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Ammonia-eliminating potential of *Gracilaria* sp. And zeolite: a preliminary study of the efficient ammonia eliminator in aquatic environment

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Abstract. High density and high feeding volume are regarded as factors causing huge accumulations of Total Ammonia Nitrogen (TAN) in an aquaculture system. An uncontrolled TAN concentration in an aquaculture system can pose a threat to the reared organisms as an abrupt escalation of the toxic form of TAN (ammonia - NH_3) may occur following the alteration of the water quality. Considering the potential of *Gracilaria* sp. and zeolite toward ammonia elimination, it would be a milestone interest to study the effect of *Gracilaria* sp and zeolite as an ammonia eliminator in aquatic environment. The result shows that *Gracilaria* sp. and zeolite significantly affect the reduction of TAN concentration ($p < 0.05$). The result shows that the reduction ranges from 0.101 to 1 ppm while the best treatment is treatment Cc (5 g/L *Gracilaria* sp. and 10 g/L zeolite) which can eliminate the TAN concentration by up to 100% (1 ppm) within 7 days. Pursuant to the result, it can be concluded that *Gracilaria* sp. and zeolite have the potential of being an efficient ammonia eliminator in an aquatic environment. Nonetheless, it is important to conduct further research with respect to the actual implementation of the ammonia eliminators in a real aquaculture system.

1. Introduction

Ammonia is an environmental factor that has been a big concern in the scope of aquaculture as well as in environmental science. This inorganic compound is actually the toxic form of Total Ammonia Nitrogen (TAN) and can pose a threat to aquatic organisms [1-3]. Despite sometimes being referred to as NH_3 (ammonia) or NH_4^+ (ammonium), ammonia concentration in an aquatic environment is commonly expressed as TAN [2]. TAN derives from the nitrogen cycle that originates from an organic material decomposition or from the excretion products of aquatic organisms. In addition, TAN may come from the carcasses of aquatic organisms or uneaten feed. The fact that aquaculture practices these days have been implementing both high density and high feeding volume may cause an escalation in the TAN concentration in aquatic systems [4-7].

An uncontrolled TAN concentration can cause a huge problem in the aquatic environment as the toxicity of TAN may suddenly increase following the alteration of the water quality factors, such as pH, temperature, ionic charge, salinity and dissolved oxygen (DO) [8-14]. If it exceeds the tolerance threshold, the toxic form of TAN – ammonia (NH_3) – can inhibit the growth of aquatic organisms and can even result in mortality as the compound disrupts oxygen binding in the blood, changes the blood pH and affects enzymatic reactions and membrane stability in aquatic organisms [15-18]. The lethal concentration (LC_{50}) of ammonia ranges from 1.10 to 22.8 ppm for invertebrates and from 0.56 to 2.37 ppm for fish within 24 – 96 hours of exposure [3]. At 0.04 ppm, ammonia can also result in 5% mortality and 20% growth impairment for cultured fish [19]. Thus, to minimize the adverse effect of ammonia particularly in aquaculture settings, the knowledge of how to control the TAN concentration is of importance.

There have been many studies conducted concerning TAN elimination in aquatic systems. The most common method used for controlling TAN concentration in aquaculture systems is using microorganisms [20]. Ammonia stripping through pressure, magnesium – ammonium – phosphate precipitation, SHARON (Single Reactor High Activity Ammonia Removal over Nitrite) and ANNAMOX (Anaerobic Ammonium Oxidation) are other methods that can be implemented in curbing the TAN concentration [21]. Nevertheless, due to affordability, the high complexity of their application and how time-consuming they are, it is inefficient to implement the above-mentioned methods [22].

On the one hand, the use of seaweed as a bio-filter for TAN, while also serving as a shelter from sunlight as well as a by-products of aquaculture, has been implemented successfully [23-28]. The integrated cultivation of *Gracilaria* sp. along with other aquaculture commodities, cannot be separated from its capability to absorb TAN in aquatic system [29-30]. This species is the best ammonia eliminator [31]. Beside of its high tolerance to a wide range of environmental conditions and efficiency in terms of cost [32], *Gracilaria* sp. is suggested to be better than other seaweed species, such as *Sargassum* sp. It is also cultivable all year round [33-34]. However, the duration of the ammonia's effect on the native organisms is more rapid than the TAN absorption by *Gracilaria* sp. [3, 34].

