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The effect of salinity on waste activated sludge alkaline fermentation and kinetic analysis

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ABSTRACT

The effect of salinity on sludge alkaline fermentation at low temperature (20°C) was investigated, and a kinetic analysis was performed. Different doses of sodium chloride (NaCl, 0–25 g/L) were added into the fermentation system. The batch-mode results showed that the soluble chemical oxygen demand (SCOD) increased with salinity. The hydrolysate (soluble protein, polysaccharide) and the acidification products (short chain fatty acids (SCFAs), NH_4^+–N, and PO_4^{3-}–P) increased with salinity initially, but slightly declined respectively at higher level salinity (20 g/L or 20–25 g/L). However, the hydrolytic acidification performance increased in the presence of salt compared to that without salt. Furthermore, the results of Haldane inhibition kinetics analysis showed that the salt enhanced the hydrolysis rate of particulate organic matter from sludge particulate and the specific utilization of hydrolysate, and decreased the specific utilization of SCFAs. Pearson correlation coefficient analysis indicated that the importance of polysaccharide on the accumulation of SCFAs was reduced with salt addition, but the importance of protein and NH_4^+–N on SCFA accumulation was increased.

Introduction

More than 30 million tons of waste activated sludge (WAS) with 80% moisture content is produced annually in China (Duan et al., 2012), and the sludge treatment expense is about 60% of the operating cost in wastewater treatment plants (Canales et al., 1994). Diverse approaches have been developed to reduce the sludge volume and recover the useful resources from WAS, such as the production of fuel byproducts through sludge melting or sludge pyrolysis and extraction of useful chemicals (Waste, 1999; Sachdeva et al., 2000; Spinosa, 2001; Soares et al., 2010).

Sludge anaerobic digestion is a complex reaction process (Gupta et al., 2012), which involves hydrolysis, acidogenesis, and methanogenesis. Hydrolysis is the first stage and the limiting step for sludge fermentation, where polymeric materials in the sludge are decomposed into smaller molecules (Demirel and Scherer, 2008; Eastman and Ferguson, 1981). Acid-consuming bacteria such as methanogens utilize short chain fatty acids (SCFAs) to generate methane. Adjusting to alkaline pH (pH = 10) was found to improve the hydrolysis rate, accelerate the extraction of carbon sources from WAS (Chen et al., 2007) and restrain the activity of methanogens. But only 45% of the biological carbon source was extracted (Chang et al., 2002) and 25% of WAS was reduced under alkaline conditions (Dytczak et al., 2007). In addition, some methanogens (Vallero et al., 2003) and hydrogen-producing bacteria (Yokoi et al., 1995) still existed in the alkaline fermentation system that could

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consume SCFA production. Therefore, further enhancing the WAS hydrolysis and controlling the growth of SCFA-consuming bacteria were the key issues for WAS fermentation.

In recent years, the seawater has been used widely in flushing toilets, which has increased the salinity of sewage. About 0.6%–1.6% salt was contained in municipal sewage (Chen and Leung, 2000), and the salt level of sludge or water may be higher after precipitation and enrichment. The salt of the concentrated water might reach about 10.12 g/L–24.75 g/L after regeneration treatment (Sun et al., 2015). Therefore, the wastewater treatment plants experience substantial variations of salinity (Reid et al., 2006). High salinity has an effect on microorganisms by changing the microbial communities and the physical and biochemical properties of microorganisms (Moussa et al., 2006; Vyrides et al., 2010). Previous studies have found that anammox microorganisms could produce large amounts of extracellular polymeric substance (EPS) under high salinity conditions (Ma et al., 2012). Enhanced WAS digestion and methane production at high salinity have been investigated extensively (Vallero et al., 2003; Hao et al., 2006; Kim et al., 2009; McCarty, 1964; Dimroth and Thomer, 1989). However, there have been few reports about enhancing SCFA accumulation by using NaCl.

Previous studies developed various mathematical models to describe the fermentation process, where the hydrolysis step and biomass decay conformed to first order reaction kinetics (Batstone et al., 2002; Gavala et al., 2003; Siegrist et al., 2002). Monod type kinetic models could be used to describe the conversion rate of soluble hydrolysate to SCFAs and the transformation of SCFAs to methane (Siegrist et al., 2002; Martin et al., 1994). In recent years, Haldane inhibition kinetics has been applied to describe the inhibition factors for SCFAs (Bernard et al., 2001), which combined the hydrolysis, SCFA generation and consumption, and the kinetic model has been used to analyze the SCFA accumulation phenomena during the fermentation process at different pH values and temperatures (Zhang et al., 2010a). An integrated model combining Monod and Haldane models was used to evaluate the kinetic coefficients using methane accumulation curves (Lokshina et al., 2001; Siles et al., 2008). Furthermore, a better fit was obtained using Haldane models and their exponential approximations in Upflow Anaerobic Sludge Blanket (UASB) biomass (Siles et al., 2008). Therefore, the Haldane model could be considered a comparatively ideal model to describe the sludge fermentation process. The main objective of this study was to estimate kinetic parameters by using the Haldane model, and demonstrate the feasibility of enhancing fermentation performance by adding NaCl. The Pearson correlation coefficient (PCC) is a statistical metric that measures the strength and direction of a linear relationship between two random variables (Pearson, 1896; Amin, 2012; Rodgers and Nicewander, 1988). It has been widely used in many applications such as time-delay estimation (Carter, 1987), pattern recognition (Chien, 1974), and data analysis. This paper used PCC to analyze the effect of the fermentation by-products on SCFA production, which had not been comprehensively analyzed before.

