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# Effect of Inoculated Baijiu Pit Mud on Anaerobic Digestion of Food Waste

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**Abstract:** In the process of anaerobic digestion (AD) of food waste (FW), anaerobic sludge is the most commonly used inoculant, but the effect of hydrolysis and acidification of FW only with anaerobic sludge as inoculant needs to be improved. There are abundant hydrolytic and acidifying microorganisms and methanogenic archaea in the Baijiu pit mud (PM), which have high compatibility with each stage of AD. In addition, the PM contains yeast, which can use the substrate to produce ethanol in the event of hypoxia, and can strengthen the methanogenic archaea to produce methane through direct intermediate electron transfer. In this paper, the effects of different proportions of PM and anaerobic sludge mixed inoculants on the AD of FW were studied. The introduction of PM as inoculants in the AD system of FW could effectively improve the hydrolysis effect of FW. Compared with the blank group, in the experimental group with anaerobic sludge =1:1, the degradation rate and hydrolysis rate of VS reached 27.93% and 38.36%, respectively, which increased by 11.63% and 8.48% compared with the blank group. This study provides a new way for the selection of FW AD vaccinations, and has important theoretical and practical significance for improving the AD performance of FW.

**Keywords:** Food Waste, Anaerobic Digestion, Baijiu Pit mud, Mixed Inoculum.

## 1. Introduction

With the development of Chinese economy and society, the output of food waste(FW) in China is increasing day by day. In 2022, the output of FW in China will account for about 120 ~ 140 million tons[1], and FW is an important part of urban solid waste, accounting for about 60%[2].The components of food and FW in different regions are basically characterized by high water content, high organic content (mainly carbohydrates, crude protein and crude fiber, etc.), high oil and fat, and have high resource utilization value[3-4].

At present, the treatment methods of FW in China mainly include landfill, heat treatment, anaerobic digestion(AD) and aerobic composting[5]. At present, landfill is a relatively traditional method to treat food and FW. This method has a large scale of disposal and convenient operation, but landfill covers a large area, which is easy to form leachate to cause secondary pollution and produce a large number of polluting gases[6-7]. Heat treatment has the advantages of large processing capacity and generating heat energy for power generation, but this method has certain requirements on the moisture content and calorific value of FW, which is easy to cause the problem of high treatment cost of FW[8]. Aerobic compost has the advantage of high resource utilization rate, but it also has disadvantages such as long compost treatment period and incomplete treatment[9]. AD is a green resource treatment technology for waste. It refers to the process of hydrolyzing and acidifying organic matter in food and FW by anaerobic microorganisms and amphoanaerobic microorganisms in an anaerobic environment to generate volatile fatty acids, and then generating methanogenic archaea to finally produce gas mixture with methane as the main component[10-11]. AD can recover biogas from biomass energy, realize the energy utilization of FW, and then further purify biogas. Renewable energy represented by biogas will occupy an important position in the future energy market.

In the process of AD of FW, the current common inoculation is anaerobic sludge. FW is rapidly hydrolyzed by the hydrolytic acidifying bacteria in the anaerobic sludge, resulting in a large amount of substances such as VFAs and ammonia nitrogen, which will lead to acid inhibition, ammonia inhibition and other problems, seriously affecting the metabolic activity of methanogenic archaea. Moreover, anabolism was further prevented by interspecific hydrogen transfer (IHT)[12], and the AD performance of FW was poor due to the inoculation of anaerobic sludge only. Baijiu pit mud(PM) is an anaerobic microbial community in the process of baijiu production, which mainly plays the role of hydrolysis and acid production in the process of baijiu production. At the same time, microorganisms such as yeast in the mud of baijiu cellar can produce ethanol by anaerobic respiration under anaerobic conditions. Studies have shown that ethanol can stimulate the interspecific electron transfer (DIET) of methanogenic archaea, and the establishment of DIET is an effective method to promote the anabolic metabolism of methanogenic microbial communities[13-14]. Therefore, inoculation of PM in the AD process of FW can not only improve the abundance of hydrolytic acidifying bacteria and methanogenic archaea in the AD system, but also strengthen the interaction between microorganisms of different sources to further improve the hydrolytic acidification efficiency of FW. In addition, anaerobic ethanol-producing microorganisms in PM can effectively alleviate the inhibitory effect of organic acids and ammonium nitrogen on the metabolic activity of methanogenic archaea, and stimulate methanogenic microorganisms to carry out DIET to enhance the methanogenic performance of AD of FW.

