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Investigation of factors influencing the hydrolysis of brewery waste activated sludge

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ABSTRACT

In this study, inoculated sludge was used to enhance the hydrolysis of brewery waste activated sludge and determine the effects of pH and stirring intensity. The production of volatile fatty acids (VFAs) and release of total phosphorus (TP) and ammonia nitrogen (NH_4^+-N) were measured everyday during the experimental process. The maximum production of VFAs was 947 mg/L with inoculated sludge dosage of 40%, the released TP was 42 mg/L, and the released NH_4^+-N was 257 mg/L. The production of VFAs in the alkaline conditions was more than that in the acidic conditions, and the largest VFAs concentration obtained was 903 mg/L at pH 10 on the 6th day. The optimal stirring intensity for the production of VFAs and the release of TP and NH_4^+-N was 60 rpm. Experimental results show that dosing with seed sludge promoted brewery sludge hydrolysis, which is beneficial for the anaerobic digestion of sludge in the next sludge treatment step.

Keywords: Hydrolysis; Volatile fatty acids (VFAs); Total phosphorus (TP); Ammonia nitrogen (NH_4^+-N)

1. Introduction

Brewery industry developed rapidly in China. With the number of factories and yearly production continuously increasing, large numbers of brewery wastewater treatment stations were constructed, and the scale was broadened in recent years. Most of the brewery wastewater stations use activated sludge as their primary biological process, which generate high volumes of waste activated sludge [1–3]. The large number of brewery waste activated sludge produced results in more strict effluent quality regulations. The conventional disposal of the brewery waste activated sludge includes land-filling, land-application, and incineration [4], all of which cause secondary environmental pollution problems. Moreover, brewery waste activated sludge contains more carbon and less nitrogen and phosphorus than sewage sludge [5–9]. Therefore, developing the most sustainable and environment-friendly disposal option has become a research focus.

Anaerobic digestion is commonly used in sludge treatment [10–12]. This process not only reduces the amount of waste activated sludge, but it also increases the proportion of recycled materials. The pathways in anaerobic digestion are shown in Fig. 1. During the anaerobic digestion of sludge, organics are degraded by microorganisms into methane, which could be

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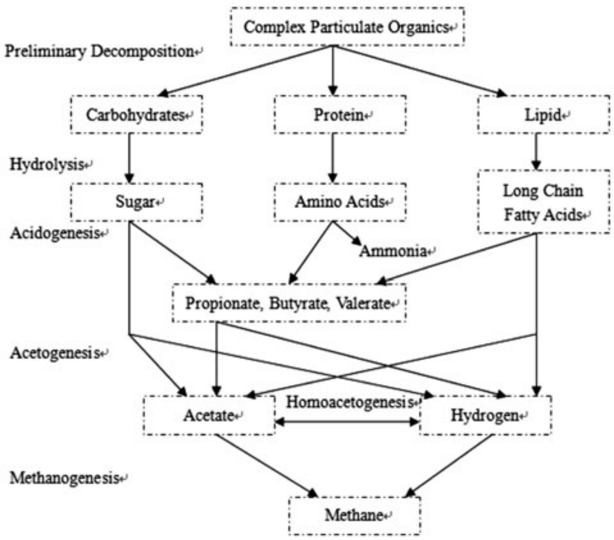

Fig. 1. Degradation pathways of complex particulate organics in anaerobic digestion [20].

applied as energy to replace fossil fuels and reduce greenhouse effect. This is an eco-friendly and energy-saving method of utilizing waste sludge. Digested sludge is also used in agricultural, construction and ceramic materials, activated carbon, and electricity generation after incineration [13–18]. The supernatant of hydrolysis is also used as the carbon source in wastewater treatment [19].

Anaerobic digestion can be divided into stages, and the hydrolysis stage in this study refers to the primary decomposition, hydrolysis, and acidogenesis process as shown in Fig. 1. The hydrolysis stage is the pivotal and limiting step for anaerobic digestion [21,22]. It degrades refractory macromolecule substrates into compounds ready to be used by microorganisms. The hydrolysis step breaks the sludge flocs, disrupts the cell wall, and degrades extracellular polymeric substances. In previous studies, the methane production was affected by the condition of hydrolysis. Lim and Wang found that microaeration pretreatment enhanced hydrolytic activities and resulted in 21 and 10% higher methane yield [23]. Alkali pretreatment improved the rate of hydrolysis by changing the physiochemical characteristics of asparagus stem and resulting in the highest production of methane, which was 38.4% higher than the control [24]. However, the opposite research was also observed, such as Zhen et al. proposed that the increased hydrolysis rate had insignificant effect on methane potential; the production of methane may have been equally influenced by the type and dose of chemical applied, as well as the composition of soluble organics released after pretreatment [25].

The factors that significantly affect waste sludge hydrolysis include temperature, pH, C/N ratio, hydraulic retention time, and pretreatment [26–29]. High hydrogen partial pressure affects hydrolytic activity and reduces overall substrate degradation [30]. The concentration of ammonia nitrogen (NH_4^+-N) is also effected by the hydrolysis process [31]. Gen et al. presented an Anaerobic Digestion Model No. 1 (ADM1)-based anaerobic co-digestion model which proposed that different mixes of residues can influence hydrolysis in anaerobic digestion [32].

The quantity and composition of sludge [33], disintegration technology [34], and operational conditions in the reactor [35,36] have been extensively investigated, but studies on the influence of inoculated sludge in hydrolysis are few. Dosing inoculated sludge in original sludge can increase the number of hydrolytic acidification bacteria and improve the acid production of the waste activated sludge [37,38]. In this study, the dosage of inoculated sludge, pH value, and stirring intensity were investigated to determine their effects on brewery waste activated sludge hydrolysis. First, the concentration and type of volatile fatty acids (VFAs) were measured to characterize the acid product in hydrolysis. Secondly, the amount of nitrogen and phosphorus released in hydrolysis were surveyed in this work to lay the foundation for the supernatant to be used as external carbon source in future.

