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Inhibitory effects of Cu (II) on fermentative methane production using bamboo wastewater as substrate

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ABSTRACT

The toxic effects of Cu (II) present in bamboo industry wastewater (BIWW) upon its anaerobic biodegradability of organic content were investigated. The analysis through the Modified Gompertz model indicated that the optimum chemical oxygen demand (COD) concentration for digestion was 22,780 mg L^{-1} with a maximum R_m (maximum CH_4 production rate) value of 2.8 mL h^{-1}, corresponding to a specific methanogenic activity (SMA) of 2.38 mLCH_4 gVSS^{-1} h^{-1}. The inhibitory effects of Cu (II) on cumulative methane production depended on its concentration and contact time. Low concentrations (5 mg L^{-1}) of Cu (II) showed a stimulating effect on methanogenesis. Methane was not detected when the Cu (II) concentration was increased beyond 300 mg L^{-1}. The IC_{50} value of Cu (II), the Cu (II) concentration that causes a 50% reduction in the cumulative methane production, was 18.32 mg L^{-1} (15.9 mg Cu (II) gVSS^{-1}).

1. Introduction

Bamboo industry is a traditional industry in China with a history of about one thousand years, generating many important bamboo products that are used today [1]. However, production of these products involves boiling and dyeing processes which generates tonnes of bamboo industry wastewater (BIWW). The wastewater produced from boiling organics out of the bamboo material contains a bulk of organics mainly consisting of plant residues like carbohydrates and organic acids, and wastewater produced from dyeing the bamboo material usually contains organic dyes and high concentrations of Cu (II). The wastewater is characterized by a high chemical oxygen demand (20,000–50,000 mg L^{-1}), low pH values (2.5–5), high concentrations of Cu (II) (800–2000 mg L^{-1}) and strong color content. Direct discharge of BIWW to the environment could be hazardous to aquatic life. Therefore, the treatment of BIWW is a challenging task which should be accomplished with urgency.

Anaerobic treatment is an effective and widely used technology for decomposition of high strength wastewater containing organics [2–5]. However, the anaerobic bio-treatment is affected by substrate concentration, toxic materials, microbial biomass and contact time [6]. Moreover, a suitable substrate concentration plays an important role in the stable operation of anaerobic reactors. Many studies have suggested that the presence of heavy metals causes instability or operation failure of anaerobic reactors [7–9]. The main reason for this instability is attributed to disruption of enzymatic structure and function due to metal binding with thiol and other groups present in protein molecules or by replacing naturally occurring metals in their prosthetic groups [10].

Copper, being one of most prevalent heavy metals in BIWW, imparts potentially negative impacts on anaerobic microorganisms. Previous studies suggested variable concentrations of Cu (II) had inhibitory effects on methanogenesis. Mori et al. [11] indicated that 63.5 mg L^{-1} of Cu (II) could completely inhibit the activities of methanogens. Meanwhile, Lin [12,13] reported that the half-inhibition concentrations were 12.50 mg L^{-1} and 130 mg L^{-1}, respectively. Such huge variations in the inhibitory concentrations were partly due to differences in the precipitation and adsorption of soluble metals during the assays [14] and the different wastewaters used in their studies.

Since none of the data has been reported in relation to the treatment of BIWW, the present study was aimed to conduct a series of batch experiments based on the Modified Gompertz model to explore the biodegradability of BIWW. The inhibitory effect of its Cu (II) content on the methanogenesis and some important kinetic parameters were investigated. The investigations would provide guidelines for treating high strength BIWW.

