Different chemical activation of humic acid from weathered coal affect the growth and development of tomatoes and cabbages

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Abstract. Different chemical activation of humic acid from weathered coal affect the growth of tomatoes and cabbage: Crop productivity is the basis of certain nutrients for human life which depends on amount of available nutrient in the soil. Humic acids (HA) are the major constituent of organic oxidation products and are formed on coal surface with various molecular weights for different oxidation conditions, and they are soluble in alkaline solutions but become insoluble in acidic solutions. HA activated coal is the new production of humic preparations to be used in the branch of agriculture in recent years. Two commonly used methods (Nitric acid oxidation method and Potassium hydroxide extraction method) were used to activate the weathered coal to find out the best method of making humic acid. We find that coals of Inner Mongolia and Shanxi after activation have more strong effect than raw materials.

1. Introduction
Weathered coal is a naturally formed brownish black, oxidized lignite, contains 50-75\% organic matter, and humic acid is the most basic component, the content of which changes from 30\% to 80\% [1-5]. Weathered coal not only can provide a variety of nutrients, but also improves the effectiveness of nutrient elements in different soil types [6-9]. It can reduce the PH and the loss of nutrients in root system, improve the availability of difficult nutrients, and reduce the loss of nutrients in the soil at the same time [10]. Weathered coal can increase the amount of cation exchange in soil and the permeability of roots. Therefore, it can be used as an accelerant for crop growth to stimulate the development of plant [11-16].
Humic acid is a kind of industrial products containing a variety of elements, which can improve the effectiveness of soil fertility and nutrient elements. Sener (2015) illustrates that humic acids can increase the uptake of nutrients by crops [17], and more important is that it can combine with trace elements (Cu, Zn, Fe, Mn etc) combining to improve the absorption, therefore, it can affect the growth and yield of crop. At the same time, it is often used to transfer and reduce the negative effects of chemical fertilizers and some chemical elements in the soil [18-20]. The application of humic acid can significantly improve the content of organic matter in soil and improve the water holding capacity of soil. Humic acid has different effects on crop yield, elemental absorption and utilization according to its origin, composition, mode of application and variety of plant varieties [21].

2. Materials and methods

2.1. Experimental sample
In this experiment, weathered coal from Inner Mongolia (IM- Innor mongolia) and Shanxi (SX-Shanxi) were subjected to acid activation and alkali activation treatment respectively. The activation treatment steps are described as follows: ① Acid treatment was used 50ml 1mol/L HNO₃ to soak the weathered coal for 1 day, then, with 300ml 0.1mol/L HNO₃ extraction, shake for 1 day, adjust the pH to 7 by analytical KOH, and put into the basin to dry; ② Alkali treatment was used 350ml 0.1mol/L KOH for extraction, then shock for 1 day, adjust the pH to 7 by HNO₃, place in the basin to dry; Content of NO₃⁻ in all sample was regulated at the same level after drying. The soil was from the Tropical Research and education center of University of Florida, soil type is calcareous marl soil (Calcareous Marl Soil), soil pH 7.32, Ec 98.29 us cm⁻¹, K 93.09mg kg⁻¹, P 98.29 mg kg⁻¹, Mg 162.87 mg kg⁻¹, Ca 21.64g kg⁻¹, SO₄²⁻ 22.83 mg kg⁻¹. Tomato varieties: Rally (Sakata); Cabbage varieties: Late Flat Dutch (Henry Fields).

Base Fertilizer: Compound Fertilizer 8-16-16 (Helena Chemical Company, FL, USA), Potassium Sulfate 0-0-50 (Crse Production Services, FL, USA). Liquid nitrogen fertilizer: N-pact (26-0-0, Loveland products, Inc., USA).