On the other hand, zeolite is the most common material used for controlling pollutants in a wide range of aspects [35-37]. Zeolite is an aluminosilicate mineral that is generally used for filtering molecules and catalyzing [38-39]. Furthermore, zeolite has been utilized as an absorbent to minimize the concentration of ammonium ions in the water [40]. The relatively short time absorption of ammonium by zeolite [41-42] can become an essential point when it comes to combinng this material with *Gracilaria* sp. as a means of eliminating TAN concentration in aquaculture settings.

Despite many studies in the literature being available regarding TAN elimination by using *Gracilaria* sp. [29, 31, 34, 43] and zeolite [20-22], there has been no study so far on discovering how these two materials can be combined to eliminate TAN concentration in aquatic systems. Hence, this study aims to find out the effect of the ammonia-eliminating potential of *Gracilaria* sp. and zeolite on TAN reduction in the aquatic environment. It is hypothesized that *Gracilaria* sp. and zeolite are capable of diminishing the amount of TAN concentration effectively and efficiently.

2. Material and method

2.1 Material

Thirty tanks (40x20x25 cm) filled with 10 liters of saltwater (23 ppt; 1 ppm of TAN) were treated using *Gracilaria* sp. and zeolite over 7 days in the laboratory of the Faculty of Fisheries and Marine in Airlangga University, Surabaya, Indonesia. The concentration of TAN, pH, dissolved oxygen (DO), temperature and salinity were monitored daily while the nitrogen concentration of *Gracilaria* sp. was measured before and after the treatment during the 7-day trial. *Gracilaria* sp. (1 month old) was obtained from seaweed farmers in Kelurahan Medokan Ayu, Kecamatan Rungkut-Surabaya while the zeolites (clinoptilolite; diameter: 0.5-4 cm) were brought in from Pasar Ikan Gunung Sari, Surabaya.

2.2 Method

2.2.1 Experimental design

A completely randomized design was applied in this study to test whether *Gracilaria* sp. and zeolite are effective in eliminating TAN in an aquatic system. The *Gracilaria* sp. consisted of three levels (A, B and C which is 0, 2.5 and 5 gram/L respectively) was cross-combined with zeolite that consisted of five levels (a, b, c, d and e which is 0, 5, 10, 15 and 20 grams/L respectively). Therefore, there were 15 treatment combinations that were tested with 2 replicates each (as the control treatment). In such circumstances, thirty tanks were filled with a mixture of sterile seawater and sterile freshwater (10 L; 23 ppt), and in each tank, 10 ml of ammonium hydroxide (NH_4OH) 20% was added to concentrate the water with 1 ppm of TAN. On the first day (day 1) of the trial at around 7 am, each of the combinations of *Gracilaria* sp. and zeolite were put into each tank, and in the afternoon at around 3 pm, the concentration of TAN and other water quality parameters was measured. The afternoon measurement was repeated in the same way until the end of the trial period (day 7). The nitrogen concentration of *Gracilaria* sp. was measured only at the beginning (day 1) and at the end of the experiment period (day 7).

2.2.2 Sampling

The sampling of the water for the TAN measurement was carried out by taking 10 ml of water from each tank using a glass pipette and put it into a falcon tube. Meanwhile, temperature, pH, DO and salinity were measured using a thermometer, pH meter, DO measurement kits and refractometer respectively. The sampling of *Gracilaria* sp. for nitrogen analysis was done by collecting approximately 2 grams of the seaweed before and after the 7-day trial, and putting it into a plastic container filled with sterile saltwater (23 ppt).