2. Materials and methods

2.1. Wastewater and sludge

The raw wastewater, which contains no Cu (II), was collected from one of biggest and typical BIWW producing factory (Anji County, Zhejiang Province, China). The COD of the wastewater

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was 36,650 mg L^{-1}, while its COD:TN:TP was 190:4:1. The seed sludge was obtained from a full-scale Up-flow Anaerobic Sludge Bed (UASB) treating pig slurry (Hangzhou, Zhejiang Province, China). Volatile suspended solid (VSS) of the sludge was 58.7 g L^{-1}, accounting for 71% of total suspended solids (TSS). The essential nutrients fed to the reactor for the growth of microorganisms were (mg L^{-1}): $MgCl_2{\cdot}7H_2O$ (400), KCl (400), $CaCl_2$ (38), $FeCl_2{\cdot}4H_2O$ (40), $COCl_2{\cdot}6H_2O$ (10), KI (10), $Al_2(SO_4)_3$ (0.3), $MnCl_2{\cdot}4H_2O$ (0.5), $CuCl_2{\cdot}2H_2O$ (0.5), $ZnCl_2$ (0.5), $NaMoO_4{\cdot}2H_2O$ (0.5), H_3BO_3 (0.5), and $NiCl_2{\cdot}6H_2O$ (0.5).

2.2. Batch tests

The batch tests were performed in two experiments. Since high organic concentrations are typically encountered with raw wastewater, the first experiment was aimed to study the effects of the COD concentration on the biodegradability of the wastewater. The dyeing wastewater commonly has high concentrations of Cu (II), which can inhibit the anaerobic process. Therefore the second experiment was conducted to investigate the inhibitory effects of Cu (II) on methanogenic activity.

During the first experiment, raw wastewater was diluted with deionized water into 8 concentration gradients. Batch studies were performed to analyze the methane production during the treatment of each wastewater concentration.

In the second experiment combined raw wastewater containing COD = 6300 mg L^{-1} as substrate with different Cu (II) concentrations as $CuCl_2{\cdot}2H_2O$ (5, 20, 70, 130, 200, 250 and 300 mg L^{-1}) had been treated. Batch studies were performed to analyze methane production of each combination during treatment. Control units were operated without any Cu (II) dosage.

All batch tests were conducted in a 150 mL glass serum vials, and each vial was filled with 20 mL anaerobic sludge and 100 mL wastewater along with nutrients. Although the pH values of raw wastewater was in the range of 2.5–5, the recycle effluent of anaerobic reactor can increase it to 6–8. Therefore, the initial pH values of the study were adjusted to 7.0 ± 0.2 using NaOH (2 M) or HCl (2 M) solutions [15]. All vials were then capped with butyl rubber and incubated in a thermostatic water bath at $30 \pm 0.5\,^{\circ}C$. All vials were shaken 8–10 times a day, and CH_4 production was measured until it leveled off. Quadruplicate parallels were set for each batch test.

2.3. Analytical procedures

Methane production was monitored during the assays using a displacement system. Biogas was allowed to move into the Smith fermentation tube containing 15% (w/v) NaOH solution where CO_2 and H_2S were absorbed, and the remaining gas was CH_4, as indicated by the readings of fermentation tube. COD, TN, TP, VSS and TSS were measured according to the Standard Methods [16].

2.4. Kinetic modeling

The Modified Gompertz model (Eq. (1)) has been widely used in the studies of fermentative hydrogen production [17–20] and to predict rates of fermentative gas production processes [21,22]:

$$H_{(t)} = P \exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where $H_{(t)}$ is cumulative CH_4 production (mL), λ is lag time (h), P is CH_4 production potential (mL) and R_m is the maximum CH_4 production rate (mL h^{-1}) and $e = 2.718282828$; values of $H_{(t)}$, λ, P and R_m for each batch were estimated using Origin 9.0 for nonlinear regression analysis.

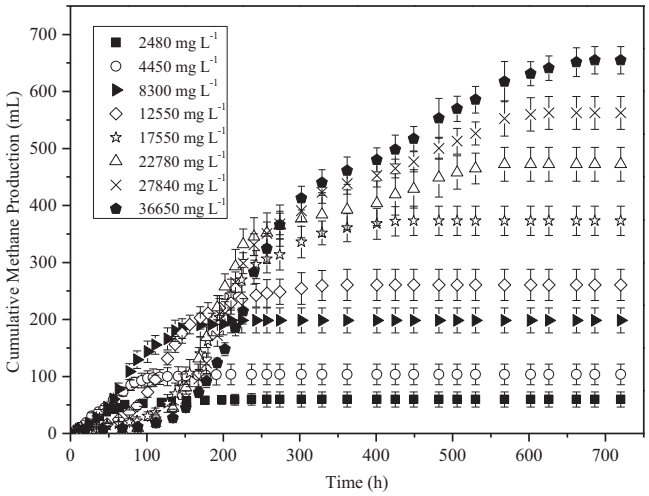

Fig. 1. Cumulative CH_4 production at different COD levels.