2.2. Experimental method
The field test was placed in Homestead (FL, USA), the average annual temperature is 28 ℃ and the rainfall was 1500mm. There were 7 different fertilizer treatments: CK (blank experiment), LIM (Inner Mongolia weathered coal), LIM-KOH (Inner Mongolia weathered coal activated by KOH), LIM-HNO₃ (Inner Mongolia weathered coal activated by HNO₃), LSX (Shanxi weathered coal), LSX-KOH (Shanxi weathered coal activated by KOH), LSX-HNO₃ (Shanxi weathered coal activated by HNO₃). Each test repeated four times and had 54 processing area, every area was random arrangement. Various treatments had the same application of N, P, K fertilizers, and just had the difference on the rates of HAs. Planting was single row, the distance among plants were 45 cm. In addition to the CK, the rest treatments were applied 5g IM, LIM-KON, LIM-HNO₃, LSX, LSX-KOH, LSX-HNO₃ respectively. N, P₂O₅ and K₂O application were 224, 168, 246 kg ha⁻¹. Basal fertilizer is 0.68 kg per plot, potassium sulfate is 0.1 kg per plot. In accordance with the customs of the local farmer, top-dress the liquid nitrogen in order to meet the demand of crops. The distance among plants were 45 cm. In addition to the CK, the rest treatments were applied 5 g IM, LIM-KON, LSX respectively. N, P₂O₅ and K₂O were 224, 168, 246 kg ha⁻¹. Basal fertilizer is 0.68 kg per plot, potassium sulfate is 0.1 kg per area. In accordance with the customs of the local farmer, the plants were dressed with liquid nitrogen in order to meet the demand of crops (Table1, 2).

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Table 1. Programme of fertilization in tomatoes and cabbages

| Treatments | Application of weathered coal (g·hill⁻¹) | Basal fertilizer | Top-dressing |
|------------|------------------------------------------|------------------|--------------|
|            | N-P₂O₅-K₂O (kg·plot⁻¹) | Compound fertilizer (8-16-16) (kg·plot⁻¹) | K₂SO₄ (0-0-50) (kg·plot⁻¹) | Percentage of nitrogen application (%) | Liquid nitrogen fertilizer (26-0-0) | Percentage of nitrogen application (%) | Plots | Duplications |
| Tomatoes   |                                           |                  |              |                                        |                                |                                |      |              |
| CK         | — —                                      | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 3            |
| LIM        | 5                                        | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 4            |
| LIM-KOH    | 5                                        | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 4            |
| LIM-HNO₃   | 5                                        | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 4            |
| LSX        | 5                                        | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 4            |
| LSX-KOH    | 5                                        | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 4            |
| LSX-HNO₃   | 5                                        | 224-168-246      | 0.68         | 0.1                                     | 37.52                          | 62.48                          | 4    | 4            |
| Cabbages   |                                           |                  |              |                                        |                                |                                |      |              |
| CK         | — —                                      | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 3            |
| LIM        | 5                                        | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 4            |
| LIM-KOH    | 5                                        | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 4            |
| LIM-HNO₃   | 5                                        | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 4            |
| LSX        | 5                                        | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 4            |
| LSX-KOH    | 5                                        | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 4            |
| LSX-HNO₃   | 5                                        | 196-168-168      | 0.68         | — —                                     | 42.88                          | 57.12                          | 4    | 4            |

3. Sample collection and measurement

3.1. Tomato experiment indicators and measuring method

The determination of pant height and chlorophyll of tomato were tested at seedling time, medium-term and harvest time for three times. Tomatoes were counted down from the top to the third branch blade, and to determine N, P, K, Ca, Mg etc, harvested the tomatoes met the requirement of diameter larger than 7 cm at harvest time. The adoption automatic grading equipment (Kerian Machines, Inc.) was used to grade the tomatoes to four levels: small size (<5cm), medium size (5-6cm), large size (6-8 cm), super-size (>8cm). The weight of them was weighted and the number of red and green tomatoes were counted in the end. The sugar content was measured by handheld sugar tester (Hand Held Brix Refractometer, Fisher Science), at last the whole pant was taken out, fresh weight of root and plant were weighted, measured the root diameter, separated above ground and underground parts, and dried
them into the oven. The content of N, P, K, Ca, Mg and other elements were tested, and recorded the fruit production and economic production (size>5cm).