2. Materials and methods

2.1. Sludge sampling

The sludge was collected from the secondary sedimentation tank in the brewery wastewater treatment station in Nanjing, China. The biochemical technology of the wastewater treatment station involved A^2/O process. Anaerobic procedures utilized internal circulation anaerobic reactor (IC). The activated sludge was applied in the aerobic procedure. The samples were stored for no more than 48 h in a refrigerator at 4℃ before experimentation. The characteristics of the sampling sludge are shown in Table 1. The brewery waste activated sludge contained approximately 12,800 mg/L of total suspended solids (TSS), 14,000 mg/L of total chemical oxygen demand (TCOD), and 1,086 mg/L of soluble chemical oxygen demand (SCOD). The VFAs content was 8.3 mg/L, and the total phosphorus (TP) concentration of the raw sludge was 4.78 mg/L.

2.2. Inoculated sludge

The inoculated sludge used was the sludge sample that had settled for 24 h and the supernatant was

Table 1
Characteristics of brewery waste activated sludge

Item	Average	Deviation
pH	7.43	0.02
TSS (mg/L)	12,808	324
VSS (mg/L)	9,893	187
TCOD (mg/L)	14,017	127
SCOD (mg/L)	1,086	21
TP (mg/L)	4.78	0.21
VFAs (mg/L)	8.3	0.38

removed. The settled sludge was added into the anaerobic reactor and fed with synthetic solution. The synthetic solution contained glucose, starch, potassium dihydrogen phosphate, and urea [39,40]. Ultimately, the COD concentration of the synthetic solution was approximately 4,000 mg/L. Total nitrogen was about 55 mg/L, and TP was 12 mg/L. After acclimating for 120 d, the pH of the supernatant was reduced to 4, and the VFAs content reached 800 mg/L. The sludge was extremely capable of producing VFAs at this moment. Inoculated sludge and raw sludge were mixed in different proportions and then the combined sludge was applied for studying.

2.3. The hydrolysis process of combined sludge

The beakers with 1 L capacity were used as the hydrolysis reactors in this research. Five different dosage radios of domesticated sludge (0, 20, 40, 60, and 80%) were used. To evaluate the effect of pH on sludge hydrolysis, the initial pH of combined sludge was set up to 5.0, 7.0, 8.0, 9.0, and 10.0, by adding 2 mol/L HCl and 2 mol/L NaOH. The agitation by magnetic stirring was feasible in this study. Various stirring intensities (0, 60, and 120 rpm) were applied to demonstrate its influence. All experiments were carried out at an ambient temperature (25˚C). The hydrolysis reactor was run for 15 d and sampled every day. Sludge samples were centrifuged for 15 min at 6,000 rpm, and the supernatants were further filtered through 0.45-μm pore-sized membrane disks. The concentration of VFAs, TP, and NH_4^+-N of the supernatant was measured.

2.4. Analytical methods

The majority of brewery waste activated sludge characteristics were analyzed by Standard Methods (APHA, 1998), and the closed reflux method was employed for COD [41]. For the analysis of TP, samples were digested using the alkaline persulfate digestion method [42], whereas Nessler's reagent spectrophotometry was applied to determine the concentration of NH_4^+-N [43]. VFAs were measured by gas chromatography (Agilent Technology, US).

3. Results and discussions

3.1. Effect of dosing ratio of inoculated sludge on hydrolysis

The color of the inoculated sludge was gray, whereas the raw sludge was gray black. The color of the combined sludge was lightened with the increased dose of the inoculated sludge. Furthermore, the lower the dosage of the seed sludge, the higher was the possibility for aerogenesis to occur. This indicated that the combined sludge contained methanogenic bacteria, but the high number of seed sludge inhibited its activity.

The addition of inoculated sludge increased the production of VFAs (Fig. 2(a)) and the release of NH_4^+-N (Fig. 2(c)), but the TP (Fig. 2(b)) was reduced in contrast. This may indicate that the seed sludge was beneficial in the production of VFAs and NH_4^+-N. The VFAs produced in raw sludge were under 100 mg/L for the first eight days, and the maximum output was 350 mg/L on the 11th day followed by a sharp decline. These appearances could have been due to the combined effect of hydrolytic and methanogenic bacteria. The high dosage ratio favored the rapid production of VFAs, and the external environment inhibited the hydrolytic bacteria, but the accumulation of VFAs was promoted to be applied by methanogenic bacteria. After the addition of seed sludge, the highest production of VFAs (947 mg/L) occurred on the 4th day at the dosing radio of 40%. The concentration of VFAs was not consistent with the increased dosing radio; when the dosing radio was 40%, the overall production of VFAs was three times higher than the others. The production of VFAs was affected by the number of microorganisms and concentration of organics. The inoculated sludge influenced the amount of microbes and determined the rate of hydrolysis. Moreover, the organic content was dependent on the raw sludge, also related to the output of VFAs. When the inoculated sludge dosage was appropriate, the hydrolysis started efficiently and ensured the VFA production (Fig. 2).

The concentration of TP was increased rapidly in the first six days, after which concentration remained constant. This result demonstrated that the content of the released phosphorus was in accordance with the production of VFAs, which provided the energy for phosphorus accumulating organisms (PAOs) to release phosphorus together with glycogen in cells. The quantity of released NH_4^+-N was linear with the hydrolysis time. As shown in Table 2, the initial NH_4^+-N concentration increased along with dosage, which demonstrated that the inoculated sludge contained NH_4^+-N. However, the rate of hydrolysis was reduced when the dosage was increased. Increasing the dosage of seed sludge may have reduced the protein content in the combined sludge, which in turn reduced the hydrolysis rate of protein reduced (Fig. 2).