To describe the biodegradability of organic content of wastewater and inactivation of anaerobic culture by Cu (II), cumulative CH_4 production curves with respect to time were initially obtained from the methane production experiments. Then, the Modified Gompertz Equation was applied to quantify the methane production. The SMA (mLCH_4 gVSS^{-1} h^{-1}) was calculated by dividing the R_m by the initial VSS in the serum vial.

3. Results and discussion

3.1. Biodegradation of organics

Cumulative CH_4 production of raw wastewater, which contains no Cu (II), at different supplied COD levels is shown in Fig. 1. It was obvious that the lag time and CH_4 production generally exhibited a linear relationship with increasing COD, whereby CH_4 production increased from 60 to 655 mL when COD was increased from 2480 to 36,650 mg L^{-1}. At low COD concentrations (<8300 mg L^{-1}), the reactor operation was stable without any obvious lag time, but further increase in COD concentration delayed CH_4 production, which might be due to the fact that unacclimated microbial communities might have required longer duration to adapt concentrated substrates.

In order to evaluate the effects of COD on methanogens, the experimental data obtained from cumulative CH_4 production at different COD concentrations were fitted to Modified Gompertz Equation with $R^2 > 0.98$. The kinetic parameters obtained from the experiment are summarized in Table 1. It was evident that the lag time λ and methanogenesis potential P increased with the increasing COD concentrations. The respective increases in lag time and methanogenesis potential P were in the range of 2.1–130.3 h and 59–639 mL when the COD was increased from 2480 to 36,650 mg L^{-1}. The maximum methanogenesis rate R_m initially increased from 0.8 to the maximum 2.8 mL h^{-1}, corresponding to a SMA range of 0.68 to 2.38 mLCH_4 gVSS^{-1} h^{-1}, for the COD increase from 2480 to 22,780 mg L^{-1}. The methanogenesis subsequently decreased to 2.6 mL h^{-1} with a corresponding SMA of 1.87 mLCH_4 gVSS^{-1} h^{-1}, when the COD was increased to 36,650 mg L^{-1}. Theoretically, 1 gCOD can produce 350 mL of methane [23], while unit methanogenesis for COD was in range of 178–241 mLCH_4 gCOD^{-1} during the present investigation which was 50.9–68.9% of theoretical methane production.

The efficiency of methanogenesis is highly dependent on the optimal control of substrate to biomass (SX^{-1}) ratio. This ratio significantly affects the metabolic and kinetic characteristics of

Table 1
Kinetic parameters of cumulative CH_4 production at various COD concentrations.

COD ($mg\,L^{-1}$)	λ (h)	R_m ($mL\,h^{-1}$)	P (mL)	Methane production per unit COD ($mLCH_4\,gCOD^{-1}$)	SMA ($mLCH_4\,gVSS^{-1}\,h^{-1}$)	R^2
2480	2.1	0.8	59	241	0.68	0.99535
4450	9.5	1.4	104	232	1.19	0.9975
8300	24.4	2.0	199	239	1.70	0.99884
12,550	62.3	2.1	259	208	1.78	0.99761
17,500	106.7	2.3	373	213	1.95	0.99653
22,780	114.4	2.8	453	207	2.38	0.98595
27,840	116.7	2.4	540	202	2.04	0.9874
36,650	130.3	2.2	639	178	1.87	0.9912

λ: lag time; P: CH_4 production potential; R_m: maximum CH_4 production rate.
SMA: calculated by dividing the R_m by the initial VSS (1.175 g).