2.2.3 Total ammonia nitrogen (TAN) analysis

The calculation of the TAN concentration was accomplished by first measuring the ammonium absorbance through the Nessler method. A 3 ml sample was collected and added to 0.06 ml of the Nessler reagent prior to being left undisturbed for 10 minutes. Afterwards, the sample was put into a cuvette tube to measure its absorbance in a spectrophotometer-UV (Spectro Health HSP 788) at 425 nm. The determination of the ammonium concentration was done by making a standard curve. The standard curve was made by using a TAN solution standard from NH_4OH 20% stock. The absorbance of the standard solution was then measured with a spectrophotometer at 425 nm. The value of TAN itself was determined from the sum of the ammonium and ammonia concentration by previously converting the ammonium value into ammonia with a formula written in Aqueous Ammonia Equilibrium-Tabulation of Percent Un-Ionized Ammonia [44]. The concentration of the Desired Form (CDF) in this case is what is going to be found out (ammonia – NH_3) while the Concentration of Expressed Form (CEF) is what was obtained from the measurement (ammonium – NH_4^+). The value of the Conversion Factor is dependent on temperature and pH [44] as the equilibrium of the NH_3 and NH_4^+ concentration in water always changes depending on both temperature and pH [45]. Therefore, CF was obtained from Table A-1 in Aqueous Ammonia Equilibrium-Tabulation of Percent Un-Ionized Ammonia by [44].

2.2.4 Nitrogen analysis

The calculation of the nitrogen concentration was carried out by measuring the protein absorbance of *Gracilaria* sp with the UV-Absorption 280/260 nm method. The protein was extracted with 0.7N NaOH solvent [46]. The seaweed sample was crushed using a pestle and mortar, 2 grams of which was dissolved in 0.7N NaOH. The solution was heated in an oven at 50°C for 90 minutes before being filtered with filter paper. The filtrate absorbance was observed at 260 and 280 nm, and the protein concentration was calculated using the following formula from [47]. Based on a study conducted by [48], the nitrogen-protein conversion factor for *Gracilaria* sp. is 5.40.

2.2.5 Statistical analysis

The data analysis in this study was carried out using Analysis of Variance (ANOVA) with an error rate of 0.05 to see whether or not the treatments affect the response variable (TAN concentration). Post-hoc analysis was accomplished by using Tukey's test to identify which treatments were different. The statistical analysis was conducted by using software R version 3.50.

3. Results

3.1 Total ammonia nitrogen (TAN) concentration

The reduction of the TAN concentration ranged from 0.101 to 1 ppm in which Cc (5 g/L *Gracilaria* sp. and 10 g/L zeolite), Cd (5 g/L *Gracilaria* sp. and 15 g/L zeolite) and Ce (5 g/L *Gracilaria* sp. and 20 g/L zeolite) showed the highest reduction of TAN concentration over the 7 days (Fig. 1). In accordance with the calculation of nitrogen concentration, the increasing amount of nitrogen concentration of *Gracilaria* sp. ranged between 0.368 and 0.745% in which treatment Cb (5 g/L Gracilaria sp. and 5 g/L zeolite) showed the highest increasing percentage of nitrogen concentration. During the study, the pH was between 7.1 and 8.4 while the temperature ranged from 29 to 31 °C. DO was noted to be between 5 and 8 ppm, and the salinity remained stable during the study at 23 ppt. The reduction range of the TAN concentration was between 0.101 and 1 ppm, and the statistical test (ANOVA) also showed that there was a significant effect from the treatments on the reduction of the TAN concentration ($p < 0.05$). The highest reduction of the TAN concentration was Cc (5 g/L *Gracilaria* sp. and 10 g/L zeolite), Cd (5 g/L *Gracilaria* sp. and 15 g/L zeolite) and Ce (5 g/L *Gracilaria* sp. and 20 g/L zeolite) in which they were able to eliminate 100% of the TAN concentration in the aquatic system within the 7-day trial.

