Waste activated sludge hydrolysis and acidification: a comparison between sodium hydroxide and steel slag addition

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Keywords
addition, sludge, sodium, hydrolysis, hydroxide, steel, slag, acidification, activated, comparison, waste, between

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Waste activated sludge hydrolysis and acidification: A comparison between sodium hydroxide and steel slag addition

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Abstract

Alkaline treatment with steel slag and NaOH addition were investigated at different pH conditions for the fermentation of waste activated sludge. Better performance was achieved in steel slag addition scenarios for both sludge hydrolysis and acidification. More solubilization of organic matters and much higher (volatile fatty acids) VFAs production at a shorter time can be achieved at pH 10 adjusted by steel slag. Higher enzyme activities were also observed in steel slag addition scenarios under same pH conditions. Phosphorus concentration in supernatant increased with fermentation time and pH in NaOH addition scenarios while in contrast most phosphorus were released and captured by steel slag simultaneously in steel slag addition scenarios. These results suggest that steel slag can be used as a substitute for NaOH in sludge alkaline treatment.

Key Words

Waste activated sludge; alkaline treatment; sodium hydroxide; steel slag

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1. Introduction

The number of wastewater treatment plants (WWTPs) worldwide has increased dramatically in recent decades due to rapid urbanization and population growth, resulting in a significant increase of waste activated sludge (WAS) production. Typically, WAS contains a large amount of organics, heavy metals and pathogens, which post great threats to the environment, and its disposal cost can account for 20-60% of capital and operating cost of WWTP (Fang et al., 2014; Su et al., 2013). Therefore, environmental friendly and cost-effective means for sludge disposal are in great need. Anaerobic digestion is one of the most widely used methods for activated sludge stabilization. Worldwide attention has been drawn to this technique due to its capability of simultaneously achieving sludge mass reduction and generating valuable products including methane which is regarded as an important energy source and volatile fatty acids (VFAs) which could be used as carbon sources to enhance biological nutrient removal in WWTPs (Chen et al., 2007; Su et al., 2013). However, the applications of anaerobic digestion were often featured by long retention time (20-50 days), large digester volumes and low overall degradation efficiency (20-50%) (Kim et al., 2010). Among the well-known three steps involved in anaerobic digestion, the hydrolysis step has been identified as the rate-limiting step (Higgins and Novak, 1997). And the major reasons for the less digestibility are the microbial cell walls containing glycan strands cross-linked by peptide chains and the existence of a large amount of extracellular polymeric substances (EPS) (Devlin et al., 2011). In order to improve the hydrolysis
efficiency and digestibility of sludge, various pretreatment methods also referred as
sludge disintegration techniques have been investigated, including chemical treatment
using alkaline, acid or ozone(Devlin et al., 2011; Li et al., 2012; Zhang et al., 2009),
mechanical treatment with ultrasound, high pressure homogenizer or ball milling(Apul
and Sanin, 2010; Wett et al., 2010; Zhang et al., 2012), thermal treatment(Xue et al.,
2015), additional enzymes treatment(Yang et al., 2010) and some of their
combinations(Kim et al., 2010; Shehu et al., 2012). These pretreatment can normally
destruct sludge flocs and cell walls, release intracellular and extracellular organic
matters, and consequently accelerate sludge hydrolysis.
Alkaline treatment is a widely examined sludge disintegration method which is believed
to be very effective in terms of solubilizing cellular substances and EPS (Fang et al.,
2014; Li et al., 2008). Compared to other methods, alkaline treatment holds the
advantages of simple devices, easy operation and lower energy demand. In addition,
alkaline conditions are particularly favourable to the production of short-chain fatty
acids (SCFAs) or VFAs which are suitable carbon sources for biological nutrient
removal processes and phosphorus recovery with MAP (magnesium ammonium
phosphate hexahydrate) or HAP(calcium phosphates)(Tong and Chen, 2007). NaOH
was the most commonly used chemical for alkaline treatment and has been found to be
more effective in terms of sludge disintegration than lime (Ca(OH)₂)(Li et al., 2008).
However, the consumption of NaOH involves a higher chemical cost and thus will
increase sludge treatment expense. New alternative chemicals with low cost must be
further explored.

