 45.55 g/l, soluble COD of 44.18 g/l, pH of 3.62, SS of 3.27 g/l, and VSS of 2.46g/l.

The reactor was inoculated with a methanogenic granular sludge obtained from a pilot-scale anaerobic filter to treat wine vinasse. SS, VSS, and VSS/SS ratio of the inoculum sludge were 36.8 g/l, 20.36 g/l and 0.5, respectively.

Trace element supplementation strategy in the study showed in Table 1, which can be divided into four series from day 178 to 232 with consideration of the added trace element combination.

Analytical methods

pH was measured immediately after sampling with a WTW 537 pH meter. Samples for analyzing soluble COD and VFA were centrifuged at speed of 13000 rpm and relative centrifugal force of 16600×g for 10 min in a J2-MC Super-speed refrigerated (Beckman, USA) , and the supernatant was used for both analyzing. Total VFA and individual acids (acetate-AA, propionate-PA, isobutyrate-IBA, butyrate-BA, iso-valerite-IVA, valerite-VA) were measured by a gas chromatography (GC 8100 Fisons Instruments) with a flame ionization detector (FID). Biogas production was monitored by using a simple water displacement gas meter, where a counter registered a unit after a certain volume of the solution containing Na_2SO_4 and H_2SO_4 was displaced[17]. Biogas composition was analyzed by a gas chromatography (Shimadzu GC-8A) with catharometer, coupled with Shimadzu CR-3A integrator. Two stainless steel columns were used in chromatography, one was Hayesep column packed with Silica gel (80-100 mesh) for separation of CO_2 (2 m in length and 3.175mm in diameter), and another packed with molecular sieve 5 angstrom (80-100 mesh, 2m in length and 3.175 mm in diameter) for CO_2, N_2, O_2, and H_2 separations. Carrier gas was argon (300 kPa). Oven, detector and injection temperatures were 30ºC, 100ºC and 100ºC, respectively. SS and VSS of sludge were measured using Standard Methods[18].The attached biomass amounts on supports was calculated by drying them in an oven at 110°C for 2h.

The total trace element concentrations in the attached and suspended biomass were analyzed with the methods described by the methods[11] using ICP-MS (Elan 6000, Perkin-Elmer) after microwave destruction of the sample.

RESULTS AND DISCUSSIONS

Reactor performance

The reactor was started immediately after inoculating with 15 litter seed sludge, and then the reactor was run for 232 days.

Figure 1 shows the performance of the reactor in terms of applied OLR, soluble COD removal, effluent PA concentration and biogas production rate during the whole experimental period.

During the first 49 days of start-up operation period, the reactor was fed with the low strength and easily digested vinasse. The reactor adapted very quickly to the feed at the operation period, and digestion of VFA and other organic matters in wastewater mainly depended upon the inoculated suspended anaerobic sludge in the reactor. Soluble COD removal efficiency was high at this period, which was 73.6-90.1% at an organic loading rate of 1.36-3.57 g COD/l d and HRT of 4.66-7.74 days. VFA and propionate levels in effluent were less than 1.1 g/l and 0.70 g/l, respectively. Biogas production rate was increased with OLR increasing, ranging from 0.45 to 1.33 l/l d.

The reactor was fed with high strength vinasse from day 50 onwards. From day 50 to 116 soluble COD removal efficiency was low and only up to 30.6-65.7% at HRT of 3.34-4.03 days and OLR of 3.69-8.25 g COD/l d. VFA and propionate level ranges in effluent were 2.13-11.05 g/l and 0.85-5.59 g/l, respectively. Biogas production was slightly increased with OLR increasing, ranging from 0.97 to 2.31 l/l d. The performance of the reactor became worse during this 66 operation days, which was probably caused by overloading and influent pH fluctuating, giving rise to accumulation of effluent VFA, especially propionate, and finally inhibited methanogenic activities. The narrowed pH differences between effluent and influent may indicate that the pH buffering systems had not been established in this period.

Table 1. Trace element supplementation strategy

Essential elements	Compound forms used	Supplementation concentration (mg/l)			
		Day 178 to 181 Series 1	Day 210 to 213 Series 2	Day 214 to 217 Series 3	Day 218 to 225 Series 4
Fe	$FeCl_2 \cdot 4H_2O$	50	50	50	50
Ni	$NiCl_2 \cdot 6H_2O$	10	not added	10	10
Co	$CoCl_2 \cdot 6H_2O$	10	not added	not added	10-20
Cu	$CuCl_2 \cdot 2H_2O$	not added	not added	not added	2
Zn	$ZnSO_4 \cdot 7H_2O$	not added	not added	not added	20
Mo	$Na_2MoO_4 \cdot 2H_2O$	not added	not added	not added	0.8

The experiment could not be further carried out from day 117 to 153, and the reactor was in the recovery period at ambient temperature without heating and feeding. VFA and propionate levels were 4.35 g/l and 2.79 g/l on day 117, respectively, while on day 153, both contents were 2.62 g/l and 2.56 g/l, respectively. This comparison showed that VFA and propionate were degraded very slowly under ambient temperature.