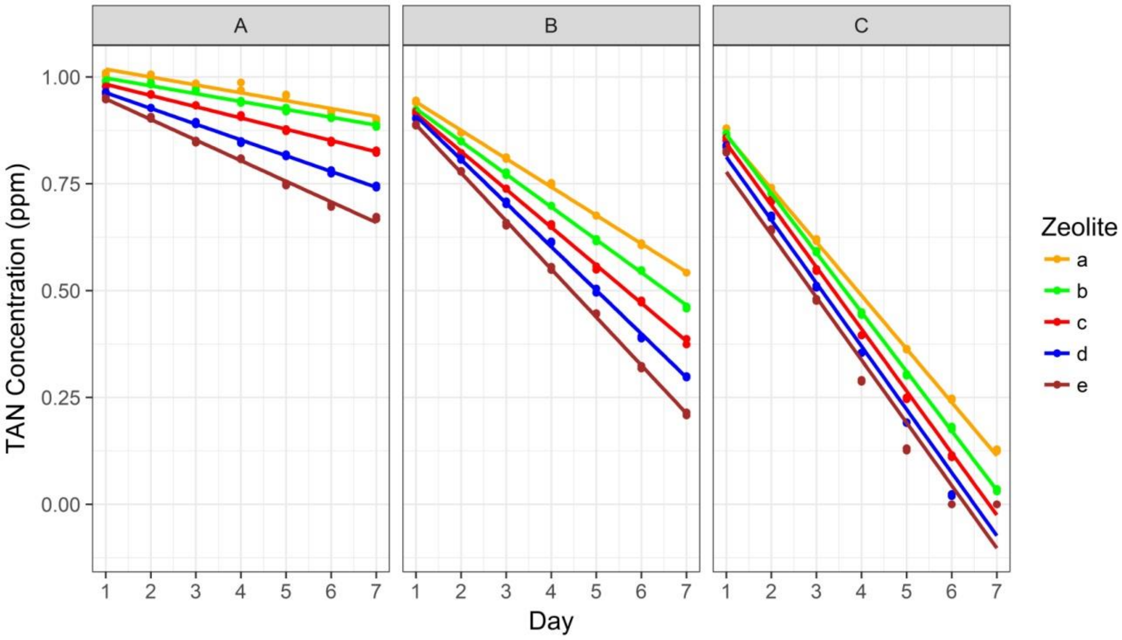

Figure 1. The concentration of Total Ammonia Nitrogen (TAN) over the 7 days of the experiment. It shows the TAN concentration after being treated with the combination of *Gracilaria* sp. and zeolite. A, B and C showed the amount of *Gracilaria* sp. to be 0, 2.5 and 5 g/L respectively while a, b, c, d and e showed the amount of zeolite to be 0, 5, 10, 15 and 20 g/L respectively. The Analysis of Variance (ANOVA) test showed the significant effect of the different treatments on TAN reduction ($p < 0.05$) while the post-hoc analysis (Tukey's test) showed that the best treatments were Cc, Cd and Ce.

3.2 Nitrogen concentration of Gracilaria sp.

Table 1. The increasing percentage of nitrogen concentration in the thalli of *Gracilaria* sp.

Treatment	The Increase of Nitrogen Concentration (%)
Ba	0.372 ± 0.0018
Bb	0.374 ± 0.0008
Bc	0.370 ± 0.0035
Bd	0.370 ± 0.0005
Be	0.371 ± 0.0011
Ca	0.734 ± 0.0052
Cb	0.745 ± 0.0002
Cc	0.710 ± 0.0033
Cd	0.653 ± 0.0031
Ce	0.608 ± 0.0028

Details: B = *Gracilaria* sp. 2.5 g/L, C = *Gracilaria* sp. 5 g/L, a = Zeolite 0 g/L, b = Zeolite 5 g/L, c = Zeolite 10 g/L, d = Zeolite 15 g/L, e = Zeolite 20 g/L

4. Discussion

The reduction range of the TAN concentration was between 0.101 and 1 ppm and the statistical test (ANOVA) also showed that there was a significant effect from the treatments on the reduction of the TAN concentration ($p < 0.05$). The highest reduction of the TAN concentration was found in Cc (5 g/L *Gracilaria* sp. and 10 g/L zeolite), Cd (5 g/L *Gracilaria* sp. and 15 g/L zeolite) and Ce (5 g/L *Gracilaria* sp. and 20 g/L zeolite), in which they were able to eliminate 100% of the TAN concentration in the aquatic system within the 7-day trial.

The significant reduction in the TAN concentration is pursuant to the study [31] that *Gracilaria* sp. has an almost unlimited capability when it comes to accumulating nitrogen in its tissue. In other words, regardless of the huge nitrogen content in the water environment, *Gracilaria* sp. will keep absorbing this element and stores it in its thalli. Indeed, *Gracilaria* sp. needs nitrogen for its growth, and the scarcity of this element will lead to the impairment of growth for the seaweed [33]. Absorbed nutrient in *Gracilaria* sp. thalli will thereafter be degraded via photosynthetic process and be assimilated to be energy [27, 31].