This study targeted the challenge of enhancing the sludge hydrolysis rate and increasing SCFA production by adding NaCl in a WAS alkaline fermentation system. The parameters of SCFA inhibition kinetics, including hydrolysis rate of particulate organic matter, the specific utilization of hydrolysate and the specific utilization of SCFAs, were measured to verify the feasibility of using NaCl. Furthermore, this work analyzed the PCC of SCFAs with protein, polysaccharide, NH_4^+–N and PO_4^{3-}–P in different salt conditions.

1. Materials and methods

1.1. Waste activated sludge source

WAS was obtained from a sequencing batch reactor (effective volume: 6.2 m^3, total volume: 8.8 m^3) treating municipal wastewater (Beijing, China), and then was washed four times before being fermented. The characteristics of WAS are listed in Table 1.

1.2. Batch experiment setup

Batch experiments were conducted in a series of identical plexiglass reactors with a working volume of 2.5 L at 20°C. The WAS concentration was controlled at 10,000 ± 100 mg/L. pH was kept at 10 ± 0.1. NaCl was added into the batch reactor and mixed well. The NaCl concentrations of fermentation systems were 0, 1.2, 2, 9, 15, 20, and 25 g/L, respectively. All reactors were sealed with rubber stoppers after flushing with N_2 for 4 min to maintain strict anaerobic conditions, and magnetically stirred at a speed of 700–750 r/min.

1.3. Analytic methods

1.3.1. Conventional detection

The analyses of total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total suspended solids (TSS) and volatile suspended solids (VSS) were conducted in accordance with Standard Methods (Crescerl et al., 1998). Polysaccharide was measured by the phenol-sulfuric method with glucose as standard (Herbert et al., 1971). Soluble protein was determined by the Lowry–Folin method with BSA as standard (Lowry et al., 1951). To determine the SCFAs produced from sludge fermentation, the fermented sludge mixture was taken from the reactors (both batch reactors) and centrifuged at 4000 *g* for 15 min, then immediately filtered through a Whatman GF/C glass fiber membrane (Yuan et al.,

Table 1 – Properties of waste activated sludge (WAS).

Indicator	Values
Total suspended solids (TSS) (mg/L)	10,000 ± 100
Volatile sludge concentration (VSS) (mg/L)	8000 ± 100
Soluble chemical oxygen demand (SCOD) (mg/L)	30 ± 5
Total chemical oxygen demand (TCOD) (mg/L)	9800 ± 100
Short chain fatty acids (SCFAs) (mg COD/L)	0
Soluble protein (as COD) (mg COD/L)	10 ± 0.5
Total protein (as COD) (mg COD/L)	6370 ± 5
Soluble polysaccharide (mg COD/L)	5 ± 0.5
Total polysaccharide (as COD) (mg COD/L)	1470 ± 5
Others (mg/L)	–
NH_4^+–N (mg/L)	5 ± 0.5
PO_4^{3-}–P (mg/L)	2 ± 0.5
Temperature (°C)	20 ± 1

2006), and the filtrate was analyzed using a gas chromatograph (GC, Agilent 7890, USA). The pH of all the reactors was monitored using a Wissenschaftlich Technische Werkstatten pH/OXi 340i. The activities of hydrolases (protease and a-glucosidase) were measured according to the methods previously reported (Goel et al., 1998).

1.3.2. Haldane inhibition kinetics model

The mathematical analytical method of this study was Haldane inhibition kinetics, which has been used to evaluate the inhibition of SCFAs under different temperatures and pH conditions (Bernard et al., 2001; Zhang et al., 2010a). Sludge alkaline fermentation can be described by Eqs. (1)–(6).

$$\frac{ds_p}{dt} = -k_m \cdot s_p + k_d \cdot X_h \cdot k_{d,v} \cdot X_v \quad (1)$$

$$\frac{ds_h}{dt} = k_m \cdot s_p - \frac{k_h}{1 + \frac{K_{s,h}}{s_h} + \frac{s_v}{K_{i,h}}} \cdot X_h \quad (2)$$

$$\frac{dS_v}{dt} = \frac{k_h}{1 + \frac{K_{s,h}}{s_h} + \frac{s_v}{K_{i,h}}} \cdot X_h - \frac{k_v}{1 + \frac{K_{s,v}}{s_v} + \frac{s_v}{K_{i,v}}} \cdot X_v \quad (3)$$

$$\frac{ds_{CH_4}}{dt} = \frac{k_v}{1 + \frac{K_{s,v}}{s_v} + \frac{s_v}{K_{i,v}}} \cdot X_v \quad (4)$$

$$\frac{dX_h}{dt} = Y_h \cdot \frac{k_h}{1 + \frac{K_{s,h}}{s_h} + \frac{s_v}{K_{i,v}}} \cdot X_h - k_d \cdot X_h \quad (5)$$

$$\frac{dX_v}{dt} = Y_v \cdot \frac{k_v}{1 + \frac{K_{s,v}}{s_v} + \frac{s_v}{K_{i,v}}} \cdot X_v - k_{d,v} \cdot X_v \quad (6)$$

The parameters of Haldane inhibition kinetics are shown in Table 2. According to Eqs. (1)–(6), we could calculate the kinetic parameters such as the hydrolysis rate of particulate organic matter (k), specific utilization of hydrolysate (k_h), decay rate of acidogenic bacteria (k_d) and specific utilization of SCFAs (k_v) of the different fermentation systems containing salt as a function of fermentation time. The maximum values were obtained and determined as the maximum hydrolysis rate of particulate organic matter (k_m), the maximum specific utilization of hydrolysate ($k_{m,h}$-), the maximum decay rate of acidogenic bacteria ($k_{d,h}$) and the maximum specific utilization of SCFAs ($k_{m,v}$) under different salinity conditions.

