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Effects of temperature on hydrolysis performance and short-chain fatty acids production during thermophilic micro-aerobic fermentation of waste activated sludge

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ABSTRACT

Rapid hydrolysis of waste activated sludge (WAS) can be achieved by improving activities of extracellular enzymes under proper temperature conditions. As short-chain fatty acids (SCFAs) accumulation is always consumed by methanogens under anaerobic conditions, and considering that the micro-aerobic condition can inhibit the activities of methanogens, in this study, effects of temperature (55–75℃) on thermophilic micro-aerobic fermentation of WAS were investigated. Results showed that the highest soluble chemical oxygen demand (SCOD) yield was obtained at 60℃ (4,407 ± 80 mg/L, 36 h), 2.0 times higher than that obtained at 75℃ (2,180 ± 40 mg/L, 36 h), the corresponding hydrolysis rate was 0.6689 d^{-1}. The highest SCFAs yield was 2,928 ± 12 mg COD/L at 60℃ and 36 h, 4.9 times higher than that obtained at 75℃ (594 ± 10 mg COD/L, 36 h). The analysis of SCFAs composition showed that acetic acid (HAc) accounted for the most percentage (>40%), followed by n-valeric (n-HVa) (20–25%), and propionic acids (HPr) (10–15%). Total suspended solids removal efficiency reached 18.7% after 192-h fermentation at 60℃. These results suggested that the optimal thermophilic micro-aerobic conditions for WAS hydrolysis and SCFAs accumulation from WAS were 60℃ and 36-h fermentation time.

Keywords: Hydrolysis; Waste activated sludge (WAS); Temperature; Short-chain fatty acids (SCFAs); Thermophilic micro-aerobic fermentation

1. Introduction

Very recently, waste activated sludge (WAS) disposal has focused on recycling resources but saving cost. As over 50% of organic materials of WAS were protein, it is potential to recover high-added productions from WAS [1]. Among existing biological treatment processes, the anaerobic digestion (AD) process is a mature process that is the most cost-effective because of renewable energy recovery and environmentally friendly impact [2]. However, organic materials in the form of particulates form a natural

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obstacle in WAS system, which leads to hydrolysis being the rate-limiting step, while AD process is selected to treat WAS and recycle resources. Thus, hydrolysis is usually employed in the digestion process for enhancing performances of AD process [3].

In order to improve WAS hydrolysis, many pretreatment methods have been investigated for improving the performances of WAS–AD. Among the various pretreatment strategies, thermophilic micro-aerobic fermentation is considered to be promising to enhance the biodegradation of WAS because of the thermophilic hydrolytic bacteria [4]. It is known that thermophilic bacteria can speed up the degradation degree of organic matters during the early stage of thermophilic aerobic digestion of WAS [3]. The solubilization of organics in the WAS occurs through the thermal effect and enzymatic hydrolysis, while thermophilic bacteria are subjected to optimal conditions for their growth [5]. With thermophilic treatment, the micro-organism converted particulate organic matters into soluble organic matters, which were more easily assimilated by the active biomass [6]. Therefore, it is interesting to study the feasibility and performance of thermophilic bacteria on WAS hydrolysis.

Meanwhile, hydrolysis of particulate organic matters could be significantly enhanced by increasing digester temperatures, especially thermophilic conditions (55–70℃), which has a rate advantage over mesophilic digestion (37℃) because of higher hydrolysis coefficient and faster reaction rates [7]. Thermophilic digestion temperature plays a primary role in organics disgestation [8]. In hyperthermy (40–60℃), the process relies on the hot stable enzyme emitted by the thermophilic bacteria in WAS to dissolve the insoluble organic substances [9]. In typical autothermal thermophilic aerobic digestion systems treating domestic sludge, the temperature range between 45 and 65℃ ensures the best degradation rates, while the temperature rise aids pathogen reduction [10]. Additional benefits include increased degradation efficiency and biogas production [11]. Previous temperature tests showed that sludge hydrolysis was the best at 55℃ and the optimum pH (8–10) for short-chain fatty acids (SCFAs) accumulation varied with temperatures (21–55℃) [12]. Watts et al. [13] reported a substantial improvement of activated sludge digestion with increased thermophilic temperature. However, processes functioning at lower temperatures (<100℃), which could represent a cost-effective selection to pretreat WAS, have not been completely explored [2].