Table 2. Programme of additional fertilizers in tomatoes and cabbages

| Topdressing | Tomatoes | Cabbage |
|-------------|----------|---------|
|             | Irrigation time(h) | Percentage of Nitrogen (%) | Amount of Topdressing (ml) | Irrigation time(h) | Percentage of Nitrogen (%) | Amount of Topdressing (ml) |
| 1           | 2.2       | 1.50%   | 227       | 2.9       | 2.00%   | 265       |
| 2           | 2         | 1.50%   | 227       | 2.5       | 3.00%   | 397       |
| 3           | 2.5       | 3.00%   | 454       | 2.4       | 4.00%   | 530       |
| 4           | 2.6       | 3.00%   | 454       | 2.5       | 4.00%   | 530       |
| 5           | 2.6       | 3.50%   | 530       | 2.5       | 4.00%   | 530       |
| 6           | 1.9       | 3.50%   | 530       | 1.7       | 4.00%   | 530       |
| 7           | 2.6       | 3.50%   | 530       | 2.5       | 3.00%   | 397       |
| 8           | 1.8       | 3.50%   | 530       | 1.7       | 3.00%   | 397       |
| 9           | 2.9       | 3.50%   | 530       | 2.7       | 5.00%   | 662       |
| 10          | 2.2       | 3.50%   | 530       | 2.1       | 5.00%   | 662       |
| 11          | 1.5       | 4.00%   | 606       | 1.3       | 5.00%   | 662       |
| 12          | 2.1       | 6.00%   | 908       | 1.7       | 5.00%   | 662       |
| 13          | 4.1       | 8.00%   | 1211      | 3.3       | 5.00%   | 662       |
| 14          | 2.5       | 8.00%   | 1211      | 1.9       | 4.00%   | 530       |
| 15          | 3.5       | 5.00%   | 757       | 3.3       | 1.00%   | 148       |
| 16          | 2.6       | 1.48%   | 224       |           |         |           |

3.2. Indicators and measuring method of the cabbage

The determination of pant height and chlorophyll of cabbage: from the apparent of cabbage, the diameter and SPAD value were test, the flower, leaf, root, vegetable and root diameter were determined. They were dried into 75°C oven, and N, P, K, Ca, Mg were tested in the end. Soil samples were measured at last term, sieved 2mm, bottled and setted aside, then measured the nutrient content.

3.3. Determination method

Vario max CNS analyzer (Elementar Analysensysteme GmbH, Germany) was used to measure the N of plant and soil. K, Ca and Mg of plant was firstly burned in muffle furnace. Shoot and root samples were also washed, dried, and analyzed for phosphorus and potassium using an Atomic Absorption Spectrophotometer (Shimadzu AA-6300, Japan). The data was subjected to statistical analysis using the SAS statistical software (version 8.0) and the mean values were compared using least significant difference (LSD) multiple range test (P ≤ 0.05) (Arancon 2006).

4. Experimental result and analysis

4.1. Effect of different treatment of weathered coal on plant height, chlorophyll, sugar content and root diameter of tomato

At the seeding of tomatoes, different treatments had a very apparent difference, the highest plants were LSX and LSX-KOH, the medium is other weathered coal treatments, the lowest was CK. This shows that LSX and LSX+KOH treatment can promote the growth of tomato seedlings better than weathered coal (activated or not activated), In the mid-term of tomato growth, the effect of each treatment on plant height was basically similar at the seedling stage, and the plant height of tomato treated with weathered coal was higher than that of CK, but the highest growth was still LSX. On the whole, the application of weathering coal can promote the mid-term growth and development of tomatoes.
During the growth and development of tomatoes, the highest height of plant was CK, but all the tomato plants treated with weathered coal were relatively short. The reason may be that all the tomatoes treated with weathered coal have more branches and grewed horizontally which was conducive to knot more fruit. However, CK treatment was still growing upward with fewer branches, which was not profitable and fruitful. Therefore, from the above results, the application of weathered coal was helpful to promote the height of tomato plant in seedling and middle stage. In the fruiting period, it helped to promote the lateral development of tomato plants and lay a foundation for the high yield of tomatoes.

