Performance of alkaline pretreatment on pathogens inactivation and sludge solubilization

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Abstract: Inactivation of pathogen indicators (i.e., faecal coliforms, *Salmonella* spp., faecal streptococcus, and helminth eggs) were investigated during alkaline pretreatment (pH=10 and 12) in this study. The performance of alkaline pretreatment on the inactivation pathogens, kinetic of pathogens inactivation and sludge solubilization was evaluated. Results of alkaline pretreatment showed that the complete inactivation periods of pathogens time were 1.5 d, 1.5 d, 2 d, 2.5 d, 3 d, 3 d and 3 d for faecal sludge total solids (TS) of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. The kinetics of pathogen inactivation can be predicted better by Weibull than the first-order model. Meanwhile, the relationship between alkaline pretreatment time and the TS content of the sludge agrees with the exponential equation (y=1.3543e$^{10.002x}$, $1\% \le x \le 8\%$) and logarithmic equation (y=3, $8\% \le x \le 12\%$). Furthermore, alkaline pretreatment can improve sludge solubilization and has a more significant effect on protein solubilization than on soluble chemical oxygen demand (SCOD).

Keywords: faecal sludge, alkaline pretreatment, pathogen inactivation, kinetic, sludge solubilization

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1 Introduction

The indiscriminate discharge of untreated faecal sludge into rivers, lakes and ground water poses great threats to the environment and human health. Previous researchers used anaerobic digestion techniques for sludge stabilization[1,2]. Compared with other methods of waste treatment, such as land filling, incineration and composting, anaerobic digestion has the advantages of reducing the amount of waste produced and generating biogas, which is a renewable energy source[3]. Moreover, the effluent of anaerobic digestion is a residue rich in inorganic elements with fertilizer values. Such effluent enriches soil with no detrimental effect on the environment[4].

Pathogens are present in high levels in faecal sludge, causing adverse effect on human health. The bacterial pathogens in waste must be maintained within acceptable levels to make reuse efforts safe[5]. Guidelines for the proper disposal and treatment of human excreta before its application in agriculture to limit the risk of pathogen infection have been proposed[6]. In several countries, the pathogen requirements for different levels of biosolid classifications (A, B and C) are defined as faecal coliforms, *Salmonella* spp., faecal streptococcus, and helminth egg[7,8].

Pathogen inactivation during anaerobic digestion is an integral component for ensuring the safe reuse of the biosolid. However, the application of mesophilic anaerobic digestion would not be enough to meet the required levels of pathogens for unrestricted use on land without further treatment[9]. Therefore, further treatment

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of faecal sludge is needed.

Alkaline pretreatment is a commonly accepted method to improve the biogas production of straw or sludge fermentation owing to its easy operation, simplicity and high efficiency[10]. But there has been a lack of research related to the effects of alkaline pretreatment to inactivate pathogens in faecal sludge. Alkaline function is based on the disruption of sludge flocs, the destruction of cell walls and membranes in several ways including the saponification of lipids, losing natural shapes of proteins, hydrolysis of RNA and the transfer of extracellular and intracellular polymeric substances into the aqueous phase by hydroxyl anions. Hence, alkaline pretreatment may be an appropriate method to inactivate pathogens.

The objective of this study is to determine the effects of total solid (TS) and holding time on the inactivation of pathogen indicators (i.e., faecal coliform, faecal streptococcus, *Salmonella* spp., and helminth eggs) during alkaline pretreatment (pH = 10 and 12). Experimental data were used to compare two models (first-order kinetics and the Weibull model) for the alkaline inactivation of pathogens. The effects of alkaline pretreatment on sludge solubilization during operation were systematically evaluated.

2 Materials and methods

2.1 Sludge sampling and characterization

The raw sludge used as substrate was obtained from one of the septic tanks in the University of Science and Technology Beijing, China. The pre-thickened sludge was stored at 4°C before pretreatment. The characteristics of raw materials are presented in Table 1.