microbial communities involved [24]. Lobos et al. [25] demonstrated that the bacterial growth is optimum at higher SX^{-1}; and in the case of low SX^{-1}, the MLVSS concentration begins to decrease. In the case of slow anaerobic bacterial growth, the microbial biomass concentration was assumed to remain virtually constant throughout the experiment (with values 1.175 mg VSS L^{-1}). Fig. 2 shows the cumulative methanogenesis per unit COD at different SX^{-1} values. It was evident that the cumulative methanogenesis per unit COD slightly decreased with the increasing SX^{-1}. Linear fitting showed a high correlation (R^2 = 0.8671) between methanogenesis per unit COD and SX^{-1}.

3.2. Effects of Cu (II) on cumulative methanogenesis

Fig. 3 illustrates the cumulative methanogenesis at various Cu (II) concentrations. The data suggested that Cu (II) concentrations of 5 mg L^{-1} have a slightly stimulating effect on the activity of methanogenic bacteria. Such behavior could be attributed to fact that the presence of Cu (II) is required for the activation or functioning of many microbial enzymes and coenzymes [15]. Similar phenomenon was reported for other heavy metals; for example, Altas [15] indicated that Zn and Ni doses of 8–32 mg L^{-1} and 0.5–16 mg L^{-1}, respectively, could increase the cumulative CH_4 production. Li and Fang [9] also found that the fermentative H_2 production could be increased by Zn and Pb respective concentrations of 80–400 mg L^{-1} and mg L^{-1}. As Cu (II) concentrations increased above 70 mg L^{-1}, the microbial activity was temporarily inhibited with obvious lag time. A significant inhibition of cumulative methanogenesis was evident at 250 mg L^{-1} with the gas volume of 35 mL which was far less than 152 mL for the control. Methanogenesis was completely inhibited, at the Cu (II) concentration of 300 mg L^{-1}, with no CH_4 detected within 250 h.

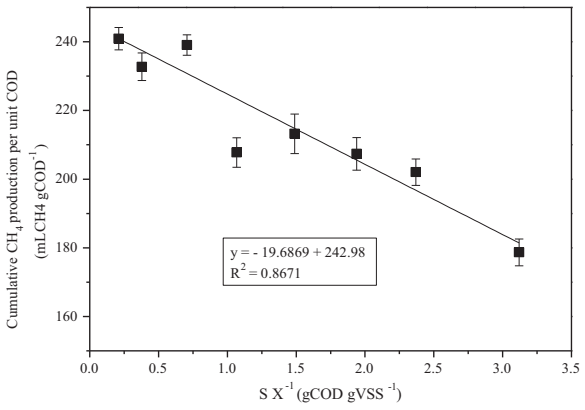

Fig. 2. Cumulative CH_4 production per unit COD at various SX^{-1} value.

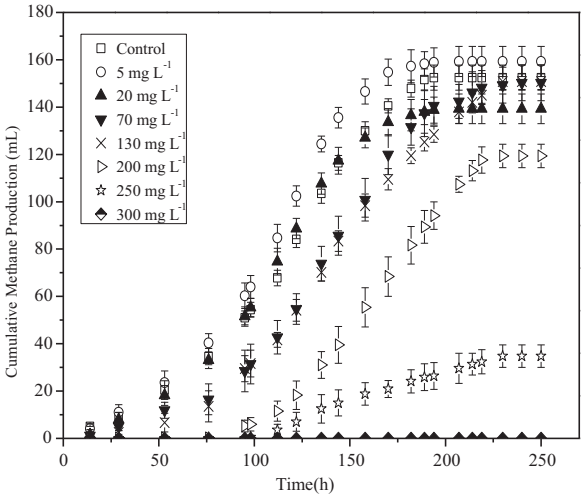

Fig. 3. Cumulative CH_4 production at different Cu (II) concentrations.