In this study, PM was introduced into the AD reaction system of FW. PM and the mixture of PM and anaerobic sludge were respectively used as inoculated objects for AD of FW. Yeast was used to compare PM to explore the influence of this mixed inoculated object on the AD performance of FW.

# 2. Materials and Methods

## 2.1. Materials

In this experiment, Chinese FW was collected from a university canteen in Sichuan Province, including leftovers, vegetable stalks, vegetable leaves and a small amount of ground meat scraps, etc., which were crushed into homogenate shape and stored at -4℃ for later use. Inoculated PM was taken from a baijiu factory in Sichuan Province. It was placed in a 35±0.5℃ water bath before use and cultured until it no longer produced gas. The inoculated anaerobic sludge was taken from the anaerobic tank of a sewage treatment plant in Sichuan Province. After the inoculated sludge was retrieved, it was placed at 35℃ until no gas was produced. The yeast used in the experiment was Angel high active dried yeast, the main component of which was saccharomyces cerevisiae. The main properties of experimental raw materials are shown in Table 1.

**Table 1.** Basic data on experimental materials

| | Catering waste | Food waste | Baijiu pit mud | Anaerobic sludge | Dried yeast |
|---|---|---|---|---|---|
| TS (%) | 25.39 | 10.06 | 26.42 | 7.77 | 97.75 |
| VS (%) | 24.9 | 8.87 | 3.56 | 3.65 | 92.31 |
| VS/TS (%) | 98.07 | 88.17 | 13.47 | 46.97 | 94.43 |
| TCOD (mg/L) | 225193.73 | 114384.50 | 84625.47 | 26123.07 | 1277913.00 |
| SCOD (mg/L) | 95594.64 | 54764.87 | 31404.53 | 477.97 | — |

## 2.2. Experimental apparatus

A Continuously Stirred Tank Reactor (CSTR) device was used in the experiment. The effective volume of the anaerobic fermentation acid production device is 1.5 L, the inner diameter is 12 cm, the height is 28 cm, the material is high borosilicate glass, and both sides are provided with holes for placing the pH probe and sampling the fermentation liquid, respectively. The stirring rate is set at 200 rpm/min. The intermittent stirring mode is adopted (run for 1 h, stop for 1 h), and the stirring rod is equipped with a liquid sealing device to ensure the tightness of the fermenter and run continuously during the anaerobic fermentation to produce acid.

## 2.3. Experimental design

CSTR with an effective volume of 1.5 L was used as the acid-producing phase reactor. Set up five groups according to different proportions of inoculants, The samples were collected from five separate reactors: $VS_{\text{pit mud}}:VS_{\text{anaerobic sludge}}=0:1$ (A1), $VS_{\text{pit mud}}:VS_{\text{anaerobic sludge}}=1:1$ (A2), $VS_{\text{pit mud}}:VS_{\text{anaerobic sludge}}=1:0$ (A3), $VS_{\text{dried yeast}}:VS_{\text{anaerobic sludge}}=1:1$ (A4) and $VS_{\text{dried yeast}}:VS_{\text{anaerobic sludge}}=1:0$ (A5). Then, according to the ratio of substrate to inoculant of 2:1(FW VS 20g/L, inoculant VS 10g/L), an equal amount of FW was added to each reactor, and water was added to the effective volume of the reactor, so that the total VS in the reactor was controlled to be about 3%, and the key physical and chemical indexes in the fermentation baijiu were determined and analyzed. The reactor was placed in a 35±0.5 ℃ water bath for fermentation.

## 2.4. Analysis Method

Appropriate amount of fermentation baijiu was taken, centrifuged at 8000 r/min for 10 min, and the supernatant was taken through 0.45 μm membrane. The conventional indexes TS and VS were used by gravimetric method. pH was measured by an on-line pH meter; Ammonia nitrogen was spectrophotometric with Nesser's reagent. COD was determined by potassium dichromate method. TVFA adopts spectrophotometric method.