Table 1 Characterization of raw sludge used in this study

Analytical parameters	Raw sludge
TS/%	12
Volatile solids (VS)/%	81.6
pH	7.0
Total chemical oxygen demand (TCOD)/mg·L^{-1}	101 300
Soluble chemical oxygen demand (SCOD)/mg·L^{-1}	49 250
Protein/g·L^{-1}	24.1
Soluble protein/g·L^{-1}	1.56
Faecal coliform/CFU·(g TS)$^{-1}$	2.79×10^{7}
Salmonella spp./CFU·(g TS)$^{-1}$	1.10×10^{7}
Faecal streptococcus/CFU·(g TS)$^{-1}$	1.24×10^{7}
Helminth eggs/Egg·(g TS)$^{-1}$	0

2.2 Alkaline pretreatment

Alkaline pretreatment was performed with initial pH of 10 and 12 to inactivate pathogens. The effects of TS and hold evaluated by obtaining samples at different TS levels (1% to 12%) and holding times (0 to 4 d) were evaluated during the experiments. A total of 200 g of sludge was used as the sample in each experiment, and NaOH solution (4 mol/L) was used to adjust the initial pH of faecal sludge.

2.3 Analytical methods

The following parameters were measured to evaluate pathogen reduction and sludge solubilization. Chromogenic agar technique was implemented to test faecal coliform, *Salmonella* spp., and faecal streptococcus according to the literature[11,12]; helminth egg was measured using direct saline smear method[13]. TS, VS, TCOD, SCOD and volatile fatty acid (VFA) were determined according to the American Public Health Association standard methods[14]. PH was measured with a pH meter (HI 9125N). Protein was determined by micro-bicinchoninic acid protein assay (Pierce, Rockford, USA), which was modified by Lowry et al.[15] utilizing a standard solution of bovine serum albumin. The soluble parameters were analyzed after filtering the sludge sample through a 0.45 μm membrane filter. Each test was reported by the mean of three replicates.

2.4 Data analysis

Pathogen concentration and time data obtained from the inactivation experiments were fitted to the first-order kinetics and the Weibull model as follows.

Pathogen inactivation is exponential with respect to time at a constant temperature (i.e., the first-order kinetics)[16,17]. The model results in the following equation:

$$\log_{10} S(t) = -\frac{t}{D} \quad (t \geq 0) \tag{1}$$

where, $S(t)$ is the survival ratio defined as the number of survivors after exposure time t, $C(t)$ divided by the initial number, C_0; D is the decimal reduction time, i.e., the time required for one log reduction in the number of cells.

An increasing number of studies indicated that microbial survival rate is not always linear. Other proposed models included logistic[18], Fermi equation[19],

the modified Gompertz equation[20] and the Weibull model[21]. The Weibull model has been used successfully to describe the nonlinear inactivation of different microorganisms under various experimental conditions. In terms of survival curve, the cumulative form of the Weibull distribution[16] is:

$$\log_{10} S(t) = -\frac{1}{2.3030}(\frac{t}{a})^{\beta} \quad (2)$$

$$\log_{10} S(t) = -bt^{n} \quad (3)$$

where, α is the scale parameter (characteristic time) and β is the shape parameter as a behavior index; n represents the shape parameter, i.e., $n=\beta$ and $b= (1/2.303)\alpha^{-n}$.

In this study, Origin 9 software was utilized for linear/nonlinear regression analysis and to determine the parameters of the first-order model/Weibull model. The effectiveness of model fit was assessed by the regression coefficient (R^2).

3 Results and discussion

3.1 Pathogen inactivation

Helminth eggs were not detected in raw sludge and during pretreatment. This is because the sludge was obtained from the septic tank of the university, where everyone had taken special medicine to prevent helminth. Furthermore, the climate condition of northern China is not suitable for helminth growth[22].

At different TS concentrations of faecal sludge of 1%, 2%, 4%, 6%, 8%, 10% and 12%, the concentrations of faecal coliform were 3.24×10^6, 6.48×10^6, 1.30×10^7, 1.94×10^7, 2.59×10^7, 2.69×10^7 and 2.79×10^7 CFU/g·TS, respectively. Similarly, the concentrations of faecal streptococcus were 1.30×10^6, 2.60×10^6, 5.20×10^6, 7.80×10^6, 1.04×10^7, 1.14×10^7 and 1.24×10^7 CFU/g·TS, respectively, where the concentrations of *Salmonella* spp. were 1.31×10^6, 2.63×10^6, 5.25×10^6, 7.88×10^6, 1.05×10^7, 1.09×10^7 and 1.10×10^7 CFU/g·TS, respectively. The effects of alkaline pretreatment on the inactivation of pathogens are shown in Figure 1. In the process of alkaline pretreatment, the TS concentrations of faecal sludge did not have a significant effect on pathogens reduction, but had a relationship with the dosage of NaOH solution used to adjust pH of faecal sludge.