It was also found out that limited or controlled presence of oxygen inside a digester is not dangerous, but may have a positive effect in some aspects [14]. The micro-aerobic conditions can be realized by dosing limited amount of air (oxygen) into an anaerobic reactor [15]. With limited oxygen, the facultative anaerobes were suggested to maintain low redox potential and supply more growth factors for the strict anaerobes [16]. Although, these studies have compared the micro-aeration intensity and effectiveness in the hydrolysis of various organic substrates, the relationship between temperature and acidification rate has not been completely understood.

Therefore, the objective of the present study was to study the effect of different temperatures from 55 to 75℃ under micro-aeration condition on the solubilization and speciation of SCFAs. In addition, the thermodynamic analysis of WAS hydrolysis was evaluated in batch assays. The outcome of this study may provide some potential technical guidance for the WAS disposal in the future.

2. Materials and methods

2.1. Source of WAS

WAS was collected from the secondary sedimentation tank of Taiping Municipal Wastewater Treatment Plant (Harbin, Heilongjiang, China). The sludge was thickened by gravity settling for 24 h at 4℃, leading to the volatile suspended solids (VSS) of 11,700 mg/L, and screened with a 1-mm sieve to remove impurities, then stored at 4℃ prior to tests. Its characteristics are listed as follows: pH 6.68 ± 0.5, total suspended solids (TSS) 18,800 ± 158 mg g/L, total chemical oxygen demand (TCOD) 12,300 ± 150 mg/L, soluble chemical oxygen demand (SCOD) 546 ± 50 mg/L, soluble protein 105 ± 5 mg COD/L, soluble carbohydrate 58 ± 15 mg COD/L, SCFAs 371 ± 15 mg COD/L, NH_4^+-N 281 ± 15 mg/L, and PO_4^{3-}-P 374 ± 12 mg/L.

2.2. Batch setup

As shown in Fig. 1, the batch fermentation experiments were conducted in a series of fermentation reactors, which were all filled with 1,000-mL WAS. Five of reactors inoculated 100 mL, the corresponding seed sludge ran at 55, 60, 65, 70, and 75℃ for 10 d at a speed of 100 rpm, respectively. The ventilation rate was 0.05 vvm (volume air per volume sludge per minute) to limit concentration of dissolved oxygen below 1 mg/L.

2.3. Sludge sampling and analysis

Sludge samples were centrifuged at 10,000 rpm after anaerobic fermentation, then supernatant samples were filtered by 0.45-μm cellulose nitrate

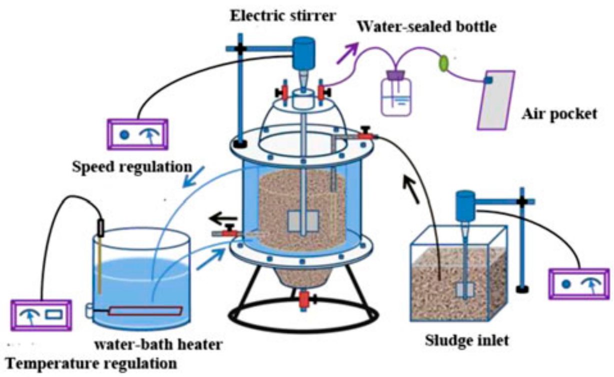

Fig. 1. Schematic diagram of batch experiments.

membrane filters, and finally filtrate samples were stored at 4°C prior to analysis. It is noteworthy that the soluble carbohydrate and protein of filtrate samples should be analyzed immediately. Analysis methods of TSS, VSS, SCFAs, SCOD, TCOD, CH_4, NH_4^+-N, PO_4^{3-}-P, and soluble carbohydrate and protein were the same as previous publications [17,18]. The SCFAs were recorded as the sum of acetic (HAc), propionic (HPr), n-butyric (n-HBu), iso-butyric (iso-HBu), n-valeric (n-HVa), and iso-valeric acids (iso-HVa).