The dosage of the seed sludge tripled the production of VFAs, reduced half of the released TP, and doubled the released NH_4^+-N. As a consequence, the added seed sludge favored sludge hydrolysis. Considering the concentration of VFAs, TP, and NH_4^+-N comprehensively, the optimal ratio of dosing was 40%.

3.2. Effect of pH on hydrolysis of combined sludge

Based on previous studies, the inoculated sludge dosage radio of 40% was employed in the following experiment. To investigate the effect of pH, a series experiments were carried out in acidic, neutral, and alkaline conditions (Fig. 3). The production of VFAs was higher in the alkaline environment, and VFAs content increased along with pH; when the pH was 10, the production of VFAs was four times than that at pH 5. This result was different from the previous study of Maspolim et al., who considered the optimal VFAs accumulation was at pH 8 [44]. The production of VFAs was higher in alkaline than acidic conditions, which may have been caused by the following reasons. First, the alkaline condition was conductive for the degradation of particulate organic matter into soluble organic matter, which was basis of acid production process. Second, the alkaline condition possibly reduced the activity of methanogen and accumulated more VFAs than the acidic condition. Moreover, previous research indicated that the accumulation of short-chain fatty acids followed first-order kinetics in waste activated sludge hydrolysis at 10–35℃ [45]. The low pH was suitable for methanogenic bacteria, so the concentration of VFAs decreased rapidly. The VFAs produced in acidic, neutral, and alkaline conditions were also measured (Fig. 4). Acetic, propionic, butyric, isobutyric, and isovaleric acid were maintained at a pH of 10. The alcohol was the major product in the acidic condition, whereas acetic acid was the main product in the alkaline condition. This result was similar to the research of Cysneiros et al. who stated that low pH inhibited the production of acetic acid [46]. This demonstrated that controlling the initial pH ensured the types of hydrolyzates.

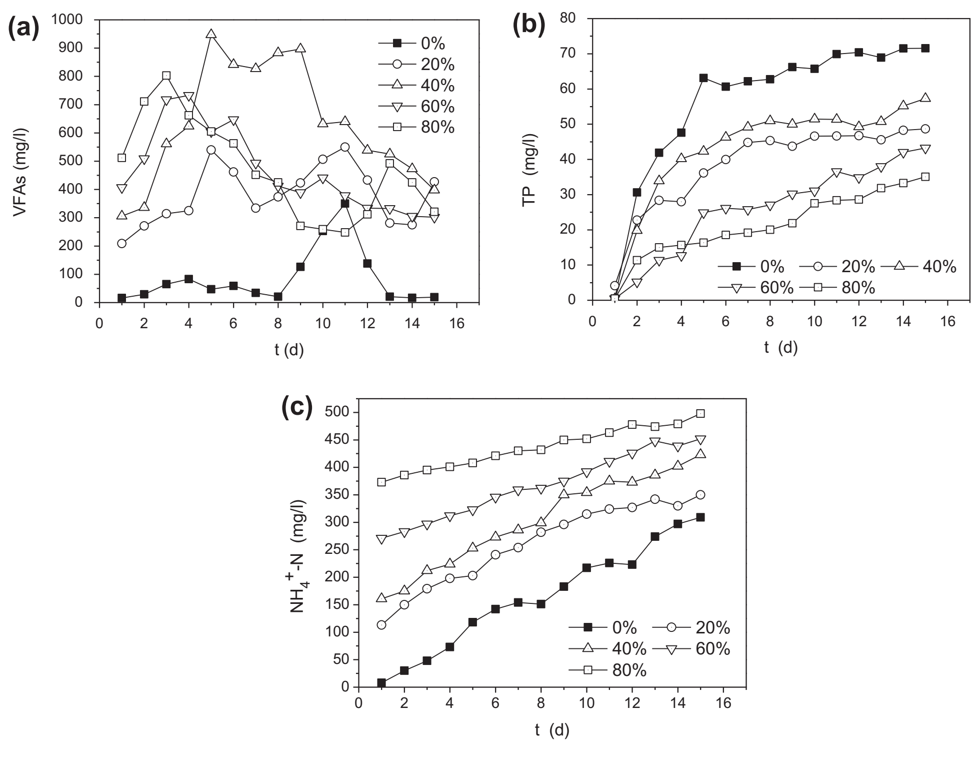

Fig. 2. Effect of inoculated sludge dosage on the production of VFAs (a) and the release of TP (b), and NH_4^+-N (c) in brewery waste activated sludge hydrolysis process.

Table 2
The linear correlation formula and coefficient in different dosage(C represents NH_4^+-N concentration; t represents hydrolysis time)

Dosage (%)	Equation of linear correlation	The linear correlation coefficient (R^2)
0	$C = 21.221t - 6.2381$	0.9836
20	$C = 16.511t + 128.18$	0.9438
40	$C = 18.764t + 152.95$	0.9784
60	$C = 13.521t + 258.23$	0.9895
80	$C = 8.5107t + 367.91$	0.9885