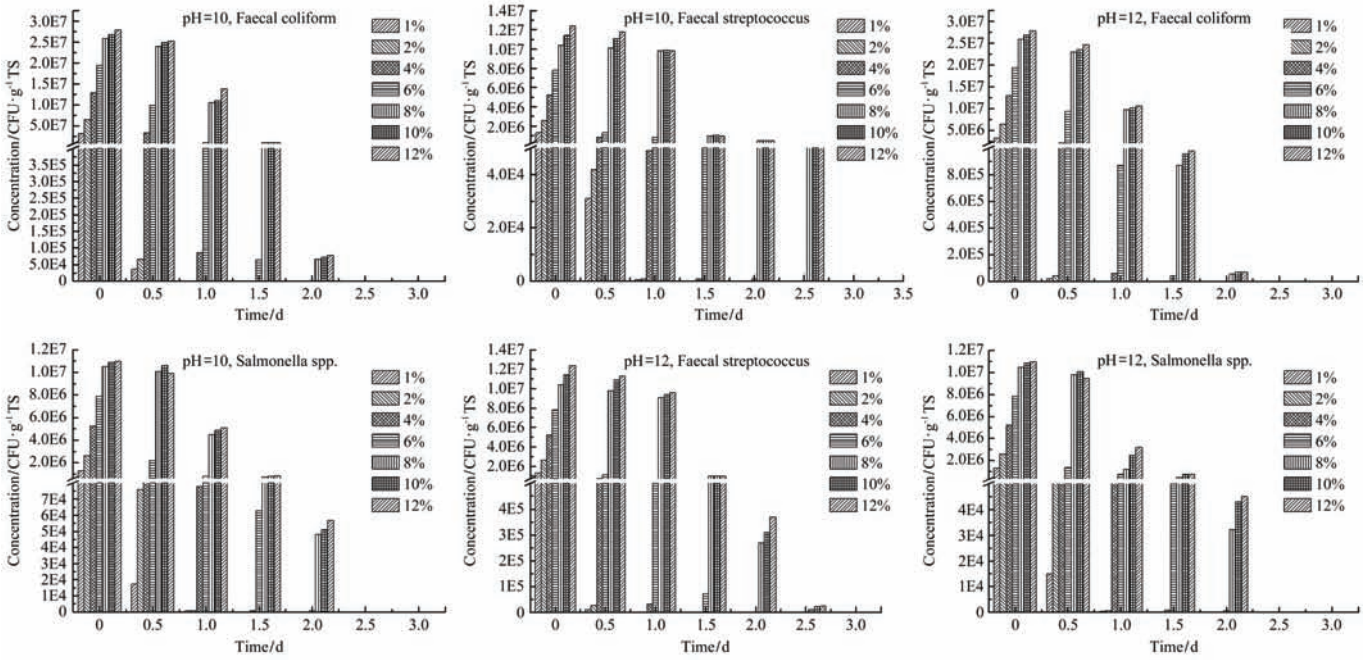

Figure 1 Effect of alkaline pretreatment on different pathogens inactivation

Regardless of the initial pH of 10 or 12, the time for complete inactivation is similar. For faecal coliform, the complete inactivation time was 1.5 d, 1.5 d, 2 d, 2.5 d, 2.5 d, 2.5 d and 2.5 d for faecal sludge with TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. For faecal streptococcus, after 1.5 d, 1.5 d, 2 d, 2.5 d, 3 d, 3 d and 3 d the pathogen was complete inactivated for faecal sludge with TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. And the complete inactivation time of *Salmonella* spp. was 1.5 d, 1.5 d, 2 d, 2.5 d, 2.5 d, 2.5 d and 2.5 d for faecal sludge with TS concentrations of 1%, 2%, 4%, 6%, 8%, 10%

and 12%, respectively. So the complete inactivation of pathogens (i.e., faecal coliform, *Salmonella* spp., faecal streptococcus) time were 1.5 d, 1.5 d, 2 d, 2.5 d, 3 d, 3 d and 3 d for faecal sludge with the TS concentrations of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively.

