

## Isolation and Preliminary Identification of Fermentation Bacteria from Activated Sludge of an AFB of Treating Slaughter Wastewater

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**Abstract.** The fermentation bacteria was obtained from activated sludge in an AFB reactor of treating slaughter wastewater and its culture conditions were researched. It showed that this fermentation bacteria grew best at 37~42°C temperature for 28d, its morphology observation, physiology and biochemistry experiment were done in the experiment. At last, this bacteria was added to the running AFB reactor, the result showed that the start-up time of the AFB reactor was shortened and the treatment efficiency increased about 18%.

**Keywords:** anaerobic fluid blanket (AFB); activated sludge; fermentation bacteria; isolation and preliminary identification Introduction

### 1. Introduction

The process of slaughter wastewater treated in an AFB (anaerobic fluid bed) reactor in fact is an anaerobic digestion process. Bryant and Zeikus [1,2] advanced the anaerobic digestion process three stage theory and four class group theories separately, and thought that the entire anaerobic digestion process may divide into 3 stages, namely hydrolysis fermentation stage, producing hydrogen and producing ethanoic acid stage and producing methane stage. At first, the organic matter produced ethyl alcohol, the propionic acid, the butyric acid, the lactic acid and so on, by the digestion function of fermentation bacterium, then be converted into ethanoic acid and H<sub>2</sub>/CO<sub>2</sub> by hydrogen producing bacteria and ethanoic acid bacteria. At last, it was converted into CH<sub>4</sub> and CO<sub>2</sub> by methanogens. The anaerobic fermentation bacteria were consisted of fermentation bacteria, homoacetogenic bacteria and methanogens in this theory. Obviously, the fermentation bacteria were the most important microorganism in early treatment of wastewater. It is great realistic significance to isolate fermentation bacteria to improve the efficiency of reactor treating wastewater.

Fermentation bacteria, also being called hydrolysis acidification bacterium, is mainly special anaerobic and facultative anaerobe bacterium, and belong to the heterotrophic bacteria. Its generation cycle is short, reproduces one generation was reproduced during several minutes. In the anaerobic digestion system, the fermentation bacterium's function is mainly degrading the macro-molecule insoluble organic matter into the smaller molecular soluble matter [3]. Early in 20 century 70 ages, the 18 fermentation bacteriums and 51 fermentation species once were reported. The cultivation conditions, producing hydrogen mechanism and the succession law, of the hydrogen producing bacteria, were researched further deeply by some domestic and foreign researchers [4-8]. However, it's till a blank that the research about fermentation bacteria of activated sludge treating slaughter wastewater in an AFB reactor.

### 2. Materials and Methods

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## 2.1. Microorganisms origin and media

The microorganisms originates from active sludge in the middle of the steady AFB reactor of treating Slaughter wastewater. Enrichment medium(1L) was consisted of 2% glucose ,0.5% specially designed, 0.05% yeast juice, 0.05% L - cysteine, 0.3% beef anoint,0.05%  $K_2HPO_4$ , 0.001%  $FeSO_4 \cdot 7H_2O$ , 0.5% NaCl, 0.05% $MgSO_4 \cdot 7H_2O$  ,0.5% $Na_2S$ ,and 20% slaughter wastewater in distilled water (w/v), Media pH values were adjusted to 7.0 prior to sterilization. The composition of separation and purification media were the same with the Enrichment media only additional to 2.5% agar powder. Physiological and biochemical identification media referenced related literature[9]. 1% resazurin sodium salt were added to the media as anaerobic indicator[9,11].

## 2.2. Enrichment cultivation

Active sludge 10ml was put in sterilized flask containing about 30-40 glass-beads and filled with nitrogen,shaked water bath for 1h at 37°C and 120rpm. In order to break bacterium group.Then the broken active sludge were put in the 500ml flask containing 90ml enrichment medium. it were cultivated until enough cells counted in microscope.