1.3.3. Pearson correlation coefficients

In order to find the strength of the relationship between protein, polysaccharide, NH_4^+–N, PO_4^{3-}–P and SCFAs, Pearson's product moment correlation coefficients were determined. Differentiating factors were searched by analysis of variance (ANOVA) with significance level 0.05. Standard statistical comparisons and graphing were performed in Microsoft Excel, and correlation analyses were performed in IBM SPSS Statistics.

The equation was the following Eq. (7):

$$r = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2 \sum_{i=1}^{n}(y_i - \overline{y})^2}}. \quad (7)$$

2. Results and discussion

2.1. Effect of salinity on the hydrolytic acidification performance

2.1.1. The hydrolysis performance

The EPSs, including protein and polysaccharides, are the active secretions of the biomass of the sludge matrix (Kavitha et al., 2013), and also were the main substances in the WAS fermentation system (Tanaka et al., 1997a; Liu and Fang, 2002). The WAS consisted of 65% protein, 15% polysaccharides and 20% unknown components on the basis of sludge TCOD, which was similar to previous studies (Chen et al., 2007). Salinity had the same effect on soluble protein and polysaccharide release (Fig. 1a and b). In this study, the protein and polysaccharide were 820.42 mg COD/L and 66.49 mg COD/L at 0 g/L salinity, and substantially increased at 0–20 g/L

Table 2 – Parameters of Haldane inhibition kinetics.

Parameter		Values	Reference
S_p (kg COD/m^3)	Hydrolysis of sludge particulate organic matter		
S_h (kg COD/m^3)	Dead biomass into hydrolysate (Soluble protein, carbohydrate and amino acid, etc.)		
S_v (kg COD/m^3)	Fermentation of the hydrolysate into SCFAs by acidogenic bacteria		
X_h (kg COD/m^3)	Acidogenic bacteria	0.30	Zhang et al. (2010a)
X_v (kg COD/m^3)	Methanogens	0.35	Zhang et al. (2010a)
S_h (kg COD/m^3)	Transformation of SCFAs into methane by methanogens		
$k_{s,h}$ (kg COD/m^3)	The half-saturation constant of acidogenic bacteria growth	0.01	Siegrist et al. (2002)
$k_{s,v}$ (kg COD/m^3)	The half-saturation constant of methanogen growth	0.01	Siegrist et al. (2002)
$k_{i,h}$ (kg COD/m^3)	The inhibition constant of SCFAs on acidogenic bacteria growth	1.50	Siegrist et al. (2002)
$k_{i,v}$ (kg COD/L)	The inhibition constant of SCFAs on methanogen growth	1.50	Siegrist et al. (2002)
Y_h (kg COD/kg COD)	The yield of acidogenic bacteria	0.05	Siegrist et al. (2002)
Y_v (kg COD/kg COD)	The yield of methanogens	0.05	Siegrist et al. (2002)
$k_{d,v}$ (day^{-1})	The decay rate of methanogens	0.01	Siegrist et al. (2002)
k (day^{-1})	The hydrolysis rate of particulate organic matter		
$k_{m,h}$ (kg COD/kg COD/day^{-1})	The specific utilization of hydrolysate		
$k_{m,v}$ (kg COD/kg COD/day^{-1})	The specific utilization of SCFAs		
$k_{d,h}$ (day^{-1})	The decay rate of acidogenic bacteria		

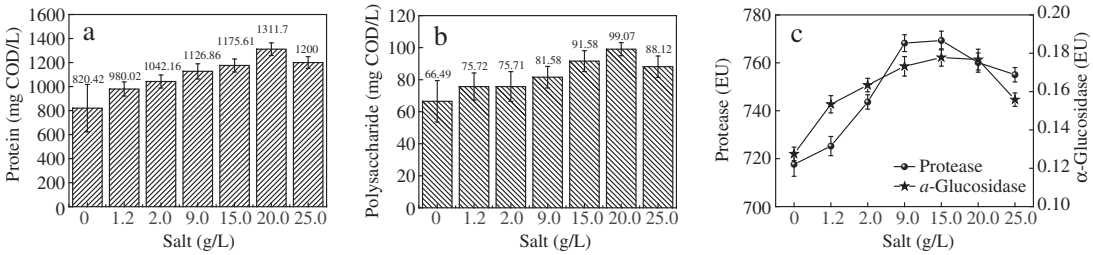

Fig. 1 – Effect of salinity on protein (a), polysaccharide (b), and hydrolase (c) at pH 10 in WAS alkaline fermentation.