3. Results and discussion

3.1. Effect of temperature on WAS hydrolysis

It has reported that the formation of SCFAs during sludge fermentation associated with bioconversion of soluble organic matter [19]. Fig. 2 showed that the temperature change significantly affected the SCOD concentrations during the first 36 h, the highest SCOD (4,407 ± 80 mg/L) reached at 60°C, followed by 65°C (4,388 ± 70 mg/L) and 70°C (4,320 ± 70 mg/L), and the lowest (2,180 ± 40 mg/L) was reached at 75°C. Substantial improvement of activated sludge digestion with increased thermophilic temperature was observed [13] and the reason was likely that a large amount of organic matter was released from dead micro-organisms into the supernatant, resulting in a rapid increase in SCOD in the early stage of the process [20]. Li et al. [9] found that thermostable enzymes enhanced the rate of degradation during the sludge digestion. In addition, thermophilic bacteria could use the released organics and achieve rapid growth [21]. This finding is supported by the significant increase in SCOD during the first 72 h. However, degradation ratio of TSS was highest under 65°C for 192 h, 25.5%, while the degradation ratio of TSS was 16.7, 21.5, 18.7, and 15.1% under 55, 60, 70, and 75°C, respectively (data not shown). The degradation ratio of TSS had no significant difference between 60 and 65°C. When the temperature was over 65°C, the microbial activity was inhibited, which resulted in un-dissolved sludge [9]. These results suggested that the organic matter in the sewage sludge degraded significantly and rapidly during the thermophilic micro-aerobic digestion process and that the thermophilic micro-aerobic digestion had a desirable TSS removal efficiency.

As protein and carbohydrate are the two predominant constituents of COD, the contents and components of SCOD were also determined (Fig. 3) [22]. It was found that at the beginning of the fermentation period, concentrations of soluble carbohydrate and protein increased sharply while the fermentation time changed from 0 to 84 h, and then remained relatively stable (84–192 h). The reason was that at higher temperature there were more soluble carbohydrate and protein, indicating that the hydrolysis of WAS was enhanced by thermophilic bacteria or thermal effects. Hery et al. [2] demonstrated that the enhancement in the biodegradation was due to the selection of thermophilic hydrolysis bacteria. The highest concentration of carbohydrate and protein was 545 ± 24 and 1,215 ± 54 mg COD/L, at 75 and 70°C for 192- and 84-h fermentation periods. Comparing Fig. 2(a) with Fig. 2(b), it can be found that the trends in the variations of soluble carbohydrate and protein concentrations with temperature range from 55 to 75°C were similar. The results showed that the soluble carbohydrate content was lower than soluble protein at all temperatures, which was similar to the previous study [23]. Mata-Alvarez et al. [24] showed that the hydrolysis of protein was more dependent on the biological activity than the hydrolysis of carbohydrate, while the hydrolysis of carbohydrate was more dependent on the thermal effect. At the temperature of which microbial activity was high, larger percent of protein was hydrolyzed, and high percent of carbohydrate was hydrolyzed by the thermal effect at high temperature, such as 75°C used in this work.

3.2. Thermodynamic analysis of WAS hydrolysis

According to the principle of reaction kinetics, temperature and concentrations of reactants are the dominant factors [25]. The cumulative effects of different processes taking place during sludge hydrolysis had traditionally been simplified to first-order kinetic equation [26]. The slopes of the curve at different temperatures in Fig. 2 were nearly invariable within the initial 36 h, indicating rapid increase of SCOD release. Subsequently, the slopes gradually approached a stationary value. According to above description, the