Alkaline pretreatment has a good performance on pathogens inactivation. On one hand, alkaline could destroy the sludge floc structure and damage the pathogens cell wall, leading to broken pathogens cells. Intracellular substances would then be released into the surrounding environment. On the other hand, alkaline can cause proteins and nucleic acids to hydrolyze, and then the enzyme system and structure of cell are damaged, therefore, the pathogens cell are inactivated[23]. Similar results were obtained by Katsiris and Alexandra[24] who observed that the increase in the sludge pH strengthened the negative charges of the bacteria surface, which causes the surface of bacteria to produce high electrostatic repulsion. This repulsion in turn results in breaking the bacteria cell. In addition, the alkaline can have a saponification reaction with the lipids in the bacteria cell wall, causing further break of the bacteria cell[25].

In this research, the variations of pH of faecal sludge were investigated during the alkaline pretreatment. PH can indirectly express the alkali consumption. As shown in Figure 2, after 4 d of alkaline pretreatment, the pH changed from an initial pH of 10 to 9.20, 9.21, 9.19, 9.18, 9.17, 9.11 and 9.06 for the TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. And for the initial pH was 12, the pH changed to 10.19, 10.18, 10.09, 10.02, 9.92, 9.82 and 9.72 after 4 d of alkaline pretreatment for TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. For the initial pH either 10 or 12, the pH rapidly decreased during the first 2 d of alkaline pretreatment. This was attributed to that most of alkaline was consumed to inactivate the pathogens in the first 2 d, and some of alkaline was consumed to disrupt the sludge flocs and cells structure, and accelerate the sludge hydrolysis. This result was consistent with the previous of this study about alkaline pretreatment to inactivate pathogens.

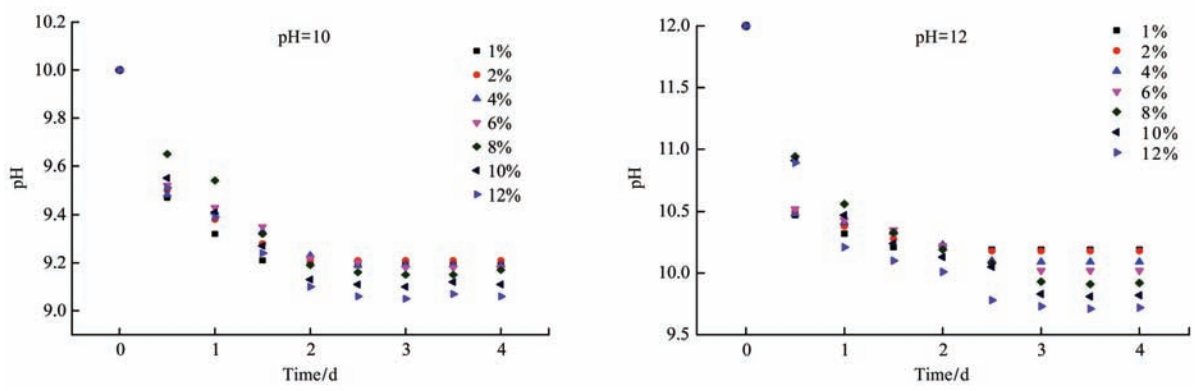

Figure 2 Changes of pH with time for different TS contents

3.2 Kinetics of pathogens inactivation

Pathogen inactivation is shown in the log-linear plots (Figure 3) using the first-order kinetics and the Weibull models. The points of complete inactivation were not considered because zero is meaningless for the Napierian logarithm.