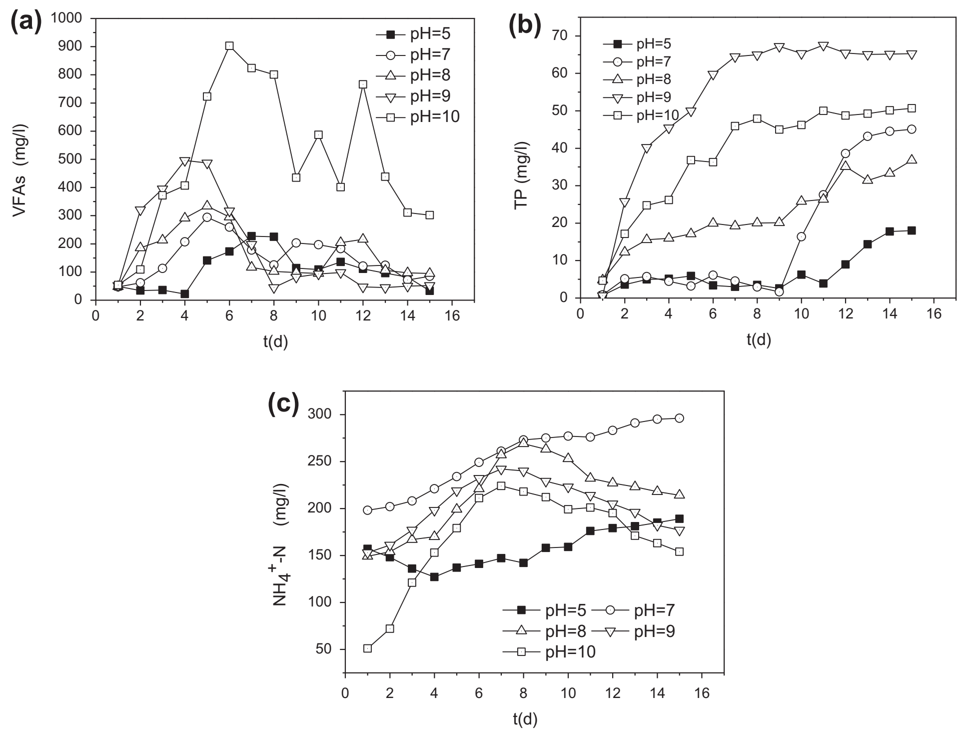

Fig. 3. Effect of pH on the production of VFAs (a) and the release of TP (b), and NH_4^+-N (c) in brewery waste activated sludge hydrolysis process.

The effect of pH on the release of phosphorus was similar to the production of VFAs. However, when the pH was 10, the concentration of phosphorus reduced, which may have resulted from the formation of precipitates with metal ions, and hydroxide. In the acidic and neutral environments, the concentration of NH_4^+-N continued to increase, but the rate of NH_4^+-N reduced after the 7th day in alkaline conditions. This demonstrated NH_4^+-N was discharged in the form of ammonia or oxide by nitrate in the sludge. Also, in alkaline condition, with the increase in pH, the maximum quantity of NH_4^+-N was reduced. This may have been caused by the increased pH, which inhibited the activity of hydrolytic enzymes like protease and peptidase.

As a summary of this section, pH 10 was optimal for the hydrolysis of the combined sludge. Under this condition, more VFAs were produced, and less TP and NH_4^+-N was released (Fig. 3).

3.3. Effect of stirring intensity on hydrolysis of combined sludge

Based on the previous research, seed sludge dosage ratio of 40% and initial pH of 10 were employed in the following experiments. Without stirring, the sludge sediment was allowed to settle under gravity, and the resulting structure was tight and gelatinous. However, the sludge floated as a result of methane production and the methanogenic bacteria applied accumulated VFAs. After the stirring sludge settled, a solid–liquid layer appeared, but the layer become vague as stirring speed increased. Moreover, a small amount of granular sludge emerged in the stirred sludge, which was 2–7 mm long and 4 mm wide. The granular sludge was formed by the centrifugal and cohesive force; however, a portion of the granular sludge was shattered by stirring, which provided new nuclear sites that promoted the formation of more granular sludge. Fig. 5 demonstrates that the surface of the granular sludge was mainly composed of spherical bacteria, which contained eyelets used to overflow gas products internally.

The production of VFAs significantly increased at the beginning of the assay, and the maximum output reached 875 mg/L on the 6th day with the stirring intensity of 60 rpm. However, the VFAs concentration subsequently decreased. The curves showed that stirring was conducive to VFAs production, which agreed with the results of Yuan et al. [47]. Three possible reasons can explain this phenomenon. First, the particle size of the large particulate matters was reduced by the shear action of mechanical stirring. Moreover, it was facilitated to contact organic with microbial even extracellular enzyme. Lastly, the concentration of dissolved solids around the hydrolyzed microorganism