Steel slag is a porous non-metallic by-product produced in steelmaking industry, the amount of which can be equivalent to 10-20% of crude steel output. Every year there would be around 100 million tons of steel slag generated in China, while only 50-60% of them can be resourcelized in proper ways, leaving a large amount of slag piled in the steel plant, which poses a great threat to the groundwater quality. Although the chemical composition of steel slag varies with different slag production processes, normally it consists of CaO, SiO₂, FeO, MgO, Al₂O₃,MnO,P₂O₅,etc.(Shi, 2004) Steel slag has been used for treating acid mine drainage since its alkaline compounds such as lime and magnesia can leach out, creating high levels of alkalinity(Simmons et al., 2002). Meanwhile, steel slag was found to be a very effective material for phosphorus removal in wastewater as a result of phosphorus adsorption onto metal oxides/oxyhydroxides throughout the porous slag matrix and on slag surface, and precipitation in forms of metal-phosphates such as Fe-phosphates and Ca-phosphates(Pratt et al., 2007). In literature, however, the enhancement of sludge hydrolysis and acidification by steel slag has never been studied although the capability of creating alkalinity and phosphorus capture make it a potential chemical for sludge alkaline treatment.

Therefore, the purpose of this study was to investigate the feasibility of steel slag addition as a substitute of NaOH for sludge hydrolysis and acidification enhancement and phosphorus recovery. As pH is a key parameter in the case of alkaline or acid treatment, the effect of steel slag addition for different pHs conditions on WAS
hydrolysis and acidification was studied in comparison with NaOH addition scenarios.

2. Materials and Methods

2.1 Sludge and Steel Slag

The waste activated sludge used in this study was collected from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai, China. The plant is operated with an EBPR (enhanced biological phosphorus removal) process. The sludge was concentrated by settling at 4 °C for 12 h and was measured after that (Table 1).

The steel slag used in this work was obtained from Baoshan Iron & Steel Co. Ltd in Shanghai, China. The Steel Slag was crushed and sifted through a 50-meshsieve before being added into the WAS. Elemental composition analysis with EDS (Energy Dispersive Spectroscopy) showed the elemental composition (expressed as atomic percentage) of the steel slag to be O 33.7%, Fe 22.4%, C 15.6%, Ca 10.9%, Mg 6.5%, Mn 3.2%, Au 2.4%, Si 2.3%, etc.

2.2 Experimental Procedure

Nine 1L glass vessels with working volume of 0.9 L were utilized as the anaerobic fermentation reactors. 0.9L of WAS were fed into each reactor. The pH in reactor 1-4 was controlled at 8.0, 9.0, 10.0 and 11.0 with steel slag and the same pH in reactor 5-8 with sodium hydroxide. The reactor 9, in which the pH was not adjusted, was set as the blank test. After 5 min nitrogen blowing, all reactors were placed in a shaking incubator maintained at the temperature of 25 ± 1 °C with a shaking speed of 100 revolutions per
minute (rpm). Samples of the mixed liquor were taken at intervals. Supernatant samples were obtained after 3min settling of the mixed liquor.

2.3 Analytical Method

Water content, TS (total solids), VS (volatile solids), and COD (chemical oxygen demand) were determined according to the standard methods (APHA et al., 2012). Sludge pH was measured with a pH meter (EUTECH Cyberscan510, Singapore). PO$_4^{3-}$-P and total phosphorus (TP) of the supernatant were also analyzed according to the standard methods (APHA et al., 2012). TP in steel slag was determined by Capacity Method of Ammonium Phosphomolybdate (Song, 2007), while TP in the sludge was measured by Vanadium Molybdate Yellow Colorimetric Method after Microwave Digestion (Wen et al., 2003). Soluble carbohydrates were determined using phenol-sulfuric acid method (Miller, 1959). Soluble protein was determined by the Lowry–Folin method with BSA (Bovin Serum Albumin) as standard (Lowry et al., 1951).