## 2.3. Separation and purification

The cultivating liquid were shifted out 1ml and added into the tube containing 9ml sterilized physiological saline and for 10 times auto-merging diluted until  $10^{-3} \sim 10^{-24}$ times. Diluted bacteria liquid 0.2ml were spreaded to Petri dishes to separate and purificate aim strains.The operation above was repeated 5-10 time until the morphology and size of bacterialcolony and individual were single in microscope by “solid-liquid” repeatedly.

## 2.4. Morphological and physiological and biochemical identification

Morphologica identification such as the morphology and size of bacterialcolony and individual were carried out. physiological and biochemical identification include Gram's staining test, sugar fermentation, methyl red test and acetyl methyl methanol test, nitrate reducing test,  $H_2S$  production test, gelatin hydrolysis test, citric acid salt growth test and indoles production test. Specific methods refer to literature[12].

# 3. Results and Discussion

## 3.1. Effect of time and temperature on enrichment cultivation

In the experimental,the optimum time and temperature were researched in bacteria enrichment cultivation.As shown in fig.1,when the temperature changed ranging of 37~42°C,the bacteria cell maximum number reached  $2.8 \times 10^8$  cell/mL.The optimum time,as shown in fig.2,were 28d.After 28d,the number of bacteria growth is not apparent, and even reduce,because the bacteria had autolysis phenomenon along with the cultivation time increased.

## 3.2. Determiation to degree of dilution

Degrees of dilution altogether 25 between  $10^{-3}$  and  $10^{-24}$  were done. Bacteria levitation liquid 0.2ml were taken to vaccinate separately on the solid medium ,cultivated for 28d at 39°C.As shown in fig.3,when the degree of dilution was in the range of  $10^{-13} \sim 10^{-18}$ ,there was single colony .so the degree of dilution was selected to separate and purificate fermentation bacterium .

## 3.3. Morphology identification

The fermentation bacterium was vaccinated to various medium such as slaughter wastewater, beef extract and PYG fermentation fluid , which grew well in each liquid medium , best especially in PYG fermentation fluid.Just shown in fig.3 , the morphology of bacterialcolony : closer dry petite colony, mostly hollow mycelium plane,and gear shape edge structure. The community growth were proliferation when the bacterium was vaccinated in basemi-solid uprights Column by puncture ways, which indicated that this mycelium had the capsule, after being dyed by wet ink , examed by microscopic, the background was gray, the mycelium was dark, there was a bright transparent circle surrounding periphery.Compared with the *Bacillus subtilis*, the bacterium was also dyed with bud spore dyeing way, the result is the same with *Bacillus subtilis*, which

showed that the bacterium had bud spore .observed in microscope,the Individual morphology,as shown in fig.4,were Bacillu ,its size was 3.1~8.2µm .

### 3.4. Physiological and biochemical identification

Compared with escherichia coli and bacillus subtilis ,when gram's staining ,the time of bacteria cultivation cannot exceed 24h, otherwise it would be false negatie. As shown in table.1,it was Gram-positie organisms,can produce acid and gas ,belonged to the kind of bacteia producing both acid and gas ,methyl red for methyl red positive, acetyl methyl methanol test negative, can use citric acid salt, gelatin using ability are relatively weak. The gases were analysed by gas chromatographic,it showed that , Among them for 7.4% H<sub>2</sub>, 1.2% CO<sub>2</sub> ,0.5 % H<sub>2</sub>S, the rest of the unknown gases stayed to analysis to identify .