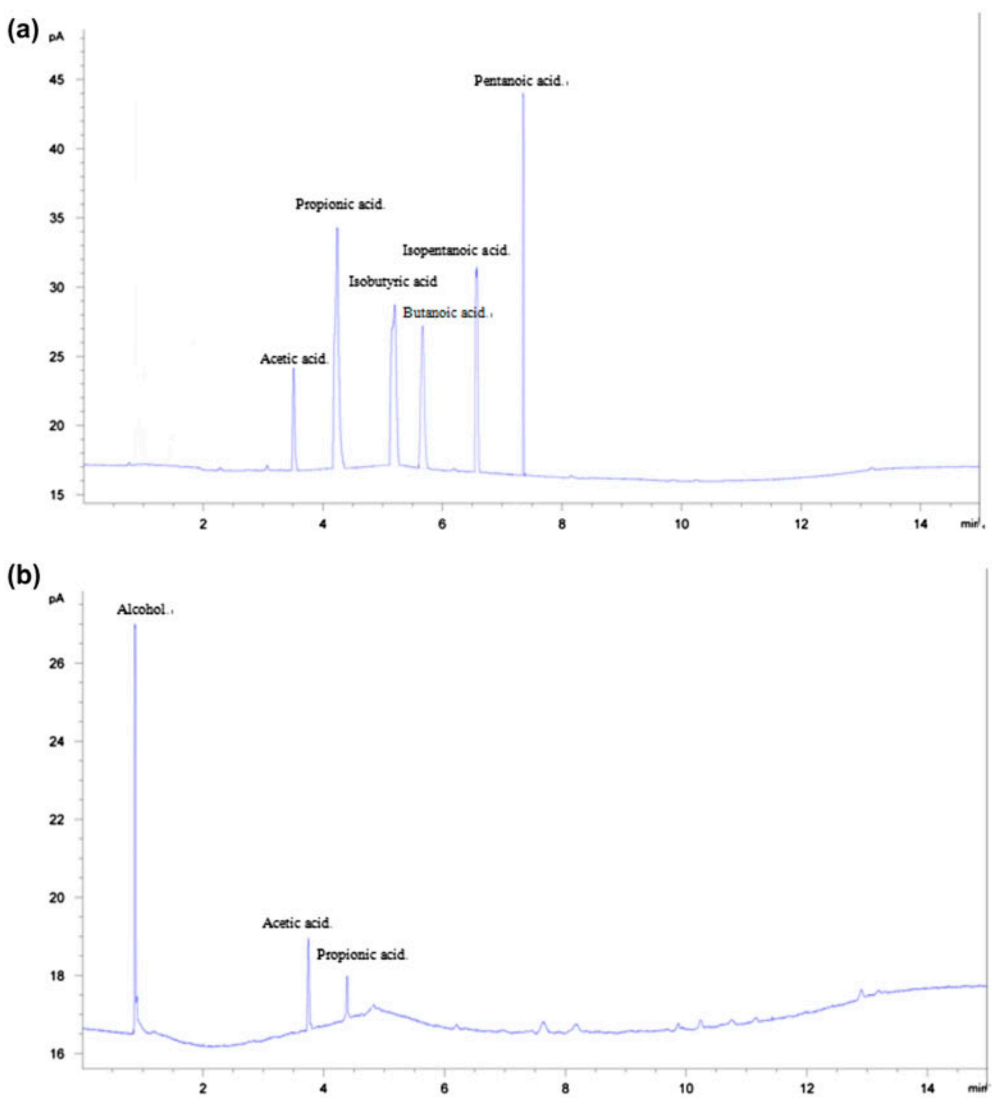

Fig. 4. Effect of pH on the type of VFAs in brewery waste activated sludge hydrolysis process: (a) Comparison chart of volatile fatty acids which measured by gas chromatography, (b) product of sludge anaerobic hydrolysis in acidic condition, (c) product of sludge anaerobic hydrolysis in neutral condition, and (d) product of sludge anaerobic hydrolysis in alkaline condition.

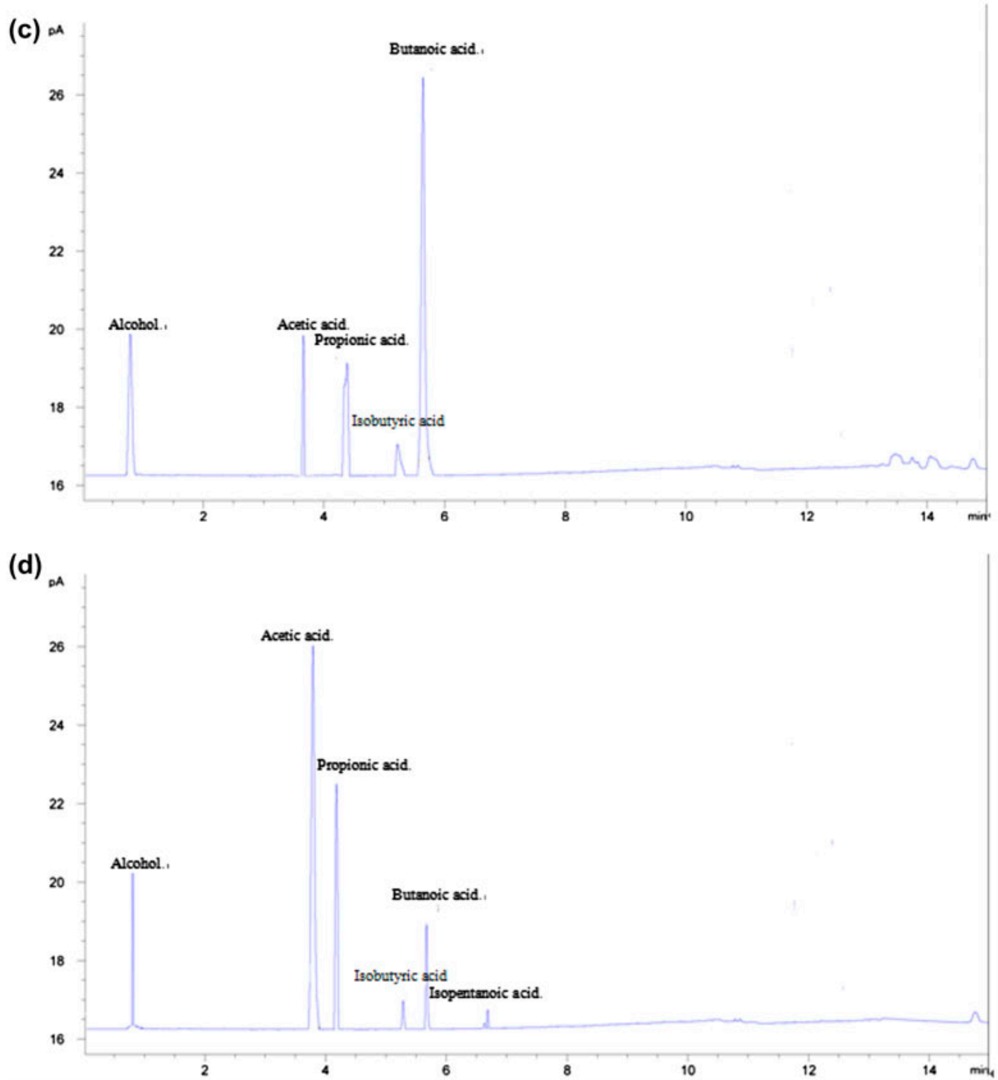

Fig. 4. (*Continued*).