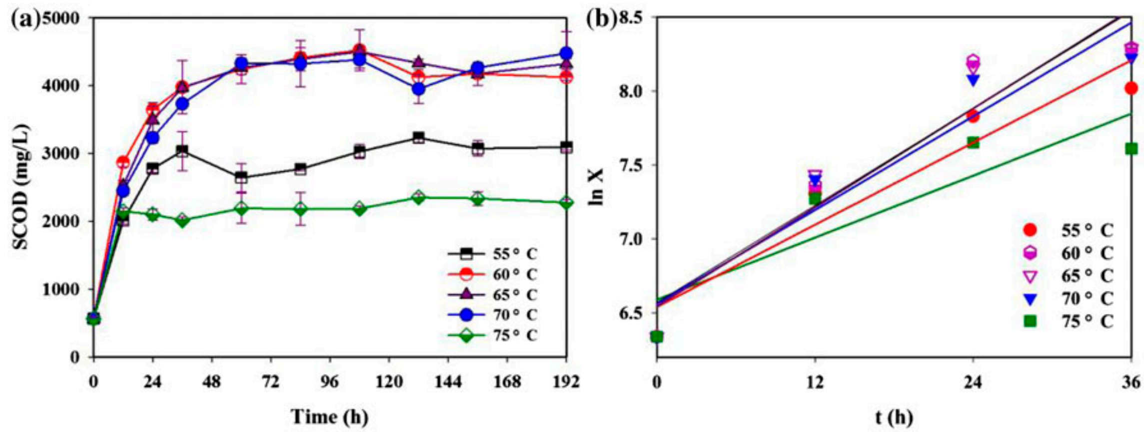

Fig. 2. SCOD concentration change in fermentation time (a) and kinetic model of WAS hydrolysis (b).

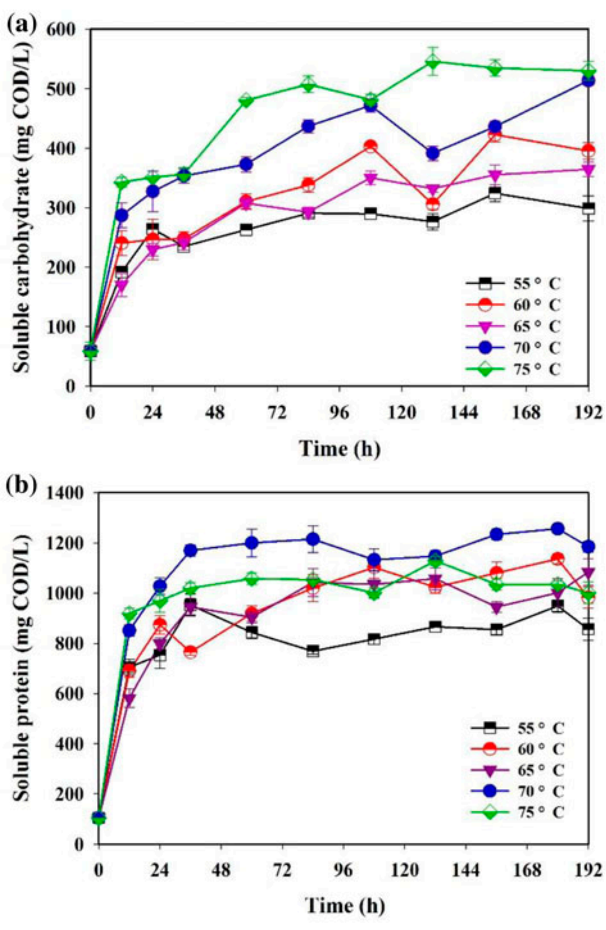

Fig. 3. Soluble carbohydrate (a) and soluble protein (b) concentration change in fermentation time.

hydrolysis of WAS within the initial 36 h could be assumed to obey the following first-order kinetics equations:

$$r = \frac{dS}{dt} = kS \quad (1)$$

$$\ln S = kt + b \quad (2)$$

where S (mg/L) was the SCOD concentration, k was the hydrolysis rate constant, and b was the constant of integration. By plotting ln S vs. t, slope and intercept could be obtained, which corresponded to the value of k and b, respectively. The ln S–t regression curves at different temperatures were illustrated in Table 1. It is clear that the goodness of fit values at different temperatures were satisfactory in the range of 0.90–0.91 (except 75℃), further indicating that the SCOD release in WAS obeyed the first-order kinetics. As shown in Table 1, at 60℃, the k value was 0.6689 h^{-1}, which was 1.2 times higher than that in the lowest (55℃) test. It is clear that the hydrolysis rate was significantly accelerated at 60℃ (Fig. 2). Moreover, the highest SCOD release was obtained at 60℃, further suggesting 60℃ was the optimal temperature for micro-aerobic fermentation of WAS.