The parameters of Weibull model (b and n), the first-order model (D) and R^2 are shown in Table 2. In this study, the value of R^2 is similar for the same TS group, so the TS content of faecal sludge has no effect on the dynamics analysis of pathogen inactivation. At the initial pH of 10, the average value of R^2 for the first-order was 0.893, 0.898 and 0.886 for faecal coliforms, faecal streptococcus and *Salmonella* spp. respectively, and the average value of R^2 for Weibull was 0.995, 0.976 and 0.991 for faecal coliforms, faecal streptococcus and *Salmonella* spp. respectively. And for the initial pH of 12, the average value of R^2 for the first-order was 0.903, 0.891 and 0.912 for faecal coliforms, faecal streptococcus and *Salmonella* spp. respectively, and the average value of R^2 for Weibull was 0.994, 0.983 and 0.971 for faecal coliforms, faecal streptococcus and *Salmonella* spp. respectively. Therefore, Weibull model exhibited a better fit with data than the first-order kinetics for pathogens inactivation. Similar results were also obtained from the inactivation curves of other pathogens[16].

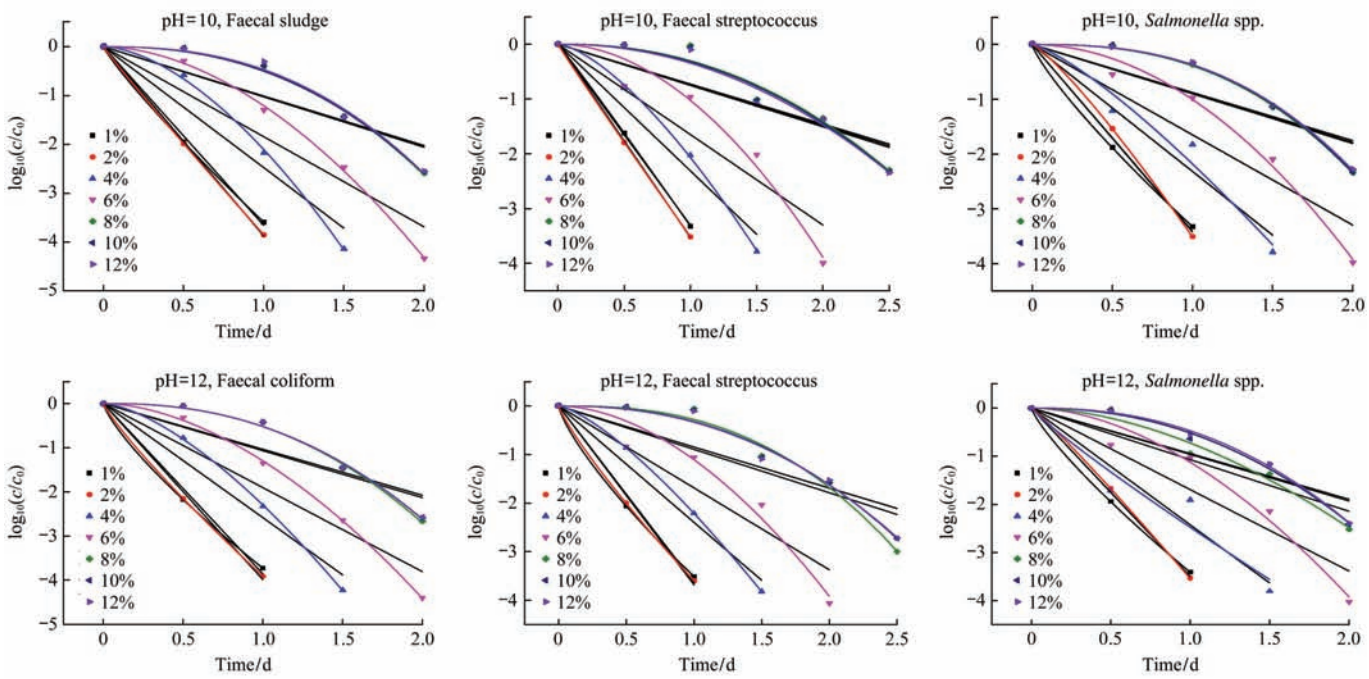

Figure 3 Kinetic curves of pathogenic inactivation fitted with two kinetic models: straight line = first-order kinetics; curves = Weibull.