Total organic Carbon (TOC) was determined using a TOC-VCPN Analyzer (Shimadzu, Tokyo). An Agilent 6890N GC with flame ionization detector equipped with a 30m × 0.32mm × 0.25μm CPWAX52CB column was utilized to analyze the composition of VFAs. Analytical Method used in the analysis was developed according to (Chen et al., 2007). The microstructures and elemental composition of the steel slag were examined by scanning electron microscopy equipped with energy dispersive X-ray spectroscopy.
Enzyme activities of four hydrolytic enzymes including protease, α-glucosidase, alkaline and acid phosphatase were measured as per (Nybroe et al., 1992). Activities of five enzymes involved in acid fermentation were examined. The measurement of acetate kinase (AK) activity and butyrate kinase (BK) activity were carried out as per (Allen et al., 1964); while phosphate transacetylase (PTA) and phosphate transbutyrylase (PTB) were carried out as per (Andersch et al., 1983); oxaloacetate transcarboxylase (OAATC) activity was carried out as per (Wood et al., 1969).

3. Results and Discussion

3.1 Sludge disintegration

Alkaline disintegration of WAS accelerates hydrolysis by disruption of flocs and sludge cells which leads to the release and solubilization of extracellular and intracellular organic matters (Doğan and Sanin, 2009; Fang et al., 2014; Li et al., 2008). Thus, the effect of steel slag and NaOH addition on sludge integration can be expressed by the change of STOC (soluble total organic carbon) which is determined by measuring TOC of supernatant. **Figure 1** shows the effect of steel slag and NaOH addition on STOC during WAS fermentation within 20 days at different pHs. It was found that the concentration of STOC under both NaOH and steel slag adjusted conditions (Abbreviations were used in following. NAC, for NaOH adjusted conditions; SSAC for steel slag adjusted conditions) increased gradually with the fermentation time. In the selected range of pH, more solubilization of organics was observed at higher pH,
coincident with previous studies (Chen et al., 2007; Doğan and Sanin, 2009) in which SCOD was used as an indicator. It is worth noting that at higher pHs (pH 10 and 11) SSAC had a significant higher value of STOC than that of NAC after 10d fermentation. The STOC was 393 and 963mg/L higher in fermentation reactors with SSAC at fermentation time 20d for pH 10 and pH 11, respectively, indicating that steel slag may be better than NaOH in the enhancement of sludge disintegration.

3.2 Protein and carbohydrate solubilization

It has been reported that protein and carbohydrate are the predominant constituents of extracellular polymeric substances (EPS) in WAS (Chen et al., 2007; Yan et al., 2010). In addition, alkaline treatment is believed to be very effective in solubilizing EPS (Chen et al., 2007; Fang et al., 2014). Thus, the solubilization of organic matters was expressed in terms of soluble protein and carbohydrate. As shown in Fig.2, the concentration of soluble protein and carbohydrate for both NAC and SSAC reactors increased with pH, which is in accordance with the change of STOC. Besides, the concentration at alkaline pH (pH=9, 10, 11) was much higher than that at near neutral pH (blank and pH=8). This is probably because the alkaline pH resulted in the dissociation of acidic groups in EPS and repulsions between the negatively charged EPS (Wingender et al., 1999). In agreement with literature (Chen et al., 2007; Su et al., 2013), the concentrations of soluble protein and carbohydrate fluctuated slightly with the increase of fermentation time in both NAC and SSAC reactors due to the competition between solubilization and degradation. Especially for carbohydrate, its concentration
showed an obvious decline after 10d, whereas the soluble protein concentration tended to remain constant after 12d. The rate of carbohydrate concentration decline was lower at pH 11 than other scenarios in both NAC and SSAC reactors, which indicated that the degradation rate of carbohydrate might be reduced under strongly basic condition. The carbohydrate concentration in SSAC was slightly higher than that of NAC. However, a remarkable difference was observed between the protein concentration of SSAC and NAC with the highest concentration 2300mg/L and 1500mg/L, respectively, further demonstrating that steel slag has an advantage over NaOH in terms of sludge solubilization, which might result from the concomitant mechanical disruption of microbial cells due to the existence of insoluble steel slag particulates.