salinity, reaching the peak values of 1311.70 mg COD/L and 99.07 mg COD/L at 20 g/L salinity, but decreased slightly at 25 g/L salinity (1200.02 mg COD/L, 88.12 mg COD/L). Comparing with other reported data (Chen et al., 2007), the values of this study were higher due to NaCl addition. It was reported that soluble protein and polysaccharides were produced by *Dunaliella* and increased concomitantly with salinity (Mishra et al., 2008). High protein concentration was caused by the protective responses of bacteria cells under salinity stress, producing more protein on the cell surface (Wang et al., 2013). High polysaccharide concentration was associated with the alteration of enzyme activities of polysaccharide biosynthetic pathways and the redistribution of the metabolic flux at high salinity (Wang et al., 2013). In addition, the mutational external osmotic pressure caused bacterial cell plasmolysis, and the cytoplasm (proteins and polysaccharides) was released into the fermentation liquor. High pH may enhance the release process (Wang et al., 1999). Kavitha et al. (2015) also found that the soluble EPSs increased with the salinity (0 g NaCl/(g·VSS): 0 EPS mg/L–0.08 g NaCl/(g·VSS): 575 EPS mg/L) and the loosely bound extracellular polymeric substances (LB-EPSs) and tightly bound extracellular polymeric substances (TB-EPSs) decreased with salinity. When the microorganisms were exposed to a higher salinity environment (25 g/L), the high concentration of Na^+ restrained the reaction pathways between enzymes (e.g., protease and amylase) and microorganisms (Rene et al., 2008). The phenomenon of physical disruption took place in microbial cells, which caused the hydrolysis performance to decline. Therefore, the proteins and polysaccharides declined at 25 g/L salt.

Extracellular enzymes (e.g., protease and a-glucosidase) play a critical role in decomposition of protein and polysaccharide in sludge; specifically, protease breaks the peptide bonds in protein molecules and a-glucosidase breaks the *a*-1,4 glucosidic linkage in maltose to release glucose (Goel et al., 1998). The data in Fig. 1c show that the activity of protease and a-glucosidase had remarkable differences in the seven fermentation tests, and increased with salt as well as protein and polysaccharide, but declined at higher salt (20–25 g/L). The reason might be that most protease was bound within pellets, with a minor fraction associated with TB-EPS and LB-EPS fractions, but α-glucosidase largely appeared in the LB-EPS fraction, with correspondingly minor quantities detected with TB-EPSs and with pellets (Yu et al., 2007). The exchange capacity of Na^+ led to the variation of extracellular enzymes being released from sludge at different salinity levels, which also resulted in differences in SCFA production in the seven fermentation systems. Anupama et al. also found a considerable increase in phosphatase activity when the concentration of NaCl was above of 15 g/L (Anupama et al., 2008).

2.1.2. *Effect of salinity on WAS acidification*

The SCFAs are the acidification products of proteins and polysaccharides by acid-forming bacteria (Liu and Fang, 2002; Tanaka et al., 1997b). As discussed above, adding NaCl had a prominent enhancing effect on the protein and polysaccharide release (Fig. 1a and b). Therefore, the salinity had a significant impact on the SCOD and SCFAs, and even the proportion of SCFAs in SCOD. In this work, SCOD concentration increased with salinity due to cell hydrolysis and EPS release (Fig. 2a). Meanwhile, the SCFA concentration and the proportion of SCFAs in SCOD increased with salinity at 0–15 g/L, but declined slightly at 20–25 g/L salinity compared with the 15 g/L salt condition (Figs. 2b and 3). The proportion of protein in SCOD was similar to the proportion of SCFAs in SCOD, but the proportion of polysaccharide had no obvious change with salt. The maximum amount of SCFAs and proportion of SCFAs in SCOD were 1893.89 mg COD/L and 43% at 15 g/L salt, while the minimum amount of SCFAs and proportion of SCFAs in SCOD were 1565.45 mg COD/L and 36.26% at 0 g/L salt at the 10th day. Meanwhile, the amount of SCFAs and the proportion of SCFAs in SCOD in 25 g/L salt (1794.31 mg COD/L, 37.93%) were higher than in the 0 g/L salt condition. The reason might be that abundant acidification substances (proteins and polysaccharides) were provided for the acidification bacteria in the moderate salt fermentation condition, and the higher activity of hydrolase had an important role in that process (Goel et al., 1998), as shown in Fig. 1c. In addition, high concentration Na^+ could inhibit the growth of methanogens (McCarty, 1964), especially in alkaline conditions (Zhang et al., 2010a; Wu et al., 2009) through interfering with their metabolism (Mendez et al., 1995). However, excessive NaCl not only suppressed the methanogens' growth but also that of the hydrolytic acidification bacteria. The same phenomenon appeared in the bio-hydrogen process with the addition of NaCl (Hao et al., 2006), which showed that excessive Na^+(>2000 mg/L) suppressed hydrogen-producing bacteria growth. The results revealed that adding NaCl could promote the hydrolytic acidification performance of fermentation sludge, but excessive NaCl was detrimental to the process. The optimum NaCl concentration for bio-acidification was 15 g/L.

2.1.3. *Effect of salinity on NH_4^+–N and PO_4^{3-}–P release*

Phosphorus (PO_4^{3-}–P) and ammonia (NH_4^+–N) in the bacterial EPSs were released during the WAS fermentation process. The

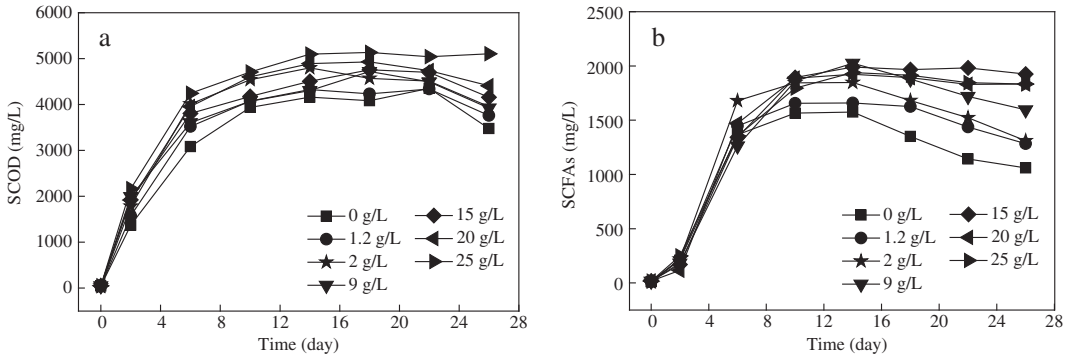

Fig. 2 – Effect of salinity on soluble chemical oxygen demand (SCOD) (a) and short chain fatty acids (SCFAs) (b) at pH 10 in WAS alkaline fermentation.