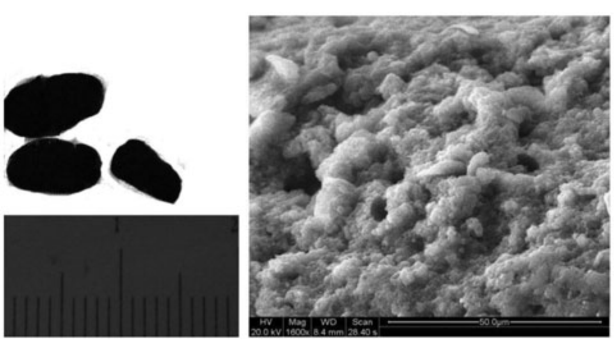

Fig. 5. The grangular sludge generated during stirring.

was decreased, which in turn reduced the inhibition of hydrolysis. As the device was not sealed, increasing the speed also increased the dissolved oxygen in the sludge, which inhibited the microbial activity. Thus, the production of VFAs at the stirring speed of 60 rpm was better than at the stirring speed of 120 rpm.

The maximum TP released was approximately 45 mg/L on the 8th day with stirring speed of 60 rpm; consequently, its concentration remained constant. Stirring prevented the accumulation of the released phosphorus, reduced the phosphorus concentration around PAOs, and promoted the release of phosphorus by PAOs. The profile of the released NH_4^+-N was similar to that of the produced VFAs, and the elevated concentration declined with long residence time. This indicated that high stirring speed promoted proteolysis and NH_4^+-N release.

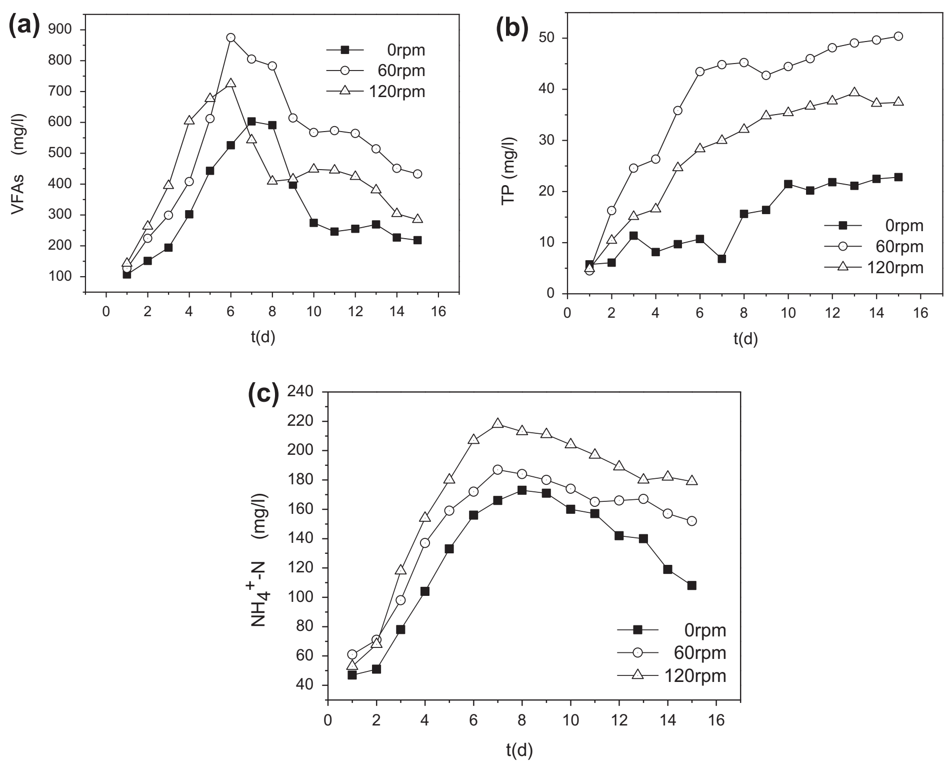

Fig. 6. Effect of stirring intensity on the production of VFAs (a) and the release of TP (b), and NH_4^+-N (c) in brewery waste activated sludge hydrolysis process.

Similar to the previous study, the stirring speed affected the geometric and rheological characteristics of sludge and the pattern of combined sludge; the sludge after stirring obtained granular properties [48]. The maximum production of VFAs was obtained at the stirring speed of 60 rpm, at which the release of TP and NH_4^+-N was highest (Fig. 6).

4. Conclusions

This work investigated the dosage of seed sludge, pH, and stirring intensity as the factors that affect brewery waste activated sludge hydrolysis. After measuring the concentration and types of VFAs, the optimum dosage of seed sludge, the optimal pH, and the optimal stirring speed for acid production in the hydrolysis process were 40%, 10, and 60 rpm, respectively. The maximum production of VFAs reached approximately 1,000 mg/L, which mainly contained acetic acid. The release of TP and NH_4^+-N changed according to different conditions, and the maximum outputs were 60 and 500 mg/L, respectively. In general, this study would benefit the practical engineering of brewery waste activated sludge hydrolysis.

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References

[1] T. Janhom, S. Wattanachira, P. Pavasant, Characterization of brewery wastewater with spectrofluorometry analysis, J. Environ. Manage. 90 (2009) 1184–1190.

[2] W. Parawira, I. Kudita, M.G. Nyandoroh, R. Zvauya, A study of industrial anaerobic treatment of opaque beer brewery wastewater in a tropical climate using a full-scale UASB reactor seeded with activated sludge, Process Biochem. 40 (2005) 593–599.