## 2.5. Calculation method

The COD conversion rate in the hydrolytic acidification experiment of FW can be characterized by Hydrolysis rate and acidification rate. The calculation formula of hydrolysis rate and acidification rate is as follows:

$$\text{Hydrolysis (COD)}=(\text{SCOD}/\text{TCODin})*100\% \quad (1)$$

$$\text{Acidogenesis (COD)}=(\text{TVFA}/\text{TCODin})*100\% \quad (2)$$

In formula (1), SCOD is the dissolved COD in fermentation broth, mg/L; TCODin is the total amount of COD added to the fermentation system, mg/L. In formula (2), TVFA is the total volatile organic acid in fermentation broth, mg/L; TCODin is the total amount of COD added to the fermentation system, mg/L.

# 3. Results and Discussion

## 3.1. TS and VS

The change rate of TS can reflect the degree of hydrolysis of FW in the reactor. The initial and final contents and degradation rate of TS in the reactor are shown in Figure 1(a). The TS in the reactor decreased from 3.76%, 6.99%, 9.08%, 3.62%, 2.34% to 3.17%, 5.30%, 7.07%, 2.54%, 1.28%, respectively. The degradation rates of TS were 15.69%, 24.17%, 22.14%, 29.83%, 45.30%, respectively. The TS degradation effect of A5 is the most significant, which may be because compared with the complex microbial community structure in other groups, the living environment of yeast in A5 is more comfortable, and the antagonism between different microorganisms is more moderate, which can effectively hydrolyze the FW in the reactor. In addition, the TS degradation rate of the mixed inoculants was more significant than that of the single inoculants, because the mixed inoculants not only increased the abundance of hydrolytic bacteria in the reactor, but also enhanced the interaction between different types of hydrolytic microorganisms. The addition of PM provides more abundant hydrolytic bacteria for the AD of FW, and strengthens the interaction between hydrolytic microorganisms existing in the anaerobic sludge. Compared with the reactor that only uses anaerobic sludge or PM as inoculants, the hydrolysis efficiency of FW is further improved. The content and degradation rate of VS at the beginning and end of each experimental group are shown in Figure 1 (b). As can be seen from the figure, VS in each group decreased from the initial 70%, 2.90%, 2.74%, 3.00% and 2.20% to 2.26%, 2.09%, 1.99%, 1.98% and 1.12%, respectively, and the degradation rates of VS were 16.30%, 27.93%, 27.37%, 34.00% and 49.10%, respectively. The VS degradation rate of A5 was the most significant, the effect of A2 and A3 was similar, and the VS degradation rate of A1 was the lowest. In this experiment, the PM and anaerobic sludge were not pre-treated before use, and the microbial metabolic activity contained in them was low. During the operation of the reactor, the nutrients in FW could not be efficiently used for growth and metabolism, while the yeast in commercially available dried yeast had high microbial metabolic activity. At the same time, due to the low antagonism of different microorganisms in the reactor, yeast can well utilize the organic matter in the FW for growth and metabolism, and achieve a high VS degradation rate. Compared with the anaerobic sludge of traditional inoculants, the introduction of PM as the inoculant for AD of FW can effectively improve the hydrolysis efficiency of FW.