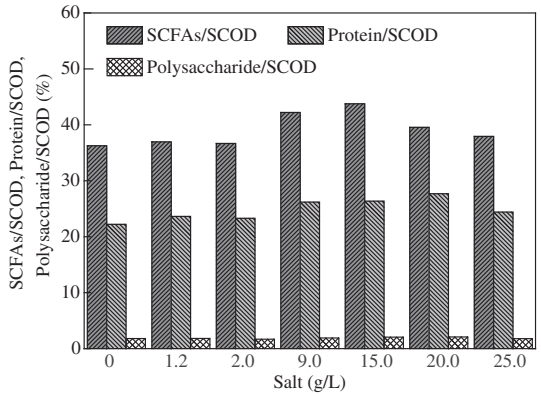

Fig. 3 – Effect of salinity on SCFAs/SCOD, protein/SCOD and polysaccharide/SCOD at pH 10 in WAS alkaline fermentation.

NH_4^+–N concentration in solution increased with salinity (0–15 g/L), and reached a peak value of 273.62 mg/L at 15 g/L, but decreased at 20–25 g/L salinity. The PO_4^{3-}–P concentration had only a small increase, with values from 45.61 mg/L (0 g/L) to 50.46 mg/L (25 g/L) (Fig. 4). In addition, the data of Fig. 4 showed that the NH_4^+–N concentration was higher than that of PO_4^{3-}–P, since ammonia was produced by the decomposition of nitrogenous substances (e.g., proteins and urea) (Chen et al., 2008) and orthophosphate was produced from organophosphorus substances (polysaccharides) (Chen et al., 2008; Banister et al., 1998; Bouallagui et al., 2004). The amount of organophosphorus substances was less than nitrogenous substances and orthophosphate, which resulted in lower PO_4^{3-}–P. Furthermore, the SCFAs/SCOD increased with NH_4^+–N, which showed that NH_4^+–N had an important effect on the SCFA accumulation. The reason might be the influence of free ammonia on acidification bacteria.

2.2. Fermentation kinetics analysis

2.2.1. SCFA inhibition kinetics analysis

As shown by the data in Fig. 5a, increasing salt from 0 to 15 g/L caused the improvement of the maximum hydrolysis rate of particulate organic matter (k_m) significantly, and the value of k_m declined at 20–25 g/L salt, which indicated that the release rate of soluble protein and polysaccharide from the granular sludge into the fermentation system increased rapidly at 0 to 15 g/L; but the release rate k_m had a declining phenomenon

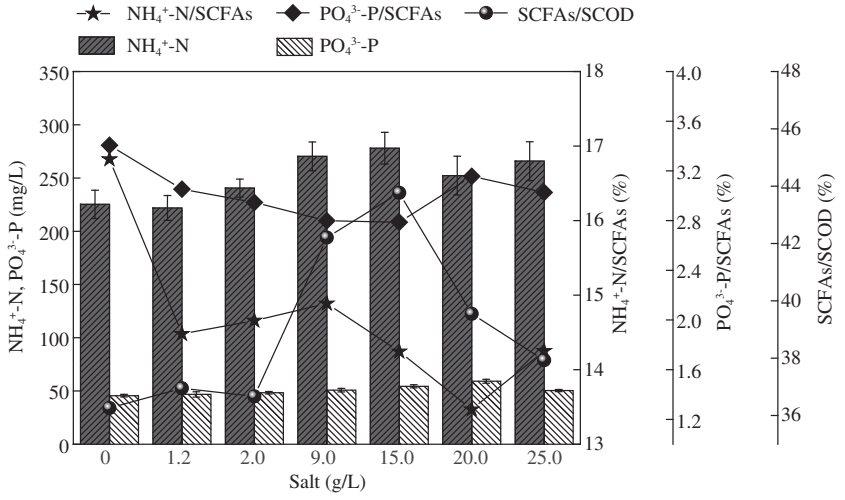

Fig. 4 – Effect of salinity on NH_4^+–N and PO_4^{3-}–P at pH 10 in WAS alkaline fermentation.

when the salt was too high. However, the release amount of soluble protein and polysaccharide was still increased with salt compared with 0 g/L salt (Fig. 1a and b), which showed that salt could improve the hydrolysis of WAS and could further enhance the SCFA generation (Fig. 2b). In the data of hydrolysis rate as a function of fermentation time, the values of *k* first increased at the early fermentation stage (0–6 days), then declined, and remained stable at the end of fermentation Fig. 5a. The maximum value appeared at the 6th day, which was early compared with the peak of soluble protein and polysaccharide (7–8 days). This indicated that the hydrolysis rate of WAS increased under a high substrate and favorable hydrolysis reaction environment. With the fermentation reaction, the competitive antagonism and shortage of substrate