Meanwhile, the difference between treatments Ab (0 g/L *Gracilaria* sp. and 5 g/L zeolite), Ac (0 g/L *Gracilaria* sp. and 10 g/L zeolite), Ad (0 g/L *Gracilaria* sp. and 15 g/L zeolite) and Ae (0 g/L *Gracilaria* sp. and 20 g/L zeolite) suggest the absorption process that occurred in the matrix of zeolite. A study performed by [40] revealed that zeolite can be applied to dwindle the ammonium concentration in the water environment. This fact then means that zeolite is frequently used as a filter for closed-circulation fishponds as this material can absorb pollutants such as ammonia due to its high affinity toward ammonium ions [37, 49]. The absorption occured due to the negative charge derived from the charge difference between Si^{4+} and Al^{3+} in zeolite's structure. The negative charge can be neutralized by cations, such as NH_4^+, Na^+, Ca^{2+} and Mg^{2+} [38, 41, 50].

The control treatment of Aa (0 g/L *Gracilaria* sp. and 0 g/L zeolite) showed a depleting amount of TAN, i.e. 0.101 ± 0.003 ppm over the 7 days. Another study [14] suggested that the depletion of TAN in the aquatic system may also be from the volatility of the ammonia. Nonetheless, it is apparent that the dwindling amount of TAN during the 7-day trial was relatively light in comparison to the other treatments. Thus, it can be asserted that the depletion of TAN was because of the treatments.

According to the post-hoc test, the best treatment to use to reduce the TAN concentration in this study was treatment Cc (5 g/L *Gracilaria* sp. and 10 g/L zeolite). This is pursuant to the previous study [18] in that the more zeolite there is, the more that the pores that can absorb ammonium ions and similarly so for *Gracilaria* sp., this seaweed needs nitrogen to grow [31].

The ammonia (NH_3) concentration in the aquatic system over the 7-day experiment was between 0 and 0.0742 ppm. The concentration tended to rise following the increase in pH and temperature in the aquatic system. The concentration tended to dwindle in line with the increasing amount of either *Gracilaria* sp. or zeolite in each treatment. This is in line with the contention of other study [45] that proved that both *Gracilaria* sp. and zeolite kept absorbing and ammonium ions in the aquatic system, so then the equilibrium reaction will automatically move to the right despite the fact that the concentration of ammonia and ammonium will never disappear but approach zero in fractions following the pH and temperature conditions [51].

The percentage of nitrogen content that increases in the tissue of *Gracilaria* sp. from treatment Ba to Ce ranged from 0.370 to 0.745%. This proves that the ammonium ions were truly absorbed by the seaweed, and not due to ammonia's volatility. This is in line with the previous study conducted by [33] which stated that *Gracilaria* sp. needs nutrients, namely nitrogen for its growth.

The nitrogen content of *Gracilaria* sp. in treatment Cb (5 g/L *Gracilaria* sp. and 5 g/L zeolite) showed the highest percentage (0.745 ± 0.0002 %) while in treatment Ca, it was around 0.734 ± 0.0052 %. The fact that Ca had more *Gracilaria* sp. and no zeolite should logically mean that it had the highest nitrogen content compared to Cb, because the Ca treatment had the higher probability of *Gracilaria* sp. absorbing more ammonia without competing with zeolite. Nonetheless, the difference is only approximately 0.011%, in which it can be assumed to be in the vicinity of the volatility range.

The higher amount of nitrogen increase in treatment Ca in comparison to treatment Cb may be due to the relatively higher ammonia volatility occurring in treatment Ca. This case is relevant to the pH and temperature status of treatment Ca that was found to be relatively higher than that of Cb. The higher pH and temperature moved the equilibrium of the ammonium-ammonia to the left, so the ammonia concentration will increase in turn [52]. This circumstance will in turn enlarge the possibility of ammonia volatility. This assumption agrees with the previous study [14], contenting that pH and temperature is a major cause of ammonia volatility in an aquatic system.