3.3 VFAs production and composition

Total VFAs production from WAS was defined as the sum of the concentrations of acetic, propionic, n-butyrlic, isobutyric, n-valeric and isovaleric acids(Su et al., 2013). The total VFAs production at different pHs adjusted by NaOH and steel slag is shown in Fig.3A and Fig.3B. In both NAC and SSAC fermentation reactors, VFAs concentration increased with fermentation time gradually, and then decreased after reaching the highest around 6–9 d at all pHs except for pH 11. At pH 11, VFAs concentration kept rising during fermentation time of 20d, which was in accordance with (Chen et al., 2007), where the initial less VFAs production was explained as ineffectiveness of acidogenic bacteria due to toxic effects of the strongly basic condition and the subsequent significant increase showed the adaptation of bacteria to the
environment. The greatest VFAs production was observed at pH 10, which was 3431mg/L and 3981mg/L for NAC and SSAC reactors, respectively. Not only did SSAC have a higher VFAs production, but also it took a shorter time (6d versus 8d) to reach the highest. As is well known, three stages are included in sludge anaerobic fermentation: hydrolysis, acidification and methane generation. The better performance of steel slag addition in VFAs production could be caused by either more soluble substrate for acidification due to higher hydrolysis rate or inhibition of methanogens activities (Yuan et al., 2006). It has been demonstrated that more soluble protein and carbohydrate were available in SSAC fermentation reactors, while methanogens activities has not been examined in this study, which should be further investigated in future work.

**Figure 3C** and **Figure 3D** shows the percentage of individual VFAs accounting for total VFAs at different pHs when total VFAs was at the highest level. All six common short-chain fatty acids in WAS (acetic, propionic, n-butyric, isobutyric, n-valeric and isovaleric acids) were detected. In agreement with literature (Chen et al., 2007; Su et al., 2013), acetic acid was the prevalent acid and its proportion tended to be greater at higher pH, probably owing to the inhibition effect of stronger alkaline conditions on methanogens and the subsequent less consumption of acetic acid. The order of individual VFAs was acetic > propionic > (isovaleric, isobutyric) > (n-butyric, n-valeric). No obvious difference was discovered between NAC and SSAC in terms of VFAs composition.
3.4 PO$_4^{3-}$ release

Figure 4 shows the variation of orthophosphate concentration at different pHs in NAC and SSAC fermentation reactors. In NAC reactor, it can be seen that orthophosphate concentration increased gradually with fermentation time as well as pHs. Highest concentration observed at pH 11 with 20d was 250mg/L, which could be explained that stronger alkaline conditions were more effective in cell lysis and more nucleic acids were released and hydrolyzed. In addition, further disruption of sludge cells at pH 11 and above 11 may lead to disruption of cell membranes composed of phospholipids bilayer and thus more phosphorus will be released (Bi et al., 2014). However, in SSAC reactors, almost no orthophosphate was detected after 1 d fermentation. Two possible reasons for this phenomenon are: the addition of steel slag significantly inhibited sludge phosphorus release; or the orthophosphate released into supernatant was captured by steel slag particles. In order to further explore the reason, the distribution of total phosphorus was examined (Fig.5). The proportion of TP in sludge declined along with fermentation time in both NAC and SSAC reactors, while the majority of TP migrated into steel slag rather than supernatant in SSAC. Also, SEM-EDX analysis was conducted to investigate morphology and elemental composition of steel slag before and after the fermentation. Although no obvious morphology difference was found between the SEM images, an apparent phosphorus percentage change in the elemental composition of steel slag surface was observed (Table 2), further demonstrating that a large amount of phosphorus was transferred to the surface of steel slag, which could be
caused by either adsorption or precipitation as mentioned above in introduction. After fermentation of 20 days at pH 11, 91.6% TP was released and mitigated to steel slag, whereas 85.0% TP was observed in the supernatant of NAC. Therefore, by steel slag addition the release of phosphorus in sludge was enhanced and simultaneously removed effectively from supernatant.