The content of chlorophyll can characterize the photosynthetic capacity and nutritional status of tomatoes to some extent. From the results of Table 3, LIM+KOH treatment can promote chlorophyll content of plant leaves at all growth stage, while the differences among the other treatments were not obvious. This showed that the changes of chlorophyll content in tomato leaves were not significantly affected by other weathered coal treatments. Among the first harvested tomatoes, there was no difference in all treatments on the sugar content of tomato fruits. But the difference was obvious in the second, except for LSX treatment, the lowest sugar content is CK. The possible reason was that when the tomatoes were picked at last, the plants treated with CK were relatively old (the naked eye can see), whereas the weathered coal treated tomatoes had relatively late anti-aging effect, which can ensure the supply of nutrients in the late stage, and was favorable to increase sugar content in tomatoes. There was no obvious rule from the effect of each treatment on the root diameter of tomatoes, only LSX and LSX+KOH treatments were the worst, and there was no prominent difference among other treatments. From the above results, the treatment with weather coal can promote the growth and development of tomato seedlings and medium-term plants as a whole, and increase the sugar content in fruits at the later stage of growth and development. The application had no obvious effect on the height of different treated weathered coal compared with CK and different treatments of weathered coal had no obvious effect at the early stage of tomato. In the later stage, LIM+KOH treatment significantly increased chlorophyll, while LIM had the worst effect. At the first harvest the treatments had no prominent effect on the sugar content, but LIM+KOH, LSX and LSX+HNO₃ significantly increased their sugar content at the second harvest, and LSX+HNO₃ treatment had the highest root diameter.

Table 3. HA affect plant height, chlorophyll content, sugar content and root diameter of Tomatoes

| Treatments | Height of plant (cm) | SPAD | Sugar content (%) | root diameter (cm) |
|------------|----------------------|------|------------------|-------------------|
|            | A        | B     | C     | A      | B     | C     | C1    | C2    | C     |
| CK         | 35.67c   | 89.18c| 99.63a| 54.67a | 44.13ab| 39.37b| 3.87a | 3.50c | 1.13bc|
| LIM        | 36.76bc  | 92.28abc | 89.75bc| 50.73a | 44.13ab| 36.10c| 3.87a | 3.60bc | 1.20ab|
| LIM+KOH    | 36.68bc  | 92.01abc| 94.26abc| 49.63a | 46.47a | 42.40a| 3.87a | 3.87ab | 1.14bc|
| LIM+HNO₃  | 37.67ab  | 90.87abc| 85.51c | 50.23a | 44.17ab| 38.87bc| 4.00a | 3.80abc| 1.25a |
| LSX        | 39.00a   | 95.67a | 95.95ab| 52.67a | 44.00ab| 39.60ab| 3.93a | 3.93ab | 1.12c |
| LSX+KOH    | 36.78bc  | 94.26ab | 92.85abc| 52.03a | 43.27b | 38.73bc| 3.83a | 3.50c  | 1.13bc|
| LSX+HNO₃  | 38.78a   | 92.94abc| 96.75ab| 51.13a | 44.63ab| 38.07bc| 4.00a | 4.10a  | 1.19abc|
| LSD        | 1.67     | 4.68  | 8.88  | 5.09   | 2.59   | 2.95  | 0.18  | 0.36   | 0.08  |

Seedling time, medium-term and harvest time is A, B and C. Means in the same column followed by the same letter are not significantly different at 0.05.

4.2. Effects of different weathered coal on Tomato Yield and economic yield
The yield of tomato treated with weathered coal was significantly different from that of CK, all of them were higher than CK (Fig1). Among them, the production of LIM+KOH was the highest, which was 37.8% higher than that of CK. The other treatment with weathered coal had no significant difference. LSX was the lowest among them, but which still 13.5% higher than CK. The above results
showed that the application of weathered coal treatments whether activated or not, they can significantly increase the yield of tomatoes, LIM+KOH tomato yield was most obviously. Economic yield is one of the most direct and effective evaluation indexes to evaluate the effect of fertilization treatment. As can be reflected from Figure 2, only LIM+KOH significantly increased the economic yield of tomato and was 36.8% higher than that of the CK, the difference between others and CK was not significant and the average yield was higher than that of CK. The results showed that the application of LIM+KOH treatment can significantly increase the economic yield of tomatoes. This provided a theoretical basis for the future development of activated weathered coal manure fertilizer.