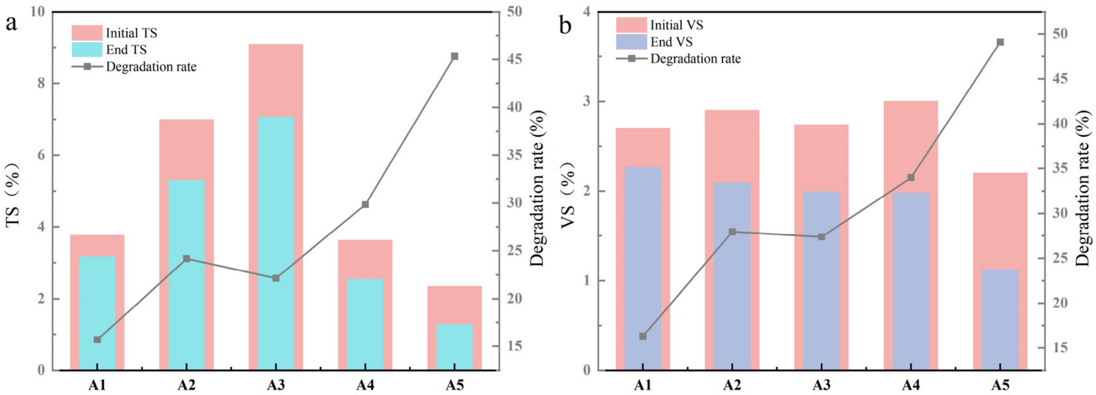


**Figure 1.** TS(a); VS(b) changes and degradation rates under different inoculum conditions

## 3.2. pH

When the pH is not in the appropriate range, it will affect the activity of microorganisms, resulting in inhibition and even death. During the experiment, the pH changes in the reactor of each group were shown in Figure 2. Except for A5, the pH changes of each group showed roughly the same trend. This was because FW was prone to acidification and decay. Acidizing microorganisms in the inoculum could use the products after hydrolysis of FW for growth and metabolism, and produce organic acids such as acetic acid and propionic acid to reduce the pH in the reactor. At the end of the reactor operation, the pH of each group was stable between 4.0-4.5. In A5, due to the lack of acidifying bacteria in the reactor, the organic matter in the FW and the ethanol produced by yeast after hydrolysis of the FW could not be further acidified, so that the pH of this group did not decrease significantly at the end of the reactor operation.

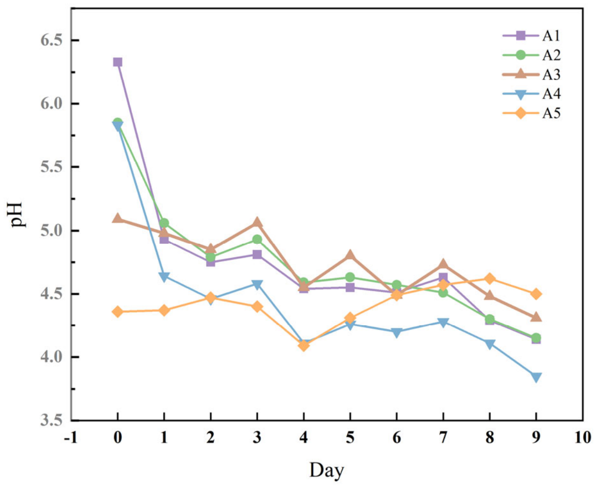


**Figure 2.** Changes in pH under different inoculum conditions

## 3.3. SCOD

SCOD concentration can directly reflect the concentration of organic matter in fermentation solution, and the change trend of SCOD is shown in Figure 3. At 1d, the SCOD of groups A2 and A3 showed a small decrease, which may be caused by the non-pretreatment activation of anaerobic sludge, and the microorganisms in the sludge consumed the organic matter in the FW to supply themselves. In the following 2-9d, SCOD of all groups showed an overall upward trend, among which the SCOD of A5 increased most obviously. It was assumed that this was due to the fact that yeast in the reactor hydrolyzed organic nutrients in FW under anaerobic conditions and produced a large amount of ethanol and $CO_2$, which greatly increased the SCOD content in the reactor. In addition, the addition of PM as the inoculant for AD of FW also has a significant effect on the improvement of SCOD in the system, which is because the hydrolytic acidifying bacteria contained in the PM improves the abundance of hydrolytic acidifying bacteria in the AD system of FW. At the same time, the interaction between different sources of hydrolytic acidification bacteria in the inoculants was established and strengthened, so that the organic matter in the FW can achieve more efficient hydrolysis and acidification.