3.5 Enzyme activities

Major constituents of WAS such as protein and carbohydrate are all biological macromolecules. Their degradation are heavily dependent on microorganisms and enzymes. Thus, the activities of key enzymes for hydrolysis and VFAs production were examined to further understand the effect of NaOH and steel slag addition on WAS fermentation. Four hydrolytic enzymes (protease, α-glucosidase, alkaline phosphatase and acid phosphatase) and five acid-forming enzymes (AK, BK, PTA, PTB and OAATC) were selected for this study. Usually, the protease breaks the peptide bonds in protein molecules, while α-glucosidase breaks the linkage in maltose to release glucose. Phosphatase hydrolyzes phosphate esters and releases the phosphate group, and acid/alkaline phosphatase differs in pH optimum for maximum activity as well as the reaction mechanisms on different substrates (Goel et al., 1998). Figure 6A~6D shows the variation of hydrolytic enzyme activities at different pHs in NAC and SSAC. It was obvious that alkaline treatment had a positive effect on hydrolytic enzyme activities, probably because of more substrate available under alkaline conditions. The highest
enzyme activities of protease, α-glucosidase, alkaline phosphatase and acid phosphatase observed were in the fifth day of pH 10 with the value of 4.93, 110.6, 83.0, 16.2 U/g VSS for NAC, and 5.88, 119.8, 90.5, 17.6 U/g VSS for SSAC. Although pH 10 may not be the optimal pH for these enzymes, this result indicates that in this process the enhancement of enzyme activity by extra amount of substrates can outweigh the negative effect of non-optimal pH. Hydrolytic enzymes in SSAC showed higher activities than that of NAC, which may result from its better performance in sludge solubilization and could contribute to more phosphorus release and VFAs production. It is noted that at pH 11 longer time was required to reach the highest enzyme activities than any other pHs and the activities were lower than that of pH 10 even with a higher organic matter solubilization at pH 11, which suggested that pH higher than 10 had a stronger inhibitory effect on enzyme activities, in accordance with the less effectiveness of pH 11 at VFAs production at the initial days of fermentation. In all scenarios, the enzyme activities decreased gradually after reaching the highest values possibly because the accumulation of products inhibited the enzymatic reaction.

In the literature, Feng et al. (2009) proposed a metabolic pathway for VFAs production from protein and carbohydrate in which AK, BK, PTA, PTB and OAATC were identified as the key enzymes to transform the hydrolytic products of protein and carbohydrate into VFAs. Fig.6E–6I shows the variation of acid-forming enzyme activities at different pHs in NAC and SSAC. The highest activities of AK, BK, PTA, PTB and OAATC were observed at pH 10, 6d and 8d for NAC and SSAC, respectively,
which was in good agreement with the highest total VFAs production mentioned above.

And the activities of key enzymes in SSAC were slightly higher (5.88, 4.30, 0.21, 0.0032, 1.41 U/g VSS) than those in NAC (4.93, 4.19, 0.18, 0.0030, 1.30 U/g VSS).

4. Conclusions
Alkaline treatment with steel slag addition showed a better performance than NaOH in the solubilization of organic matters and VFAs production. Higher enzyme activities were also observed in SSAC than NAC under same pH condition. Orthophosphate concentration in supernatant increased with fermentation time and pH in NAC while in contrast most orthophosphate released in SSAC were mitigated to steel slag. These results demonstrated that steel slag can be used as a substitute for NaOH in sludge alkaline treatment. Moreover, the steel slags which successful captured a large amount of phosphorus could be further used as efficient agriculture fertilizers.

Acknowledgements
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Table 1 Characteristics of sludge

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Table 2 Elemental composition of steel slag before and after fermentation

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List of Figures

Fig. 1 Variations of Organic matters solubilization at different pHs adjusted by NaOH (A) and steel slag (B)
Fig. 2 Variations of protein and carbohydrate concentration in supernatant at different
**pHs adjusted by NaOH and steel slag** (A and B refer to the variation of protein concentration for NAC and SAC; C and D refer to the variation of carbohydrate concentration for NAC and SSAC, respectively.)

**Fig. 3** Variations of total VFAs concentration and VFAs composition in supernatant at
different pHs adjusted by NaOH and steel slag (A and B show the variation of total VFAs concentration for NAC and SAC; C and D show VFAs composition at highest total VFAs level for NAC and SSAC, respectively.)

Fig. 4 Variations of PO$_4^{3-}$-P concentration in supernatant at different pHs adjusted by NaOH (A) and steel slag (B)
Fig. 5 Variations of TP (total phosphorus) distribution at different pHs adjusted by NaOH (A) and steel slag (B) (In these columns, B refers to the blank test, and 8, 9, 10, 11 refer to the scenarios under these pH conditions)
Fig. 6 Variations of hydrolytic and acid-forming enzyme activities at different pHs (B refers to the blank test, and 8, 9, 10, 11 refer to the scenarios under these pH conditions adjusted by NaOH, while 8s, 9s, 10s, 11s refer to the scenarios under these pH conditions adjusted by steel slag)