The size and weight of plants are important indicators for the synthesis and accumulation of tomato, and are also the basis and condition for the formation of fruit. Compared with other treatments, LIM+KOH significantly increased tomato plant growth and biomass accumulation with a relative increase of 26.8% in fresh weight compared to CK (Figure 3). Even though the difference between LIM, LSX+KOH, LSX+HNO₃ and CK was insignificant, the fresh weight of plants compared with CK increased by 26.8%, 14.4%, 18.3% and 13.5% respectively. As a whole, the treatment of weathered coal could increase the fresh weight of tomato plants compared with the blank treatment (CK), while the treatment with LIM+KOH had the best effect. The fresh weight of tomato roots was promoted by LSX+HNO₃ treatment, and relative fresh weight was increased by 14% compared with CK. In addition, the treatment of other weathered coal was different from that of CK, but the difference was not prominent.

![Fig. 1 HA affect the yield of Tomato](image)

Fig. 1 HA affect the yield of Tomato
**Fig. 2** HA affect economic yield of Tomato

**Fig. 3** Humic acids affect fresh weight of Tomato
4.3. Effects of different weathered coals on the diameter, chlorophyll and root diameter of cabbage

All the cabbage hearts with weathered coals were significantly larger in diameter than CK, and which reached a significant level (Table 4). The diameter of the cabbage treated with LSX was the biggest, and the ratio was 7.2% higher than that of CK. For root diameter, except for LSX+HNO$_3$, the average diameter of roots was significantly increased, but the difference between the other treatments was not significant. In addition, the treatments of weathered coal could significantly improve the chlorophyll content of cabbage compared with CK at seedling stage, and LSX+HNO$_3$ had the best effect. However, only chlorophyll content of LSX was higher in the mid-term of cabbage growth, while there was no significant difference among other treatments, but the treatments did not affect the chlorophyll content of cabbage at the late growth stage. The results show that the application of weathered coal can obviously improve the diameter of cabbage and the content of chlorophyll in the prophase of growth development.

Table 4. HA affect diameter, chlorophyll and root diameter of Cabbage

| Treatments    | Diameter (cm) | Root diameter (cm) | SPAD   |
|---------------|---------------|--------------------|--------|
|               | C             | C                  | A      | B      | C      |
| CK            | 18.83c        | 1.715ab            | 39.87c | 53.47b | 62.17a |
| LIM           | 20.14b        | 1.652bc            | 50.73a | 58.90ab| 63.93a |
| LIM+KOH       | 20.20ab       | 1.704b             | 46.3ab | 57.50ab| 63.92a |
| LIM+HNO$_3$   | 20.14b        | 1.695b             | 50.23a | 57.73ab| 60.13a |
| LSX           | 21.20a        | 1.603c             | 44.17bc| 59.53a | 60.58a |
| LSX+KOH       | 20.28ab       | 1.678b             | 48.70ab| 57.63ab| 62.10a |
| LSX+HNO$_3$   | 19.86b        | 1.776a             | 52.30a | 58.90ab| 59.13a |
| LSD           | 1.02          | 0.072              | 6.41   | 5.94   | 6.14   |

Seedling time, medium-term and harvest time is A, B and C, Means in the same column followed by the same letter are not significantly different at 0.05.
4.4. Effect of Different Treatment of Weathered Coal on Total Fresh Weight, Brassica campestris, Leaves and Root Weight of cabbage