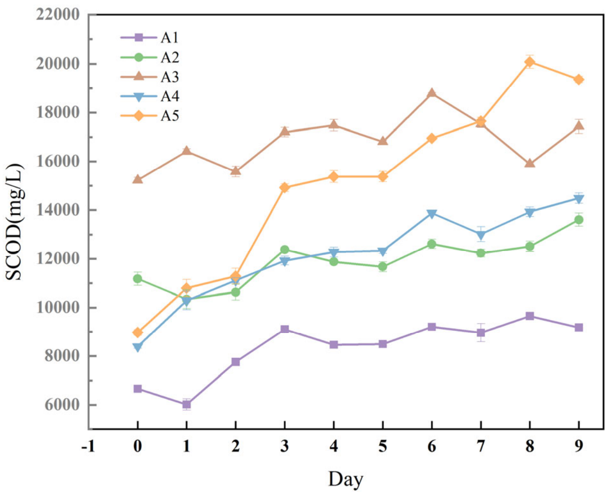


**Figure 3.** Variation of SCOD concentration under different inoculum conditions

## 3.4. $NH_4^+$-N

In the AD process of FW, most of the nitrogen in the system is degraded and converted into ammonia nitrogen in the fermentation liquid by microorganisms in the process of hydrolysis and acidification, and a small part is converted into microbial cells. The concentration of ammonia nitrogen has an important impact on the AD process, and when the concentration is lower than 200 mg/L, it can promote the efficiency of AD to a certain extent. When the concentration exceeds 1500 mg/L, the system will be inhibited[15-16]. As can be seen from Figure 4, in this experiment, except for A4 and A5, the ammonia nitrogen concentration of the other groups did not increase significantly, which was inconsistent with the expected results. However, the ammonium nitrogen concentration of each group did not reach 1500mg/L, which would not have an inhibitory effect on the AD system of FW.

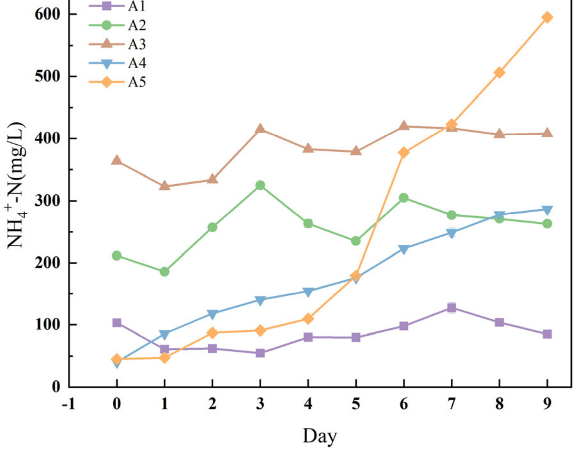


**Figure 4**. Changes in ammoniacal nitrogen content under different inoculum conditions

## 3.5. STVFA

Volatile fatty acids (VFAs) are one of the important indicators of the operation of the acid-producing reaction system. As shown in Figure 5, TVFA content in all groups increased, and at 9d, TVFA content in all groups reached 4600.42mg/L, 5471.43mg/L, 3753.69mg/L, 6457.26mg/L and 3174.36mg/L, respectively. The TVFA content of A4 was significantly improved, because microorganisms in yeast and anaerobic sludge well hydrolyzed and acidified organic matter in FW, and produced organic matter such as ethanol, acetic acid, propionic acid and butyric acid[17], which greatly increased the TVFA content in the reactor. A5 has the lowest TVFA content, which is due to the lack of acidifying bacteria in the reactor, and the ethanol produced by yeast hydrolysis of FW cannot be further acidified, which is consistent with the result of higher SCOD content and lower TVFA content. The effect of adding PM as an AD inoculation for FW is similar to that of anaerobic sludge as a single inoculation for FW. The reason may be that PM and anaerobic sludge have not been activated, and the microbial activity in them is not high, and the organic matter in FW cannot be well utilized to produce VFAs.