Table 2 Parameters of the first-order and Weibull models

pH	Pathogens	TS /%	First-order kinetics		Weibull model		
			R^2	D	R^2	b	n
10	Faecal coliforms	1	0.997	0.2733	1	3.5964	0.8802
		2	0.999	0.2580	1	3.8522	0.9564
		4	0.932	0.4033	0.9983	2.1099	1.6747
		6	0.904	0.5421	0.9984	1.2095	1.8361
		8	0.812	0.9711	0.9923	0.4948	2.4061
		10	0.813	0.9747	0.9908	0.5001	2.3812
		12	0.797	0.9881	0.9848	0.4701	2.4676
	Salmonella spp.	1	0.993	0.2929	1	3.3257	0.8210
		2	0.993	0.2920	1	3.5106	1.1904
		4	0.955	0.4309	0.9519	2.1799	1.2769
		6	0.885	0.6066	0.9855	1.0059	1.9622
		8	0.796	1.1075	0.9987	0.38980	2.5907
		10	0.788	1.1183	0.9985	0.37446	2.6440
		12	0.792	1.1371	0.9990	0.37102	2.6292
	Faecal streptococcus	1	0.999	0.3025	1	3.3215	1.0335
		2	0.999	0.2832	1	3.5173	0.9731
		4	0.962	0.4323	0.9991	2.0735	1.4710
		6	0.877	0.6067	0.9561	1.0270	1.9197
		8	0.806	1.3733	0.9567	0.3045	2.2130
		10	0.817	1.3495	0.9615	0.3204	2.1683
		12	0.828	1.3266	0.9582	0.3463	2.0874
12	Faecal coliforms	1	0.989	0.2597	1	3.7263	0.7789
		2	0.996	0.2508	1	3.9082	0.8632
		4	0.957	0.3870	0.9997	2.2956	1.5077
		6	0.915	0.5253	0.9993	1.3003	1.7601
		8	0.818	0.9388	0.9935	0.52174	2.3711
		10	0.824	0.9642	0.9931	0.52051	2.3278
		12	0.822	0.9629	0.9711	0.51994	2.3325
	Salmonella spp.	1	0.992	0.2859	1	3.4021	0.8110
		2	0.999	0.2865	1	3.5270	1.0778
		4	0.925	0.4135	0.8911	2.4895	0.8984
		6	0.897	0.5920	0.9653	1.1216	1.8048
		8	0.892	0.9338	0.9901	0.72560	1.7767
		10	0.846	1.0383	0.9972	0.51519	2.2082
		12	0.834	1.0585	0.9511	0.46970	2.3375
	Faecal streptococcus	1	0.988	0.2751	1	3.51188	0.7689
		2	0.994	0.2715	1	3.60206	0.8464
		4	0.976	0.4177	1	2.21002	1.3524
		6	0.885	0.5929	0.9476	1.10652	1.8247
		8	0.776	1.1181	0.9804	0.28041	2.5835
		10	0.805	1.1815	0.9761	0.32363	2.3285
		12	0.812	1.1830	0.9739	0.33362	2.2858

In the first-order model, D value indicates the time required for one log reduction in the number of cells. At the initial pH of 10, the average value of D was 0.630, 0.811 and 0.712 for faecal coliforms, faecal streptococcus and *Salmonella* spp. respectively. For the initial pH of 12, the average value of D was 0.613, 0.720 and 0.658 for faecal coliforms, faecal streptococcus and *Salmonella* spp., respectively. The results of D indicated that the sequence of alkali resistance from high to low was: faecal streptococcus, *Salmonella* spp., faecal coliforms, and the efficiency of pathogens inactivation was a litter better when the initial pH was higher.

The shape factors (n) of Weibull model indicated that the survival curves of the pathogens fitted with this model

were downward concave (n>1) at the initial pH of 10 and 12 as shown in Figure 3. Although Weibull model is empirical in nature, a link can be established with the microbial inactivation[16]. Downward concavity (n>1) indicates that the remaining members are increasingly damaged, whereas upward concavity (n<1) indicates that the remaining members can adapt to the applied stress[26]. Therefore, the downward concavity (n>1) survival curves of the pathogens fitted with this model can be interpreted as an evidence of a high resistance of the pathogens to alkaline pretreatment. Alkaline pretreatment destroys pathogens at a relatively fast rate, leaving behind survivors with increased resistance[16]. In other words, during alkaline pretreatment, alkali is gradually consumed, alkalinity becomes less and less, the ability of inactivating pathogens using alkalinity becomes weaker and weaker.