To evaluate the effects of Cu (II) on methanogenesis, the experimental data obtained from cumulative CH_4 production at different Cu (II) concentrations were fitted to the Modified Gompertz Equation and its kinetic parameters are summarized in Table 2. All estimated values of the kinetic parameters for Cu (II) concentrations of 5 mg L^{-1} were larger than that of control, except the lag time λ which was 51.8 h compared to 54.3 h of control. Cu (II) concentrations of 5 mg L^{-1} showed a stimulatory effect on methanogenic activity. The results presented in Table 2 showed a number of irregularities which may be due to the complexity of the real wastewater used in the batch test as a substrate. The lag time λ for 70 and 130 mg L^{-1} of Cu (II) were 77.8 and 74.5 h, respectively, which were obviously higher than that of the control keeping other parameters constant. It was indicated that the inhibitory effect of certain concentrations of Cu (II) (70–130 mg L^{-1}) could be eliminated by a longer adaption time. It was previously reported that metals are toxic to microbial activity when they exist in free soluble state [26–28]. Moreover, microorganisms secrete extracellular polymeric substances (EPS) after enough acclimation time, which can adsorb or precipitate the soluble or free Cu (II) to eliminate its inhibitory effects [29,30]. Compared to the control, 200 mg L^{-1} of Cu (II) had a higher lag time λ (110 h) and lower methanogenesis potential P (139 mL). The maximum methanogenesis rate R_m and SMA values were close to each other. The lag time λ for Cu concentrations of 250 mg L^{-1} (99 h) was shorter than that for 200 mg L^{-1}, the reason behind that phenomenon was not clear. However, the λ, R_m, P and SMA values were far less than those of the control for 200 mg L^{-1} which further suggested that the methanogenic reaction was strongly blocked.

3.3. The recovery effects and IC_{50} of Cu (II)

The inhibitory effects of Cu (II) on methanogens could be judged by relative activity (RA), which could be calculated by the following formula:

$$RA = \frac{\text{cumulative } CH_4 \text{ production of testing at particular time}}{\text{cumulative } CH_4 \text{ production of control at the same time}} \times 100\%$$

From Fig. 3 we can see that the RA of 5 and 20 mg L^{-1} increased within 112 h, and then deceased until 194 h; the maximum RA for 5 and 20 mg L^{-1} Cu (II) concentration were 110% and 125% (at 112 h), respectively. All RA values for 5 mg L^{-1} were above 100%, which implied that the Cu (II) concentration played a stimulatory

Table 2
Kinetic parameters of cumulative CH_4 production at various concentrations of Cu (II).

Cu (II) ($mg\,L^{-1}$)	λ (h)	R_m ($mL\,h^{-1}$)	P (mL)	Methane production per unit COD ($mLCH_4\,gCOD^{-1}$)	SMA ($mLCH_4\,gVSS^{-1}\,h^{-1}$)	R^2
0	54.3	1.3	166	242	1.1	0.98678
5	51.8	1.6	169	252	1.4	0.98664
20	54.5	1.4	147	221	1.2	0.99044
70	77.8	1.3	165	238	1.1	0.99238
130	74.5	1.2	172	239	1.0	0.99841
200	110	1.2	139	189	1.0	0.997
250	99	0.3	40	55	0.25	0.99597
300	–	0	0	0	0	–

λ: lag time; P: CH_4 production potential; R_m: maximum CH_4 production rate.
SMA: calculated by dividing the R_m by the initial VSS (1.175 g).