As a whole, the treatment of all the weathered coals has a significant effect on the total fresh weight of cabbage (Fig. 5), in which the LIM+HNO$_3$ treatment has the greatest effect on the total fresh weight of cabbage, which is 13.6% more than CK, followed by LIM, LIM+KOH, LSX and LSX+KOH treatment, and increased by 7.9%, 11.4%, 8.6% and 7.1% respectively. Even the total fresh weight of cabbage with LSX+HNO$_3$ was 6.8% more than CK. The results showed that the application of weathered coal can increase the total fresh weight of cabbage, and the effect of LIM+HNO$_3$ was the best. Effect of different weathered coals on fresh weight of cabbage heart, of which the size is one of the most direct factors to evaluate its economic output. Compared with CK, all cabbage treated with weathered coal had a significant increase in fresh weight (Fig 6). Among them, LIM+ KOH, LIM+HNO$_3$ and LSX treatments increased greatly, which were 19.4%, 20.4% and 17.0% higher than CK respectively. In addition, the LIM, LSX+KOH and LSX+HNO$_3$ treatments also increased relative to CK by 10.1%, 7.1% and 13.2%. In conclusion, other treatments with weathered coal significantly increased the fresh weight of cabbage heart compared with CK. The statistical analysis showed that all treatments had no obvious effect on the fresh weight of cabbage leaves (Fig 7). From the experimental results, the root fresh weight of cabbage treated with LIM + KOH and LSX was significantly greater than that of others, and the differences among the treatments were not significant. This showed that the application of LIM+KOH and LSX in cabbage promoted the growth of its roots. In conclusion, the treatment of weathering coal could significantly improve the content of chlorophyll, the diameter, the fresh weight and other indicators of cabbage compared with CK, and the best application is LIM+ HNO$_3$.

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**Fig. 5 HA affect total fresh weight of Cabbage**
**Fig. 6** HA affect fresh weight of Cabbage

**Fig. 7** HA affect fresh weight of leaf in Cabbage
Fig. 8 HA affect fresh weight of root in Cabbage

5. Conclusion
Fruit yield, economic yield, fresh weight reflected that the different treatment of weathered coal has a significant increase in crop yield. LIM+KOH treatment significantly increased the late chlorophyll readings, fruit yield, fruit economic yield, plant fresh weight, chlorophyll readings and fresh weight of cabbage. Moreover, the LSX+KOH treatment can improve fruit yield, fruit economic yield, plant fresh weight of tomato, fresh weight and vegetable heart fresh weight of cabbage compared with CK. The results showed that both alkaline weathering coal and weathered coal in Inner Mongolia had significant effects on the shoot growth after alkali treatment. However, alkali-treated weathered coal in Inner Mongolia had a more obvious effect on shoot growth. The root diameter and root fresh weight of tomato were improved by LIM+HNO$_3$ treatment and LSX+HNO$_3$ treatment, and the root diameter was the most significant. The results showed that the weathered coal treated with acid remarkably promoted the growth of the root system, the roots became thicker at the same time. LIM+KOH treatment had the most remarkable impact on tomato yield and economic yield; while for fresh weight of cabbage, fresh fruit weight LIM+HNO$_3$ effect was more prominent. Inner Mongolia weathered coal after acid-base treatment could get more economic profit being relative to Shanxi treatment. Whether Inner Mongolia or Shanxi weathered coal after acid-base treatment, the effect was significantly stronger than not treated weathered coal. The application of different weathered coal treatment on tomato fruit yield, economic yield and fresh weight of cabbage heart had a prominent increase in yield compared with other treatment. LIM+KOH treatment significantly increased chlorophyll content, total fruit yield and fruit economic yield at the later stage of tomato. In contrast, the application of LIM+HNO$_3$ has the obvious advantage over other treatments in promoting the total fresh weight of cabbage. In addition, both weathered coal in Inner Mongolia and Shanxi weathered coal after alkali treatment significantly promoted the growth of crop shoots.

Acknowledgments
Funding was provided by Natural Science Foundation of Jiangsu Province (BK20170614), Jiangsu Agriculture Science and Technology Innovation Fund [CX (14)5074] and Science and Technology Agency of Jiangsu Province (BE2014342-1). We sincerely thank the University of Florida for its cooperation and assistance in implementing research, and the author would like to thank all partners who participated in this study.
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