3.3. SCFAs accumulation during fermentation

SCFAs were converted from monosaccharide and amino acid (soluble carbohydrate and protein) by acid-forming bacteria [27]. The effect of temperature on SCFAs accumulation is shown in Fig. 4(a). The SCFAs contents increased rapidly during the first 36 h, then kept stable, and did not increase with temperature. The highest SCFAs accumulation production was obtained at 60℃ (2,928 ± 12 mg COD/L, 36 h), but then dropped rapidly with temperature increasing, such as at 75℃ (594 ± 10 mg COD/L, 36 h), which was even lower than that at 55℃ (1,786 ± 15 mg COD/L,

Table 1
Kinetic data relevant to SCOD release at different temperatures

Temperature (℃)	Dynamic equation	Rate constants k (h^{-1})	Coefficient R^2
55	$y = 0.5562x + 5.9822$	0.5562	0.910
60	$y = 0.6689x + 5.8744$	0.6689	0.905
65	$y = 0.6567x + 5.912$	0.6567	0.9011
70	$y = 0.6336x + 5.927$	0.6336	0.9042
75	$y = 0.4192x + 6.1693$	0.4192	0.7864

36 h). Some researchers have also found that a proper increase of temperature could enhance SCFAs production. Also, as seen in Fig. 4(a), the times required to achieve the maximum SCFAs accumulation were 132, 84, 108, 108, and 156 h at 55, 60, 65, 70, and 75℃, respectively, which indicated that the more proper temperature, the shorter time for maximum SCFAs accumulation reached. It could be concluded that among the five temperatures, 60℃ was the optimal temperature for realizing the highest SCFAs accumulation.

As is well known, the types of SCFAs in wastewater always influence the removal efficiency of nutrients during biological wastewater treatment; HAc and HPr are regarded as two preferred carbon sources among different SCFAs used for biological phosphorus removal [28]. The percentage of individual SCFAs at different temperature at 36-h fermentation was given in Fig. 4(b). It was observed that the compositions of SCFAs did not vary significantly with changing temperature. HAc accounted for the most percentage (40–50%), followed by n-HVa (20–25%) and HPr (10–15%), and other acids (iso-HBu, n-HBu, and iso-HVa) were less than 10%. This result was similar to the previous results of batch sludge fermentation under thermophilic conditions [29], and showed that no matter under mesophilic or thermophilic conditions, the dominant SCFAs produced from WAS fermentation was HAc [30].

3.4. Changes of NH_4^+-N and PO_4^{3-}-P in the supernatant

As nitrogen and phosphorus are present in the bacterial cells, the process of biomass fermentation will lead to release of these nutrients as ammonium and phosphate [31]. The changes of NH_4^+-N in the supernatant are shown in Fig. 5(a). The concentration of NH_4^+-N in the WAS increased rapidly during the early digestion period, then decreased slightly after 132 h. The highest concentration of NH_4^+-N was 900 ± 20 mg/L at 60℃ for 132 h, which was 2.5 times higher than that at 75℃ for 132 h. The PO_4^{3-}-P concentration during the WAS fermentation has the same change as NH_4^+-N (Fig. 5(b)). The maximum PO_4^{3-}-P concentration was obtained at 60℃ for 36-h fermentation, 510 ± 15 mg/L. Phosphorus and nitrogen are the important components of cells and extracellular polymeric substance (EPS) of WAS. The results obtained