In order to better express the relationship between the complete pathogens removal time and the TS content of the faecal sludge, three different types of data regression analysis (i.e., exponential equation, linear equation and logarithmic equation) were used for statistical analysis. To ensure the fitting equation is practical, all the data must be included within the scope of the fitting equation. The fitting results are shown in Figure 4. Two determination criterions were considered for the optimal fitting equation: the effectiveness of the model fit with the higher regression coefficient, all the data is included within the scope of the fitting equation. So the relationship between the harmless treatment time and the TS content of the sludge agrees with the exponential equation ($y = 1.3543e^{10.002x}$, $1\% \le x \le 8\%$) and the logarithmic equation ($y = 3$, $8\% \le x \le 12\%$).

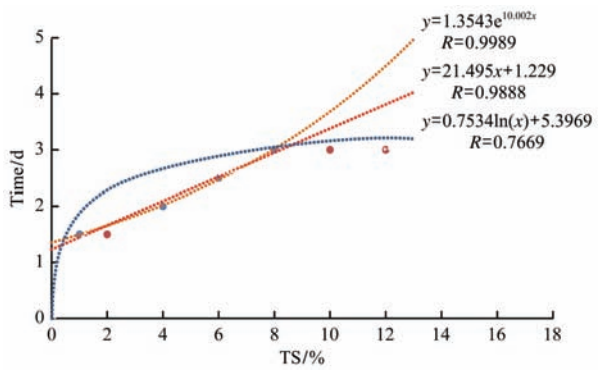

Figure 4 Relationship between alkaline pretreatment time and TS of faecal sludge

3.3 Sludge solubilization

The degree of substrate solubilization was estimated from the amount of SCOD and soluble protein. The SCOD and soluble protein values were higher in pretreated sludge than in raw sludge as shown in Figure 5, confirming the previously reported significant solubilization effects of alkaline on faecal sludge, the pretreatment can disrupt sludge flocs and cells, release inner organic matter, accelerate sludge hydrolysis[27, 28].

For the initial pH of 10, the value of SCOD increased from 3150 mg/L, 3880 mg/L, 1180 mg/L, 14 620 mg/L, 15 150 mg/L, 29 550 mg/L and 39 250 mg/L to 5670 mg/L, 7060 mg/L, 21 500 mg/L, 26 800 mg/L, 28 070 mg/L, 55 960 mg/L and 72 370 mg/L at TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. The values of soluble protein increased from 0.213 g/L, 0.427 g/L, 0.658 g/L, 0.812 g/L, 1.233 g/L, 1.340 g/L and 1.563 g/L to 1.79 g/L, 3.64 g/L, 5.90 g/L, 7.42 g/L, 11.580 g/L, 12.371 g/L and 12.902 g/L at TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. At the initial pH of 12, the values of SCOD increased from 3150 mg/L, 3880 mg/L, 1180 mg/L, 14 620 mg/L, 15 150 mg/L, 29 550 mg/L and 39 250 mg/L to 5710 mg/L, 7120 mg/L, 21 780 mg/L, 27 070 mg/L, 29 120 mg/L, 58 210 mg/L and 74 380 mg/L at TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. The values of soluble protein increased from 0.213 g/L, 0.427 g/L, 0.658 g/L, 0.812 g/L, 1.233 g/L, 1.340 g/L and 1.563 g/L to 1.86 g/L, 3.81 g/L, 6.16 g/L, 7.74 g/L, 12.21 g/L, 12.60 g/L and 13.48 g/L at TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. At similar initial pH, TS has some effects on increasing SCOD and soluble protein in faecal sludge during the alkaline pretreatment. At similar TS in faecal sludge, a higher initial pH led to increases in SCOD and soluble protein in faecal sludge, therefore more alkaline is needed during the pretreatment.

The increased levels of SCOD and soluble protein indicated the release and dissolution of organic substances from faecal sludge. After 2.5 d of alkaline pretreatment, SCOD and soluble protein in faecal sludge increased by about two and nine times, respectively. When the pretreatment duration was extended from 2.5 d

to 4 d, the increase in SCOD and soluble protein was very limited.

Alkaline pretreatment had a more significant effect on protein solubilization than on SCOD. The degradation of sludge during anaerobic digestion caused changes in the physical–chemical properties of EPS, i.e., its intracellular components were released, changing its morphology and increasing its true colloidal content[29,30].