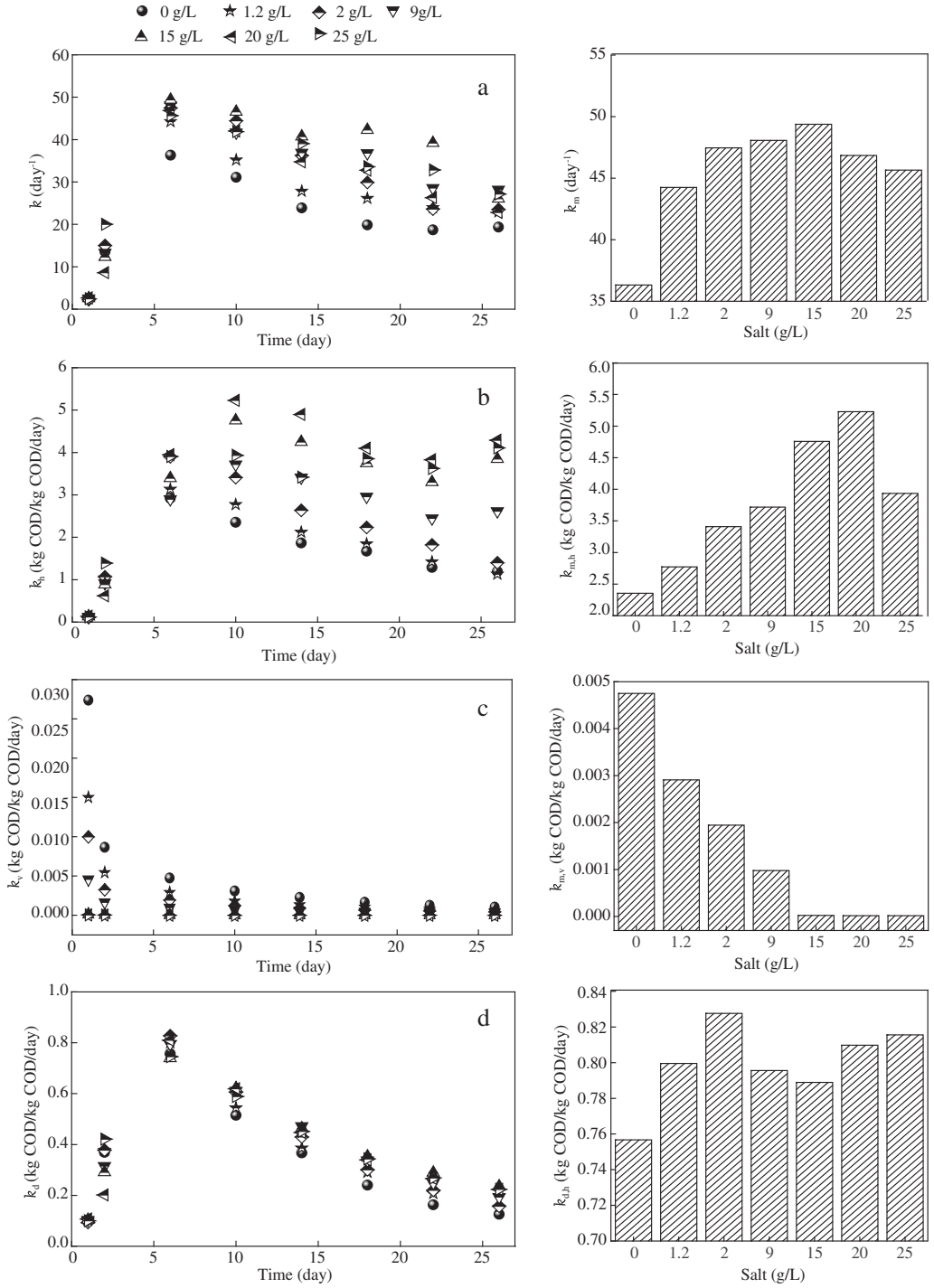

Fig. 5 – Effect of pH on the hydrolysis rate of particulate organic matter (k, k_m); b: the specific utilization of hydrolysate (k_h, $k_{m.h}$); c: the specific utilization of SCFAs (k_v, $k_{m,v}$); d: the decay rate of acidogenic bacteria (k_d, $k_{d.h}$).

made the hydrolysis rate decline (Putnam and Tang, 1986). Meanwhile, the values of k showed that the dissolution of sludge particles and release of soluble protein and polysaccharide were asynchronous. Previous studies also focused attention on the k_m (Table 3), with values of k_m ranging from 0.005 to 0.99 day^{-1} at 35–55°C, which were lower than those in this study (2–50 day^{-1}). This was probably due to the higher protein content in WAS than in PS, since protein hydrolysis is considered to be slower than carbohydrate (Zhang et al., 2010b).