The operation of the reactor was resumed starting from day 154. In the following 17 operation days the reactor was operated at a comparatively low applied OLR ranging from 2.83 to 4.63 g COD/l d, and its influent was carefully controlled at about 7.0, but the performance efficiency of the reactor was still unsatisfactory. Soluble COD removal efficiency of the reactor was low and only up to 35.26-53.8%, VFA and propionate levels in effluent were 1.98-3.53g/l and 1.64-2.33 g/l, respectively, and propionate still took the majority of total VFA.

In order to improve the degradation rate of propionate and enhance soluble COD removal efficiency, the trial for supplementing specific trace elements to enhancing the performance of the reactor was carried out from day 178 with four trace element addition series.

It can be seen from Figure 1 that soluble COD removal efficiency was increased by 7% from day 178 to 181 with OLR of 5.5-6.1 g COD/l d and HRT of 3.3 days by the first series trace element addition with Fe, Ni and Co associations, VFA in effluent was 3.5-3.9 g /l with propionate of 2.5-2.8 g/l, biogas production was 1.32-1.69 l/l d, and influent pH and effluent pH of the reactor ranged between 6.23-7.02 and 7.59-7.82, respectively. The three days addition of trace elements indicated that the trace elements can optimize the performance of the reactor.

From day 182 to day 202, soluble COD removal efficiency was increased from 67.8 % to 84 % with OLR being gradually raised from 7.90 to 11.93 g COD/l d even if there was no trace element addition in the 20 operation days, VFA content in effluent was 1.96-3.52 g/l with propionate ranging from 1.01-3.34 g/l, biogas production was 2.21-7.29 l/l d, and influent pH and effluent pH ranged between 4.05-6.52 and 7.21-8.36, respectively. The acidic feed of the reactor did not affect the performance, and soluble COD removal efficiency kept more than 80%, implying that a well-buffered pH system was established by the above mentioned three day additions of trace elements with Fe, Ni and Co associations. The VFA and propionate levels in effluent were still high in the operation time span, suggesting that Fe, Ni and Co associations can highly improve the VFA and propionate degradation rate.

From day 210 to day 213, the second series trace element addition with single Fe did not clearly enhanced soluble COD removal efficiency, on the contrary it cut down soluble COD removal efficiency to 64.5-65.9% although OLR was only slightly increased to 17.1 g COD/l d, VFA in effluent ranged from 6.0 to 6.6 g/l with propionate of 2.3-2.7 g/l, biogas production was 6.65-7.65 l/l d, and influent pH and effluent pH of the reactor ranged between 4.46-4.95 and 7.18-7.31, respectively.

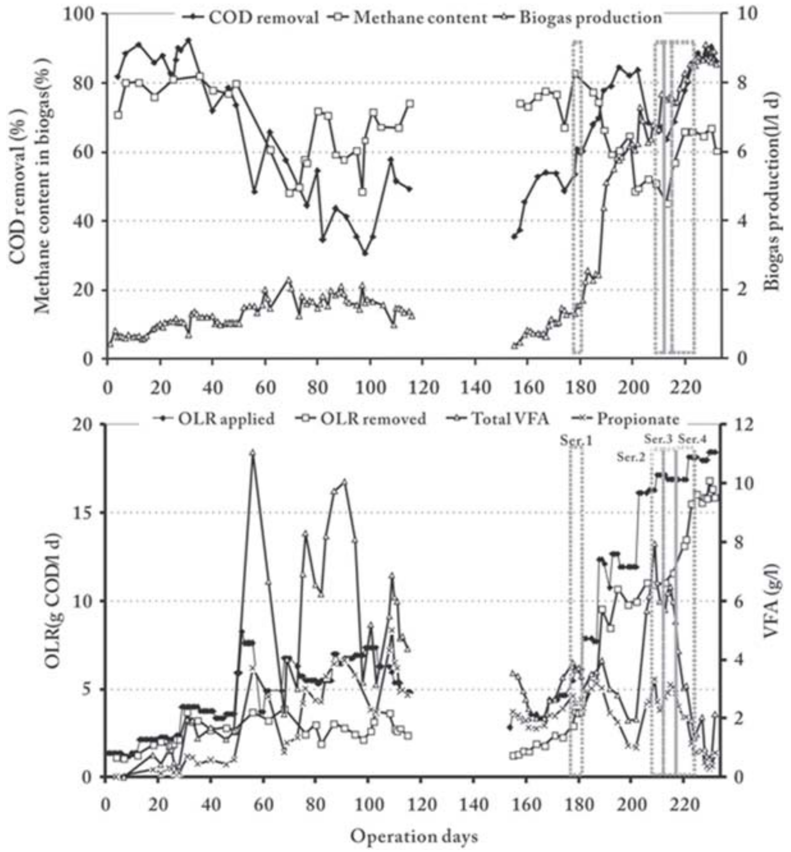

Fig. 1. Performance of the reactor during the experiment

From day 214 to day 217, the third series trace element addition with Fe, Ni associations made soluble COD removal efficiency a slight increase and up to 68% with OLR of 16.9 g COD/l d, VFA in effluent was in the range of 5.3-6.5 g/l with propionate of 2.9-3.1 g/l, biogas production was7.41-7.57 l/l d, and influent pH and effluent pH of the reactor ranged between 4.95-5.14 and 7.42-7.75, respectively.