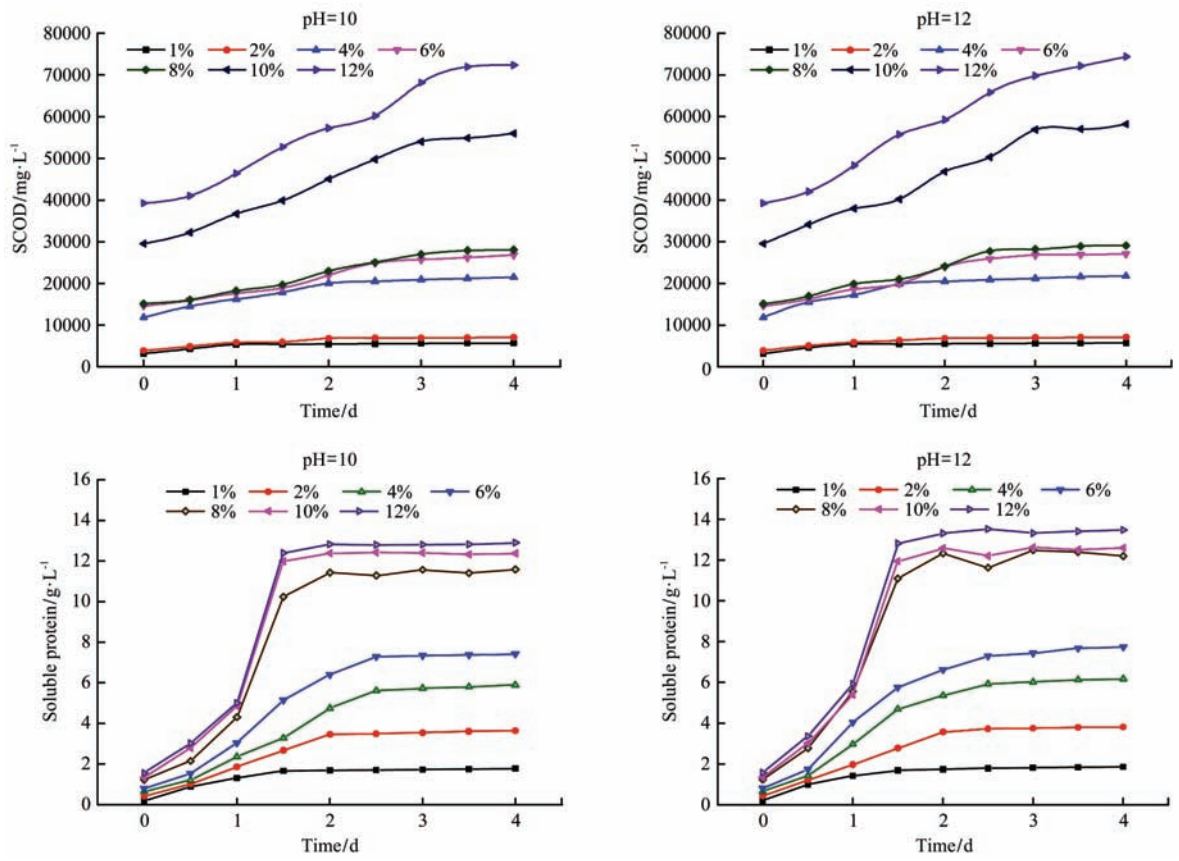

Figure 5 Changes of SCOD and soluble protein contents during alkaline pretreatment

4 Conclusions

The time durations for the complete inactivation of pathogens (i.e., faecal coliform, *Salmonella* spp., faecal streptococcus) were 1.5 d, 1.5 d, 2 d, 2.5 d, 3 d, 3 d and 3 d for faecal sludge TS concentration of 1%, 2%, 4%, 6%, 8%, 10% and 12%, respectively. Weibull model is more flexible than the first-order model.

The relationship between the alkaline pretreatment time and the TS content of the sludge agrees with the exponential equation ($y = 1.3543e^{10.002x}$, $1\% \le x \le 8\%$) and logarithmic equation ($y = 3$, $8\% \le x \le 12\%$).

Alkaline pretreatment can improve sludge solubilization and has a more significant effect on protein solubilization than on SCOD.

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