As we know, the hydrolysis product is the substrate for production of SCFAs by acid-forming bacteria. Therefore, the maximum specific utilization of hydrolysate ($k_{m,h}$) plays an important role in SCFA production. The data in Fig. 5b showed that the values of $k_{m,h}$ as well as the k_m increased in the presence of salt compared to that in the absence of salt in the fermentation system, which showed that the utilization of hydrolysate (soluble protein and polysaccharide) increased rapidly under salt levels lower than 15 g/L, but the utilization of hydrolysate declined in the 20–25 g/L salt level range compared with 15 g/L salt. The tendency was similar with SCFA production (Fig. 2d). The reason might be that NaCl can provide favorable microenvironment conditions such as cell–cell contact (bacterial populations can co-ordinate their behavior) and enhance favorable conditions for production of acid as well as surfactant (Kumar et al., 2008). The change of $k_{m,h}$ indicated that salt could improve the conversion rate of hydrolysis product, but that it was suppressed at high concentration NaCl, where the anticipated effect was the change of SCFA generation (Fig. 2b). Meanwhile, the values of k_h first increased with time (0–10 days), then decreased from the 10th to 26th days. The acidifying reaction occurred later than the hydrolysis reaction compared with the hydrolysis rate of particulate organic matter (k), and the acid-producing bacteria could produce large quantities of SCFAs by using the hydrolysis product only when sufficient hydrolysis product was present. Furthermore, the maximum specific utilization of hydrolysate ($k_{m,h}$) initially increased with the salt level (0–15 g/L) but declined at 20–25 g/L salt due to the fact that the hydrolysis rate of particulate organic matter decreased. Higher utilization of hydrolysate compared with other cases (0.002–2 kg COD/kg COD day^{-1}) (Table 3) showed that an appropriate concentration of NaCl

Table 3 – Kinetic coefficients from various studies.

Parameter	Sludge character	Temperature(°C)	pH	Values	Reference
k_m	Kitchen waste	35	Un	0.34	Liebetrau et al. (2004)
	Biowaste	35	Un	0.12	Liebetrau et al. (2004)
	Primary	35	Un	0.55	O'Rourke (1968)
	Primary	35	Un	0.99	Ristow et al. (2006)
	Secondary sludge	35	Un	0.17–0.6	Ghosh (1981)
	Sludge	T[b]	Un	0.1	Batstone (2002)
	Primary	35	Un	0.25	(Siegrist et al. (2002)
		55		0.40	
	WAS	35	Un	0.12	Zhang et al. (2010b)
	WAS + SDBS	35		0.18–0.25	
	WAS	55		0.18	
	WAS + SDBS	55		0.21–0.28	
	WAS	20	10	36.32–49.37	This study
$k_{m,h}$	WAS	20	10	4.5	Zhang et al. (2010a)
		35		13.5	
		55		45.0	
	Carbohydrates	55	Un	0.025–0.2	Christ et al. (2000)
	Proteins			0.015–0.075	
	Lipids			0.005–0.010	
	Carbohydrates	Room temperature	Un	0.5–2.0	Garcıa-Heras (2002)
	Lipids			0.1–0.7	
	Proteins			0.25–0.8	
	WAS + SDBS	35 ± 2°C	Un	6.3–12.4	Zhang et al. (2010b)
		55 ± 2°C		19.0–52.0	
	WAS	20	10	3.25–5.22	This study
$k_{m,v}$	WAS	20	10	0.01–0.02	Zhang et al. (2010a)
		35			
		55			
	WAS + SDBS	35 ± 2°C	Un	0.07–0.73	Zhang et al. (2010b)
		55 ± 2°C		0.03–0.25	
	WAS	20	10	0.001–0.0048	This study
$k_{d.h}$	WAS	20	10	0.5	Zhang et al. (2010a)
		35		1.25	
		55		3.4	
	WAS + SDBS	35 ± 2°C	Un	0.8–1.15	Zhang et al. (2010b)
		55 ± 2°C		3.2–3.5	
	WAS	20	10	0.75–0.81	This study

Un: pH uncontrolled.

acted as a catalyst that could promote the utilization of hydrolysate.

The data in Fig. 5c showed that the specific utilization of SCFAs (k_v) decreased with time, and the value of $k_{m,v}$ showed a rapid decrease from 0 g/L salt to 15 g/L salt, then stabilized at 20 g/L and 25 g/L salt, which indicated that the SCFA consumption rate decreased with salt and finally remained stable. The change of $k_{m,v}$ showed that the salt could effectively suppress methanogens, and led to the phenomenon of large accumulation of SCFAs (Fig. 2d). Meanwhile, k_v also decreased with the fermentation time. Previous studies had reported that sodium concentrations exceeding 10 g/L strongly inhibited methanogenesis (Kugelman and McCarty, 1965). Therefore, the salt further enhanced the SCFA accumulation in the WAS alkaline fermentation.

The decay rate of acidogenic bacteria ($k_{d,h}$) represents lysis and endogenous respiration processes and is believed to be an essential parameter in the description of microbiological conversion of organic matter during anaerobic sludge fermentation (Zhang et al., 2010b). The data in Fig. 5d indicated that the maximum decay rate of acidogenic bacteria ($k_{d,h}$) showed the same trend as k_m and $k_{m,d}$. Furthermore, $K_{d,h}$ increased with the salt concentration although the SCFA production increased, which was similar to the report of Zhang et al. (2010a). In Zhang's report, the decay rate of acidogenic bacteria ($k_{d,h}$) increased with increasing pH, but the SCFA production and the percentage of acidogenic bacteria increased with pH. However, k_d increased during the early fermentation time and then declined, which showed that the acidogenic bacteria did not adapt to the alkaline environment containing salt and rapidly died, and then adapted to the environment step by step.

2.2.2. Pearson correlation coefficient analysis

It has been reported that the hydrolysis step is the limiting step in sludge fermentation (Eastman and Ferguson, 1981) and that the protein and polysaccharides were the key matters for acidification (SCFA generation), especially the polysaccharides. In addition, the free ammonia was important for SCFA generation (Chen et al., 2008).