From day 218 to 225, the forth series trace element addition with Fe, Ni, Co, Mo, Cu and Zn associations can obviously optimize the performance by accelerating VFA degradation, soluble COD removal efficiency increased from 77.7% to 88.6% although OLR was enhanced from 16.9 to 18.2 g COD/l d, VFA in effluent was reduced from 4.3 g/l to 1.4g/l with propionate ranging from 2.4 g/l to 0.8 g/l, biogas production was 7.81-8.62 l/l d, and influent pH and effluent pH of the reactor ranged between 5.04-5.13 and 7.53-7.92, respectively.

From days 225 to 232, soluble COD removal efficiency was still kept at a high value of more than 86% with OLR in the ranges of 18.0-18.4 g COD/l d although no trace element was added anymore. On day 232, soluble COD was 86.1% with OLR of 18.4 g COD/l day and HRT of 2.49 days, VAF level in effluent was 2.10 with propionate of 0.8 g/l, biogas production was 8.54 l/l d, and influent pH and effluent pH of the reactor were 4.84 and 7.89, respectively.

The results obtained from the study showed the performance efficiency can only be optimized by trace element combination with Fe, Ni and Co, and the performance can be greatly enhanced by trace element combination with Fe, Ni, Co, Cu, Zn and Mo through improving degradation rate of VFA, especially propionate.

Trace element concentrations in biomass

Trace element contents in the attached and suspended biomass were measured on days 115, 205 and 232, respectively. It can seen from Fig. 2 that the trace elements in the biomass can be grouped into two types according to their content variations in biomass: the accumulated ones, which included Ni, Co, Mo, those levels in the attached and the suspended biomass were increased owing to their additions; the depleted ones, which included Fe, Cu, Zn, those contents in the attached biomass was decreased, while their contents in the suspended biomass did not changed too much. Ni, Co and Mo were easily absorbed onto biomass or chelated with some polymer in biomass as the stock of elements, which can meet the need for metabolism of anaerobic microorganism for a long time if the reactor was operated continuously; while Fe, Cu,

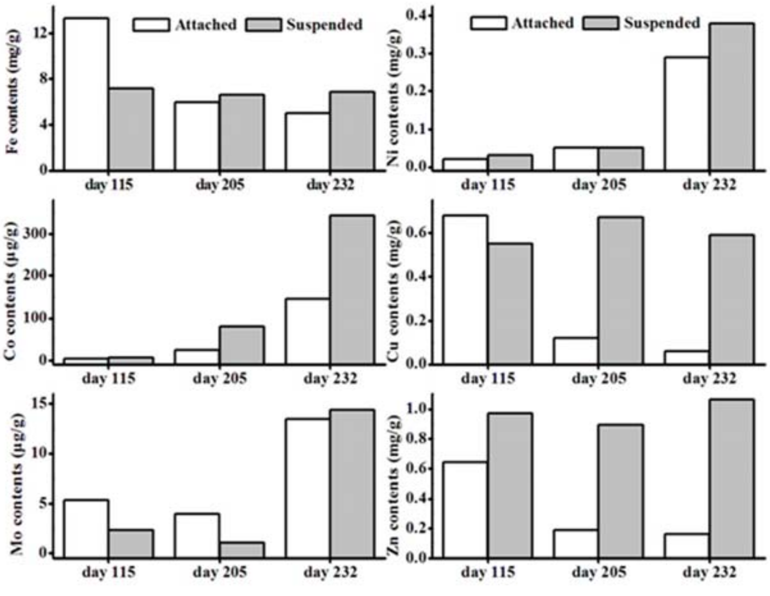

Fig. 2. Evolution of the trace element contents in the suspended and attached biomass

Zn were easily washed out the reactor and had a short retention time in sludge compared to Ni, Co, Mo, and the depleted Fe, Cu, Zn would be amended for the need for stable operation of the reactor.

CONCLUSIONS

The results obtained from the study showed the overall performance efficiency of the reactor to treat wine vinasse was inhibited by VFA, especially propionate, digestion rates. The performance of the reactor can be optimized or improved by supplementing trace element combination with the short-term dosing strategy, providing a promising method for sustainable anaerobic digestion of high strength wastewater.

Ni, Co and Mo contents in the attached and suspended biomass were accumulated with operation days, implying that these three elements may be easily absorbed onto biomass or chelated with biomass; while Fe, Cu, Zn were easily washed out the reactor, and it needs an amendment for the stable operation of the reactor.

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