role on methanogens throughout the batch tests. The initial and final RA values for Cu (II) concentration of 20 $mg\,L^{-1}$ were lower than 100%, indicating initial inhibitory effect then a stimulatory one. Such behavior may be explained in terms of adaptation that occurred after 112 h of adaption, microorganisms secreted a lot of EPS, absorbing and precipitating soluble or free Cu (II) in the wastewater that caused free Cu (II) concentrations which might have favored the growth of microbes. However, the reason for the inhibitory effect still needs further investigation. The RA values of 70, 130, 200 and 250 $mg\,L^{-1}$ increased with time and were almost unchanged after 240 h. For 70 and 130 $mg\,L^{-1}$ of Cu (II), the system could be restored to 80% of relative methanogenic activity after 194 h while the inhibitory effects could be completely eliminated after 240 h. But for 200 and 250 $mg\,L^{-1}$, the RA values for anaerobic microorganisms were far lower than 100%. Therefore, when the Cu (II) concentration was higher than 200 $mg\,L^{-1}$, a serious inhibitory effect would occur and the activity of microorganisms was hard to be recovered. The possible reason leading to this observation may be attributed to the heavy metal (Cu (II)) binding to the sulfydryl or other groups of the protein molecules causing alterations in delicate structure and subsequent function of microbial enzymes [10]. Jin et al. [31] reported that recovery was possible with a feed copper to volatile suspended solids ratio (Cu (II):VSS^{-1}) in the range of 0.011–0.022 up to 15 $mg\,L^{-1}$ of added Cu (II). However, if the Cu (II) concentration increased to more than 20 $mg\,L^{-1}$, the methanogenic activity could not be recovered even the Cu (II):VSS^{-1} was 0.015.

The inhibitory effects of Cu (II) may be expressed by its half-inhibitory concentration (IC_{50}) – the concentration of Cu (II) that causes a 50% reduction in the cumulative methanogenesis relative to the control sample over a fixed period of exposure time (250 h), expressed as $mg\,Cu\,(II)\,L^{-1}$. Therefore, the IC_{50} value of Cu (II) was calculated to be 18.32 $mg\,L^{-1}$ ($R^2 = 0.9061$) by plotting the RA values at different Cu (II) concentrations. It has been suggested that inhibition caused by heavy metals would be more comparable if metal dosage was expressed as milligram of metal per gram of volatile solids [27]. The IC_{50} value of Cu (II) for the present study was 15.9 $mg\,Cu\,(II)\,gVSS^{-1}$.

It is reported that the corresponding IC_{50} data of Cu (II) for methanogenic activities of granular sludge degrading cattail [32], benzoate [33] and volatile fatty acids (VFA) [34] were 6.4, 175 and 130 $mg\,L^{-1}$, respectively. Moreover, the SMA of granules for the degradation of starch was reduced by 50% when 180 $mg\,Cu\,(II)\,gVSS^{-1}$ was added [35], while for flocculent digester sludge for the degradation of mixed VFA the value was decreased to 23.3 $mg\,Cu\,(II)\,gVSS^{-1}$ [36]. Results indicate that the inhibition effect was dependent on substrate and granulation degree of seed sludge.

Overall, with the exception of degrading cattail, the IC_{50} values in this study were substantially lower than those reported in the literature, indicating that Cu (II) was quite inhibitive to the CH_4-producing sludge when using raw wastewater's organics as substrate. This may be attributed to the following two factors: (1) the capabilities for the precipitation and adsorption of soluble Cu (II) during individual assays [14]; (2) the concentration of EPS of the sludge, which are the microstructures of anaerobic granular sludge [37] and protect the CH_4-producing cells against the stressful environment conditions.

4. Conclusions

The anaerobic treatment of the BIWW was quite feasible; the adaptation time required for the anaerobic microorganisms increased with the increasing COD in wastewater. The optimum COD concentration for methane production was 22,780 $mg\,L^{-1}$. A continuous experiment is needed to further prove the possibility of anaerobic treatment of the BIWW.

Copper concentrations of 5 $mg\,L^{-1}$ showed a stimulatory effect which verified the Modified Gompertz model. In general, the cumulative methane production of microorganisms decreased with increasing Cu (II) concentrations from 70 to 250 $mg\,L^{-1}$. Methane-producing sludge had the ability to recover after a certain period. However, methanogenic activity was completely blocked at the Cu (II) concentration of 300 $mg\,L^{-1}$, and the IC_{50} value for Cu (II) was 18.32 $mg\,L^{-1}$ (15.9 $mg\,Cu\,(II)\,gVSS^{-1}$).

Acknowledgments

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