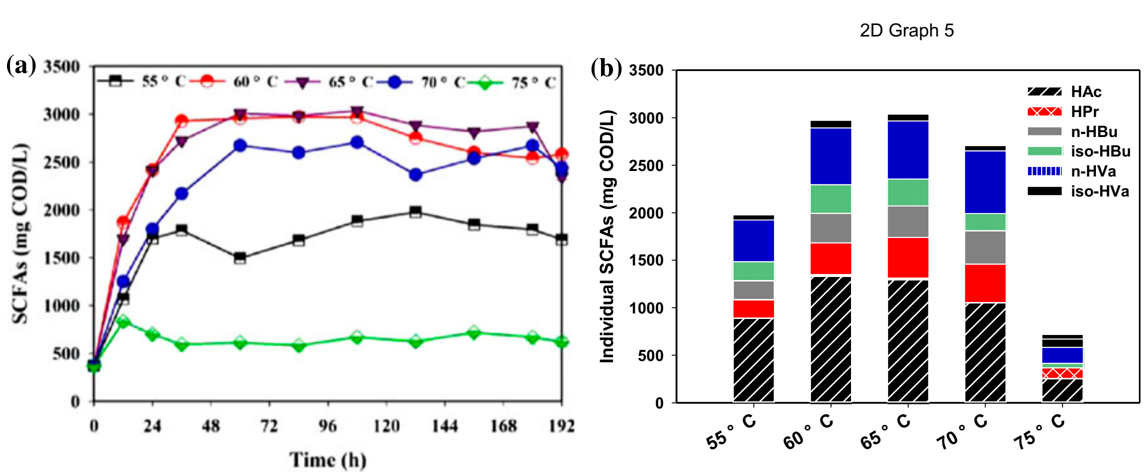

Fig. 4. Total SCFAs concentration (a), ratio of individual SCFAs (b), and the relationship between SCFAs accumulation and soluble organics consumption (c).

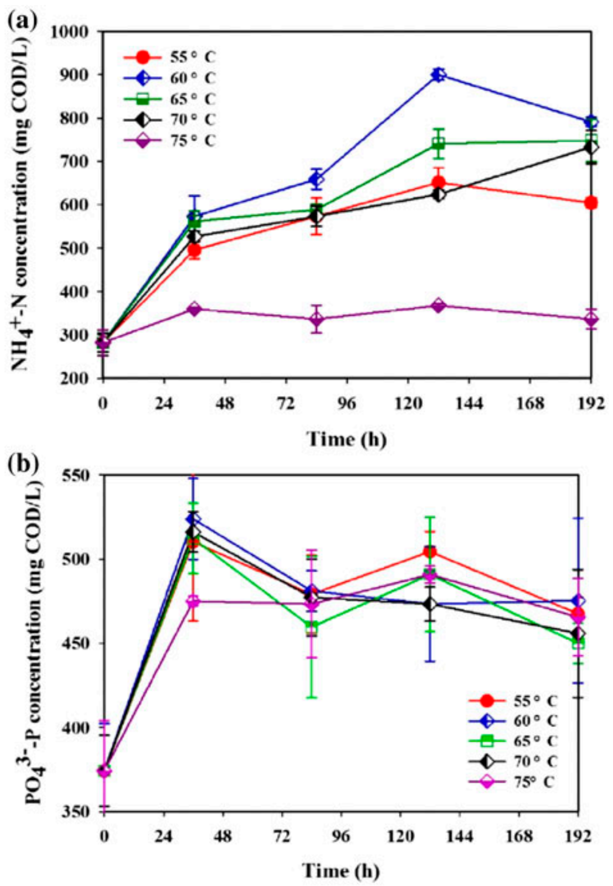

Fig. 5. The release of NH_4 (a) and total P (b) during the fermentation time.

from this work were consistent with the previous study [20]. NH_4^+-N and PO_4^{3-}-P were the products from soluble protein hydrolysis from EPS and decayed less temperature-tolerant cells [22]. In addition, NH_4^+-N was the major component of the total nitrogen in the supernatant during the digestion process. The results indicated that the organic nitrogen in the WAS was released mainly in the form of ammonia nitrogen in thermophilic micro-aerobic fermentation. Abundant ammonia nitrogen was produced in the 60℃ system, while thermophilic micro-aerobic fermentation inhibited nitrification and denitrification [32], and this system might have contributed to the appropriate temperature.

4. Conclusions

The effects of temperatures on performances of thermophilic micro-aerobic fermentation of WAS were investigated. The maximal SCOD concentration was 4,407 ± 80 mg COD/L at 60℃, and the corresponding hydrolysis rate constant was 0.6689. The highest concentrations of carbohydrate and protein were 422 ± 12 and 1,136 ± 21 mg COD/L, at 60℃ for 156- and 180-h fermentation. And SCFAs accumulation was 2,928 ± 12 mg COD/L at fermentation time of 36 h. Three main components contained in SCFAs were HAc, n-HVa, and HPr with the fraction of 45.3, 20.1 and 11.3%, respectively. The highest concentration of NH_4^+-N was 900 ± 20 mg/L at 60℃ for 132 h. The maximum PO_4^{3-}-P concentration was obtained at 60℃ for 36-h fermentation, 510 ± 15 mg/L. This work introduced a promising treatment method to enhance WAS hydrolysis/acidification, benefiting further resource recovery from WAS.

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