During the study, the pH was between 7.1and 8.4 while the temperature ranged from 29 to 31 ^{o}C. The DO was noted to be between 5 and 8 ppm, and the salinity remained stable during the study at 23 ppt. The pH range in this study was between 7.1 and 8.4 in which it tended to increase following the increase in the seaweed concentration used in each treatment. This range is the optimum pH condition for supporting *Gracilaria* sp. growth as argued by a previous study [12], stating that the optimum pH range for *Gracilaria* sp. is between 6 and 9. This increase was predicted due to the decreasing amount of CO_2 concentration in the aquatic system as a consequence of the photosynthetic process performed by *Gracilaria* sp. In other words, the pH will increase following the depleting amount of CO_2 in the water. This assumption was in line with the previous study [7] which asserted that the carbonate equilibrium will move to the right if the amount of CO2 increases. The more H^+ ions there are, the higher the pH in the water.

The temperature during the trial ranged from 29 to 31 ^{o}C, and this was tightly related to the surrounding temperature conditions. According to the previous study [9, 31], the optimum range of temperature for *Gracilaria* sp. is between 20 and 30 ^{o}C. This reveals that the temperature condition still supported the growth of *Gracilaria* sp.

During the 7-day trial, the DO concentration ranged from 5 to 8 ppm in which the treatments that had *Gracilaria* sp. in them (treatment Ba up to Ce) showed the highest DO concentration. This escalation of DO can be observed from day 4 to day 7, and it was expected to occur due to the photosynthetic process undergone by *Gracilaria* sp., as the oxygen released by the seaweed was dissolved in the water. The DO range was also considered to be optimum as a previous study [9] also argued that 3-8 ppm for the DO range can optimally support *Gracilaria* sp. growth. The salinity during the trial was stagnant (23 ppt). This concentration is regarded to be optimal for *Gracilaria* sp. growth because this seaweed can develop optimally in a range of salinity between 17 and 40 ppt [31].

Despite some studies suggesting that the scarcity of zeolite use in saltwater is due to considering the competition with other ions, zeolite is still capable of absorbing ammonium ions in favor of other ions, such as sodium, magnesium and calcium in a saltwater environment [2, 38]. In other words, salinity, to some extent, affects the affinity of zeolite toward ammonium ions. Nevertheless, other water quality factors, such as pH, temperature and DO, will not influence the affinity rate of zeolite [2, 18].

The fact that *Gracilaria* sp. and zeolite effectively reduce the amount of TAN in aquatic systems may become an interesting point of concern, particularly for those playing a role in aquaculture-related activities. *Gracilaria* sp. is known to be efficient not only due to its year-round cultivability, high capability for ammonia absorbance and high tolerance of a wide range of environmental conditions, but it is also due to the affordability in terms of cost [32-34]. The vegetative life cycle of *Gracilaria* sp. also enables the seaweed to reproduce easily without the need for a substantial labor cost [53]. Moreover, a plethora of studies have proven the benefits of the integrated farming of seaweed with other types of aquatic organism, such as fish and shrimps [23-30].

Zeolite, on the other hand, is found to be an effective water pollutant absorbent that has a wide range of uses [35-40]. Not only can zeolite be utilized in a freshwater environment, but it can also be used in brackish water or seawater [2, 38]. Besides being efficient with respect to its capacity of absorbing ammonium ions [2], [36], [41-42], [51], [54], zeolite is also efficient in terms of cost as this material can be reused due to its cation exchanger capability [55-58].

5. Conclusion

Even though many studies have been conducted to find out the effect of either *Gracilaria* sp. or zeolite on the ammonia elimination process, there has been no study proving how the two materials can be combined in reducing TAN concentration in aquatic systems. Therefore, this study suggests that *Gracilaria* sp. and zeolite can effectively and efficiently deplete the amount of TAN in an aquatic system. Despite the fact that the control treatment also experienced a depletion in TAN concentration, it is assumed to be due to ammonia volatility. Furthermore, the increasing percentage of nitrogen content in *Gracilaria sp. thalli* also suggests that there is a process of absorption of TAN in the aquatic system. Nonetheless, in spite of the fact that the absorption process occurring in the zeolite matrix is assumed to exist referring to the difference in the treatment combinations, a further study proving the occurrence of ammonium ion binding in the matrix of the zeolite is necessary to be conducted.

Based on the study, it is interesting to discover the ammonia-eliminating potential of *Gracilaria* sp and zeolite, and whether or not they – together - have an effect on the reduction of TAN in a real aquaculture system. It is recommended to separate between the water processing area and rearing area in order to see the apparent effect of the two materials on TAN concentration.

6. References

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