In order to analyze the correlation of SCFAs with the protein, polysaccharide, NH_4^+–N and PO_4^{3-}–P, Pearson correlation coefficient analysis was carried out in this study (Fig. 6). The data in Fig. 6 showed that the correlation of SCFAs with protein and NH_4^+–N increased with salt, which indicated that the protein and NH_4^+–N had an important role in the WAS fermentation with salt addition due to the hydrolysis rate of protein, and the acidification performance was enhanced. A previous study found that the effect of noncompetitive inhibition functions of ammonia on biomass was remarkable only when the concentration of NH_4^+–N was in the range of 1.7 to 14 g/L (Chen et al., 2008), and the concentration in this study was lower than the reported range. Therefore, a small amount of free ammonia not only had no inhibiting effect on SCFA production, but also had a promoting function on the process (Fig. 4). Fig. 6 also showed that the correlation of SCFAs with polysaccharide decreased with salt because of the lower concentration compared with proteins.

2.3. Evaluation and significance of sludge fermentation with salinity treatment

Through the analysis of organic material in the fermented liquid and granular sludge, it can be seen that salinity played an important role in WAS anaerobic fermentation. Salinity

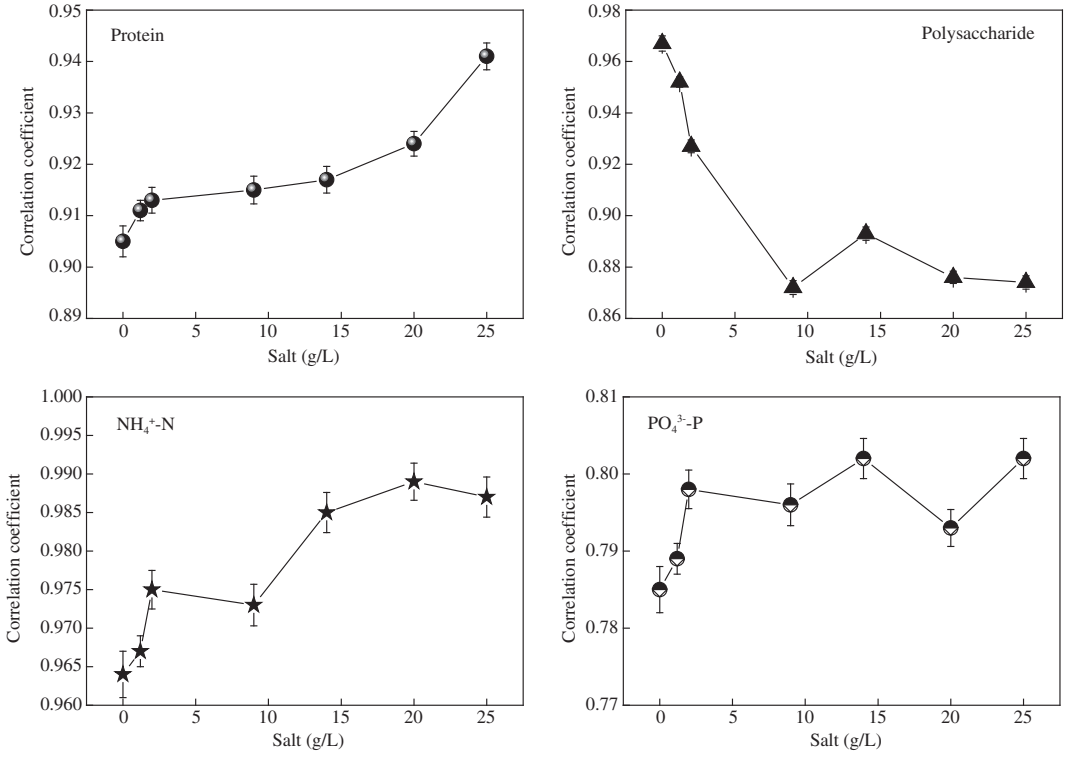

Fig. 6 – Correlation (C_i) analysis of SCFAs with protein, polysaccharide, NH_4^+–N and PO_4^{3-}–P.

mainly impacted the physical and biochemical properties of sludge, particularly the protein, polysaccharides, particle size and moisture content of fermented sludge, and sludge dehydration level (Moussa et al., 2006; Vyrides et al., 2010; Zhang et al., 2011; Shao et al., 2009). In this study, the EPS was released effectively from granular sludge, and salt enhanced this process, which provided an abundant acidification matrix for acid-forming bacteria and produced a large amount of SCFAs. This proved that salt further enhanced extraction of the carbon source compared with alkaline fermentation alone. This is beneficial to urban sewage treatment plants in which the main treatment process is the activated sludge process, especially in those treatment plants with low C/N and large amounts of WAS. In addition, the study provides a new method using high salt wastewater to enhance WAS alkaline fermentation and improve the application value of high salt wastewater.

3. Conclusions

The promoting effect of salinity on SCFA accumulation was investigated in this work. Inhibition dynamics and Pearson correlation coefficient analysis of the WAS alkaline fermentation were used to prove the feasibility of using salt.

The salt further enhanced the soluble protein and polysaccharide release and SCFA accumulation for WAS alkaline fermentation. Meanwhile, the Haldane inhibition kinetics model evaluated the feasibility of using salt for WAS alkaline fermentation, where salinity remarkably enhanced the hydrolysis rate of particulate organic matter and the specific utilization of hydrolysate, which led to a significant hydrolytic acidification effect compared with the 0 g/L salt condition. Furthermore, the specific utilization of SCFAs decreased with salt, which indicated that methanogens were suppressed effectively, enabling larger SCFA production. Meanwhile, salt decreased the dependence of SCFAs on polysaccharide, and protein may become the key substance for SCFA accumulation.

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