[3] G.S. Simate, J. Cluett, S.E. Iyuke, E.T. Musapatika, S. Ndlovu, L.F. Walubita, A.E. Alvarez, The treatment of brewery wastewater for reuse: State of the art, Desalination 273 (2011) 235–247.

[4] C. Kütük, G. Çaycı, A. Baran, O. Başkan, R. Hartmann, Effects of beer factory sludge on soil properties and growth of sugar beet (*Beta vulgaris saccharifera* L.), Bioresour. Technol. 90 (2003) 75–80.

[5] X. Ren, R. Yan, H.-C. Wang, Y.-Y. Kou, K.-J. Chae, S. Kim, Y.-J. Park, A.-J. Wang, Citric acid and ethylene diamine tetra-acetic acid as effective washing agents to treat sewage sludge for agricultural reuse, Waste Management 46 (2015) 440–448.

[6] S. Kavitha, R. Yukesh Kannah, I.T. Yeom, K.-U. Do, J. Rajesh Banu, Combined thermo-chemo-sonic disintegration of waste activated sludge for biogas production, Bioresour. Technol. 197 (2015) 383–392.
[7] M. Nikaeen, A.H. Nafez, B. Bina, B.F. Nabavi, A. Hassanzadeh, Respiration and enzymatic activities as indicators of stabilization of sewage sludge composting, Waste Manage. 39 (2015) 104–110.
[8] I. Nansubuga, N. Banadda, M. Babu, J.D. Vrieze, W. Verstraete, K. Rabaey, Enhancement of biogas potential of primary sludge by co-digestion with cow manure and brewery sludge, Int. J. Agric. Biol. Eng. 8 (2015) 86–94.
[9] N. Erdem, S.S. Ok, Effect of brewery sludge amendments on some chemical properties of acid soil in pot experiments, Bioresour. Technol. 84 (2002) 271–273.
[10] G. Yang, P.Y. Zhang, G.M. Zhang, Y.Y. Wang, A.Q. Yang, Degradation properties of protein and carbohydrate during sludge anaerobic digestion, Bioresour. Technol. 192 (2015) 126–130.
[11] J.D. McLeod, M.Z. Othman, D.J. Beale, D. Joshi, The use of laboratory scale reactors to predict sensitivity to changes in operating conditions for full-scale anaerobic digestion treating municipal sewage sludge, Bioresour. Technol. 189 (2015) 384–390.
[12] E.H. Koupaie, C. Eskicioglu, Below and above boiling point comparison of microwave irradiation and conductive heating for municipal sludge digestion under identical heating/cooling profiles, Bioresour. Technol. 187 (2015) 235–245.
[13] K.B. Dassanayake, G.Y. Jayasinghe, A. Surapaneni, C. Hetherington, A review on alum sludge reuse with special reference to agricultural applications and future challenges, Waste Manage. 38 (2015) 321–335.
[14] E. Wolff, W.K. Schwabe, S.V. Conceição, Utilization of water treatment plant sludge in structural ceramics, J. Cleaner Prod. 96 (2015) 282–289.
[15] W. Xu, J.C. Xu, J. Liu, H.X. Li, B. Cao, X.F. Huang, G.M. Li, The utilization of lime-dried sludge as resource for producing cement, J. Cleaner Prod. 83 (2014) 286–293.
[16] V. Mymrin, W. Klitzke, K. Alekseev, R.E. Catai, A. Nagalli, R.L. Izzo, C.A. Romano, Red clay application in the utilization of paper production sludge and scrap glass to fabricate ceramic materials, Appl. Clay Sci. 107 (2015) 28–35.
[17] P. Hadi, M. Xu, C. Ning, C.S. Lin, G. McKay, A critical review on preparation, characterization and utilization of sludge-derived activated carbons for wastewater treatment, Chem. Eng. J. 260 (2015) 895–906.
[18] M.C. Samolada, A.A. Zabaniotou, Comparative assessment of municipal sewage sludge incineration, gasification and pyrolysis for a sustainable sludge-to-energy management in Greece, Waste Manage. 34 (2014) 411–420.
[19] H.B. Liu, F. Zhao, B.Y. Mao, X.H. Wen, Enhanced nitrogen removal in a wastewater treatment process characterized by carbon source manipulation with biological adsorption and sludge hydrolysis, Bioresour. Technol. 114 (2014) 62–68.
[20] E. Salminen, J. Rintala, Anaerobic digestion of organic solid poultry slaughterhouse waste—A review, Bioresour. Technol. 83 (2002) 13–26.
[21] J.M. Abelleira-Pereira, S.I. Pérez-Elvira, J. Sánchez-Oneto, R.D. Cruz, J.R. Portela, E. Nebot, Enhancement of methane production in mesophilic anaerobic digestion of secondary sewage sludge by advanced thermal hydrolysis pretreatment, Water Res. 71 (2015) 330–340.
[22] Y.G. Xue, H.J. Liu, S.S. Chen, N. Dichtl, X.H. Dai, N. Li, Effects of thermal hydrolysis on organic matter solubilization and anaerobic digestion of high solid sludge, Chem. Eng. J. 264 (2015) 174–180.
[23] J.W. Lim, J.Y. Wang, Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste, Waste Manage. 33 (2013) 813–819.
[24] X.H. Chen, Y. Gu, X.F. Zhou, Y.L. Zhang, Asparagus stem as a new lignocellulosic biomass feedstock for anaerobic digestion: Increasing hydrolysis rate, methane production and biodegradability by alkaline pretreatment, Bioresour. Technol. 164 (2014) 78–85.
[25] G.Y. Zhen, X.Q. Lu, Y.Y. Li, Y.C. Zhao, Combined electrical-alkali pretreatment to increase the anaerobic hydrolysis rate of waste activated sludge during anaerobic digestion, Appl. Energy 128 (2014) 93–102.
[26] S.T. Zhang, H.G. Guo, L.Z. Du, J.F. Liang, X.B. Lu, N. Li, K.Q. Zhang, Influence of NaOH and thermal pretreatment on dewatered activated sludge solubilisation and subsequent anaerobic digestion: Focused on high-solid state, Bioresour. Technol. 185 (2015) 171–177.
[27] R. Montañés, M. Pérez, R. Solera, Anaerobic mesophilic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of pH control, Chem. Eng. J. 255 (2014) 492–499.
[28] S. Ponsá, I. Ferrer, F. Vázquez, X. Font, Optimization of the hydrolytic–acidogenic anaerobic digestion stage (55˚C) of sewage sludge: Influence of pH and solid content, Water Res. 42 (2008) 3972–3980.
[29] H.Q. Ge, P.D. Jensen, D.J. Batstone, Temperature phased anaerobic digestion increases apparent hydrolysis rate for waste activated sludge, Water Res. 45 (2011) 1597–1606.
[30] E.A. Cazier, E. Trably, J.P. Steyer, R. Escudie, Biomass hydrolysis inhibition at high hydrogen partial pressure in solid-state anaerobic digestion, Bioresour. Technol. 190 (2015) 106–113.
[31] T.V. Fernandes, K.J. Keesman, G. Zeeman, J.B. van Lier, Effect of ammonia on the anaerobic hydrolysis of cellulose and tributyrin, Biomass Bioenergy 47 (2012) 316–323.
[32] S.G. Gen, P. Sousbie, G. Rangaraj, J.M. Lema, J. Rodríguez, J.P. Steyer, M. Torrijos, Kinetic modelling of anaerobic hydrolysis of solid wastes, including disintegration processes, Waste Manage. 35 (2015) 96–104.
[33] Q. Yuan, R. Sparling, J.A. Oleszkiewicz, Waste activated sludge fermentation: Effect of solids retention time and biomass concentration, Water Res. 43 (2009) 5180–5186.
[34] C. Yang, W. Liu, Z. He, S. Thangavel, L. Wang, A. Zhou, A. Wang, Freezing/thawing pretreatment coupled with biological process of thermophilic *Geobacillus* sp. G1: Acceleration on waste activated sludge hydrolysis and acidification Bioresour. Technol. 175 (2015) 509–516.
[35] H. Xiong, J. Chen, H. Wang, H. Shi, Influences of volatile solid concentration, temperature and solid retention time for the hydrolysis of waste activated sludge to recover volatile fatty acids, Bioresour. Technol. 119 (2012) 285–292.