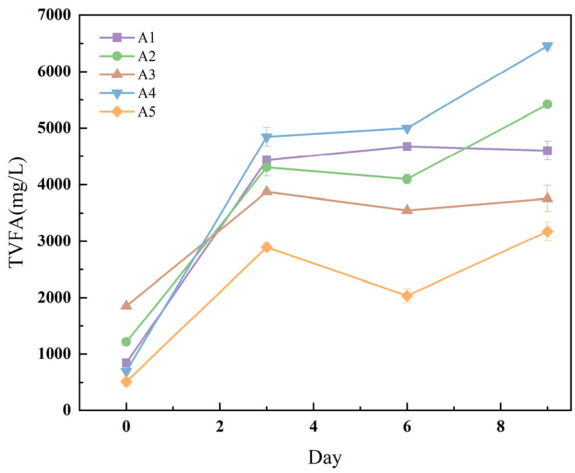


**Figure 5.** Changes in TVFA concentration under different inoculum conditions

## 3.6. Hydrolysis rate and acidification rate

As shown in Figure 6(a), the hydrolysis rates of each experimental group were 29.88%, 38.36%, 39.84%, 42.67% and 58.57%, respectively. Compared with A1, the addition of PM could significantly improve the hydrolysis rate of FW, indicating that inoculation with PM could promote the hydrolysis of FW. However, the hydrolysis rate did not increase significantly, which may be due to the fact that the hydrolytic performance of PM was limited by the part of SCOD carried by the mud. The addition of yeast has a significant effect on the hydrolysis of FW, mainly because the highly active yeast can effectively hydrolyze the organic nutrients in the FW and produce a large amount of ethanol. At the same time, yeast can further improve the hydrolysis ability of anaerobic sludge to FW and improve the hydrolysis rate of the reaction system. The acidification rates of each group were shown in Figure 6(b), which were 14.97%, 15.27%, 8.58%, 19.53% and 9.35%, respectively. The acidification rate of A2 and A1 is almost the same, while the acidification rate of A3 is significantly lower than that of other groups, which may be because although the PM can improve the AD capacity of the anaerobic sludge, there are many substances that are difficult to be acidified, resulting in the acidification difficulty of FW, so the acidification rate is low. A5 only added yeast, due to the lack of acidifying microorganisms, making the hydrolysis products of yeast acidification difficult, acidification rate is low. The inoculant mixed with yeast and anaerobic sludge can effectively improve the acidification rate of FW. This is because the addition of yeast can effectively improve the hydrolysis capacity of anaerobic sludge, and strengthen the interaction with acid-producing bacteria in anaerobic sludge to help the anaerobic sludge hydrolyze and acidify FW.

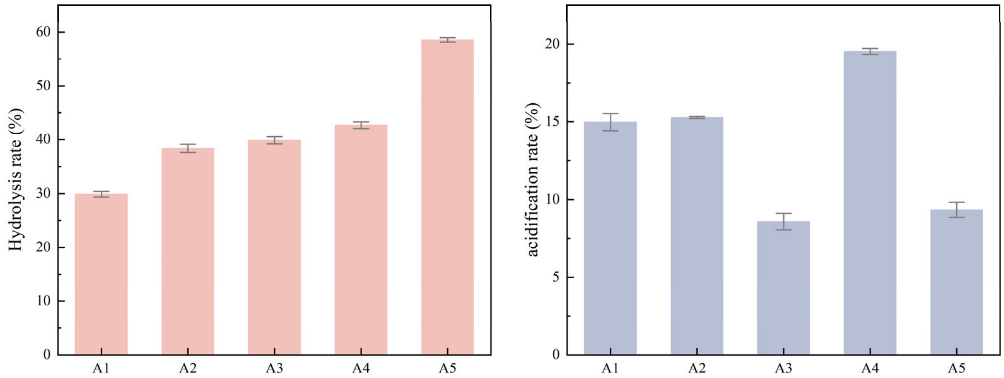


**Figure 6.** Hydrolysis and acidification rate of each group under different inoculum conditions

# 4. Conclusion

Using PM as the inoculant for AD of FW, the degradation rate of TS and VS and the hydrolysis rate of FW during AD were effectively improved. Compared with A1, the TS degradation rates of A2 and A3 increased by 8.48% and 6.45%, VS degradation rates increased by 11.63% and 11.07%, and hydrolysis rates increased by 8.48% and 9.96%, respectively.

The use of PM as the AD inoculant for FW has not played a good role in promoting the acidification of FW. Part of the reason is that the introduced PM has not been activated and its microbial metabolic activity is not satisfactory. Part of the reason is that a large number of complex organic compounds which are difficult to be acidified have been introduced into the system due to the problem of setting the ratio of PM as the inoculant. The overall acidification effect of the experimental group was poor;

A4 and A5 have more significant effect on the hydrolysis of FW than A1. Due to the lack of acidizing microorganisms in the reactor, the acidification effect of A5 group is not ideal, and the acidification rate is reduced by 5.44% compared with A1 group. Compared with A1, the hydrolysis rate of A4 is increased by 9.96%, and the acidification rate is increased by 4.56%. The introduction of yeast not only promoted the hydrolysis efficiency of FW, but also strengthened the interaction with acid-producing bacteria in anaerobic sludge, and enhanced the acidification effect of inoculants on substrates.

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