### 3.5. Performance tests of fermentation bacteria in slaughter wastewater treatment

The fermentation bacteria was thrown into the AFB reactor treating slaughter wastewater. The better effect was obtained after being domesticated for a period of time, .As showed in fig.5,the start-up time of AFB reactor was shorted greatly after adding the isolated fermentation bacterium. Treatment efficiency increases about 18%,which indicated that, this bacterium had higher adaptability,and domesticated a period time it became dominant fungi decomposing insoluble organic macromolecular of organic wastewater into small molecule soluble substances . Which prepared well for further being decomposed and metabolic of organic wastewater

Table1 Appraisal result of physiology and biochemistry of fermentation bacteria

Kinds of test	phenomina	conclusion
Gram's staining test	purple	++
sugar fermentation test	join B.C.P reagents,yellow, bubble	Producing acid and gas
methyl red test	red	++
acetyl methyl methanol test	medium color	-
nitrate reducing test	blue	-
H <sub>2</sub> S production test	A little black	-
gelatin hydrolysis test	weak	-
citric acid salt growth test	blue dimming	-
indoles production test	without rosy color Ether layer	-

Note: +: positive, ++: stronger positive; -: negative, --: stronger Negative

## 4. Conclusions

The fermentation baterium was obtained from activated sludge in AFB reactor treating slaughter wastewater by the technology of improved Hungate roll tube .it was bacillu and Gram-positie organisms, belonged to the kind of bacteia producing both acid and gas.At the temperature 37-42°C,cultured for 28d,the fermentation bacterium growthed better.which showed that the fermentation baterium adapted to the slaughter wastewater quickly when thrown into AFB reactor again.shorting the stard-up time of reactor and increasing the efficiency about 18% of treatment slaughter wastewater. Of curse ,It was important to keep the balance and metabolic synergy between the fermentation bacteria and methanogens in reactor of highest efficient treatment wastewater ,which requires further in-depth studies.

## 5. Reference

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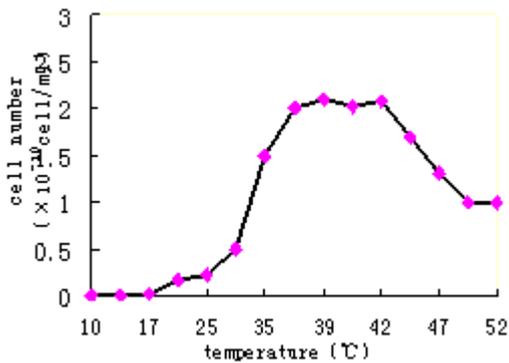


Fig. 1 The number of fermentation bacteria changed with various temperature

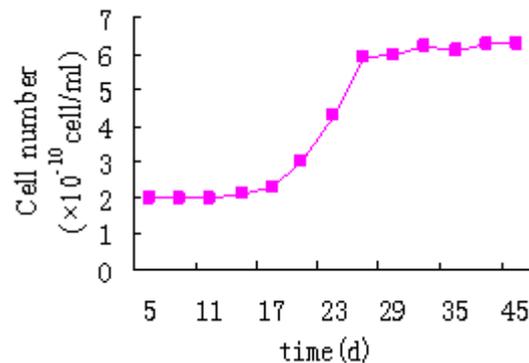


Fig. 2 Number of fermentation bacteria changed with various time



Fig3 Growth of fermentation bacteria at different microbial dilution



Fig4 Mophology observation configuration of fermentation bacteria (10×100)

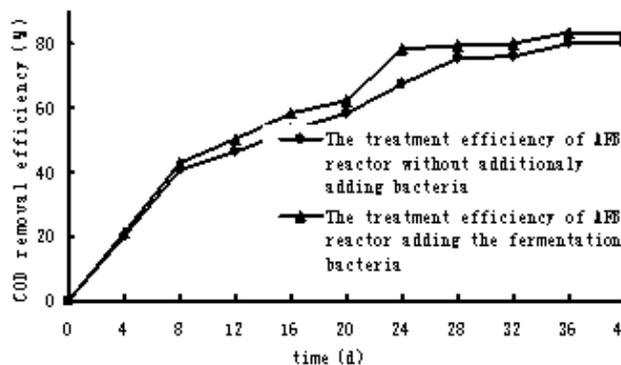


Fig. 5 Comparison of treatment efficiency of AFB reactor between adding fermentation bacteria and not adding