[36] Q. Yuan, R. Sparling, J.A. Oleszkiewicz, VFA generation from waste activated sludge: Effect of temperature and mixing, Chemosphere 82 (2011) 603–607.
[37] L.P. Wilson, L.H. Loetscher, S.E. Sharvelle, S.K. De Long, Microbial community acclimation enhances waste hydrolysis rates under elevated ammonia and salinity conditions, Bioresour. Technol. 146 (2013) 15–22.
[38] M. Kim, R.E. Speece, Aerobic waste activated sludge (WAS) for start-up seed of mesophilic and thermophilic anaerobic digestion, Water Res. 36 (2002) 3860–3866.
[39] C. Dumas, S. Perez, E. Paul, X. Lefebvre, Combined thermophilic aerobic process and conventional anaerobic digestion: Effect on sludge biodegradation and methane production, Bioresour. Technol. 101 (2010) 2629–2636.
[40] N.T. Dinh, K. Hatta, S.H. Kwon, A.P. Rollon, K. Nakasaki, Changes in the microbial community during the acclimation stages of the methane fermentation for the treatment of glycerol, Biomass Bioenergy 68 (2014) 240–249.
[41] G. Anjali, P.C. Sabumon, Development of enhanced SNAD process in a down-flow packed bed reactor for removal of higher concentrations of NH_4–N and COD, J. Environ. Chem. Eng. 3 (2015) 1009–1017.
[42] M. Takashima, Y. Tanaka, Acidic thermal post-treatment for enhancing anaerobic digestion of sewage sludge, J. Environ. Chem. Eng. 2 (2014) 773–779.
[43] X.Y. Zhang, F. Zhu, L. Chen, Q. Zhao, G.H. Tao, Removal of ammonia nitrogen from wastewater using an aerobic cathode microbial fuel cell, Bioresour. Technol. 146 (2013) 161–168.
[44] Y. Maspolim, Y. Zhou, C.H. Guo, K.K. Xiao, W.J. Ng, The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation, Bioresour. Technol. 190 (2015) 289–298.
[45] F.L. Yu, Y.Y. Yuan, C.Y. Guang, Kinetic analysis of waste activated sludge hydrolysis and short-chain fatty acids production at pH 10, J. Environ. Sci. 21 (2009) 589–594.
[46] D. Cysneiros, C.J. Banks, S. Heaven, K.G. Karatzas, The effect of pH control and 'hydraulic flush' on hydrolysis and Volatile Fatty Acids (VFA) production and profile in anaerobic leach bed reactors digesting a high solids content substrate, Bioresour. Technol. 123 (2012) 263–271.
[47] Q. Yuan, R. Sparling, J.A. Oleszkiewicz, VFA generation from waste activated sludge: Effect of temperature and mixing, Chemosphere 82 (2011) 603–607.
[48] Y.L. Wang, S.K. Dentel, The effect of high speed mixing and polymer dosing rates on the geometric and rheological characteristics of conditioned anaerobic digested sludge (ADS), Water Res. 44 (2010) 6041–6052.