CHEMICAL ENGINEERING TRANSACTIONS

VOL. 59, 2017

A publication of AIDIC

The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Zhuo Yang, Junjie Ba, Jing Pan
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ISBN 978-88-95608- 49-5; **ISSN** 2283-9216

DOI: 10.3303/CET1759031

The Conditions Optimization of Separation Screening and Degradation for Formaldehyde Degrading Bacteria In Soil

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In order to isolate five kinds of bacteria which can degrade the formaldehyde and optimize the degradation conditions, the paper has done the following works: the formaldehyde degrading bacteria were selected in Yanshan campus of Guilin University of Technology after enrichment culture, screening, re-screening and purification. The strain which had the best degradation ability was used for fermentation conditions optimization by single factor experiment. The optimum fermentation conditions of a strain formaldehyde degrading bacteria isolated on purpose are as follows: the inoculation amount is 0.20%, the temperature is 30℃, the initial pH value is 7.0, aldehyde addition is every 100ml medium with 0.020ml formaldehyde and speed is 140r/min. Conclusion: under certain fermentation conditions, the formaldehyde degrading bacteria has a certain ability of formaldehyde degradation.

1. Introduction

In today's society, formaldehyde as a binder is widely used in a variety of decoration materials. Thus increasing the harm of formaldehyde on the environmental pollution and humans (Huang et al., 2009). Formaldehyde with a pungent odor is a colorless, soluble gas. Formaldehyde can be absorbed through the respiratory tract and its aqueous solution can be absorbed through the digestive tract. This can cause great harm to the human body. In addition, formaldehyde is high toxic substances. It ranks the top priority list of toxic chemicals in China. Furthermore, the International Cancer Research Institute also identified formaldehyde as suspicious carcinogens. It can cause air pollution or addition, oxidation, reduction and polymerization reactions (Amato et al., 2007). The contact parts could absorb and degrade the formaldehyde when the human body contact it. It reacts with proteins and nucleic acids when formaldehyde enters the human body. Then it not only damage DNA, but also can produce the following phenomena that mutations of nuclear gene, formation of DNA single strand, binding between DNA and protein as well as inhibition of DNA damage reparation. Besides, formaldehyde can combine with amino resulting in the changes and coagulation of internal protein structure. So the metabolism of human cells are disrupted (Cao et al., 2010).
At present, the research on transformation of formaldehyde microbial is abroad and more progress. Low concentration formaldehyde conversion bacteria have been reported in early times. In recent years, high concentration formaldehyde conversion bacteria have been reported occasionally. However, the study of formaldehyde conversion mold is very rare. It is reported that most of the formaldehyde degrading bacteria isolated from nature are bacteria and a small number of fungi (Saeed et al., 2005). The types of formaldehyde degrading bacteria are Pseudomonas aeruginosa, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Pseudomonas testosterone, Methylotrophic bacteria, etc. They can grow in the environment of formaldehyde as the sole carbon source and energy. And some bacteria have very strong ability to degrade formaldehyde. In addition, nitrifying bacteria and denitrifying bacteria can also degrade formaldehyde under the conditions of adding methanol. In the high concentration of formaldehyde environment, formaldehyde tolerance of fungi can grow and reduce the concentration of formaldehyde to a very low or tend to zero. The fungi that are capable of degrading formaldehyde are separated yellow race, Yurimoto etc. The research is rare in treating waste gas of high concentrations industrial formaldehyde. The currently reported strains focus on its ability to detect the ability of degrading formaldehyde wastewater (Ou et

Please cite this article as: Ziyuan Li, Haiyun Li, Huixiang Zhang, Xiuli Wang, Liangmao Wei, 2017, The conditions optimization of separation screening and degradation for formaldehyde degrading bacteria in soil, Chemical Engineering Transactions, 59, 181-186 DOI:10.3303/CET1759031

al., 2008; Wang et al., 2007). Now, there are many ways to purify formaldehyde exhaust gas, such as: chemical absorption method, adsorption method, photocatalytic oxidation method, ozone oxidation method, discharge plasma method, formaldehyde capture agent and other purification methods. However, these methods cannot be very good promoted and applied because of poor processing effect, high cost, secondary pollution and other factors. It can be seen, the purification of formaldehyde exhaust gas is still a very hot issue and problem. Air pollution control technology research has received great attention in the domestic and international countries.

2. Research methods

2.1 Experimental materials and reagents

Instrument: U-1800S visible spectrophotometer, biochemical incubator and so on.
Soil samples: from the Yanshan campus of Guilin Technology University.
Formaldehyde, sodium chloride, ethanol, acetylacetone, glacial acetic acid is analytical grade.
Beef extract, peptone, agar powder are biochemical reagents.
Formaldehyde stock solution: take 0.42ml formaldehyde, diluted with distilled water to 150ml.
Formaldehyde solution: temporary use, the formaldehyde standard stock solution diluted with water to 10μg/ml formaldehyde (Yang et al., 2009; Teng et al., 2010).

2.2 Experimental methods

(1) Dilution of soil
Weight 10g soil samples accurately, add 90ml sterile water, shake 15min, stand for 20s in the aseptic operation room.
(2) Preliminary screening of formaldehyde degrading bacteria
The 0.1ml soil supernatant was uniformly applied to the formaldehyde mediums which contain 0.1ml, 0.08ml, 0.06ml, 0.04 ml and 0.02 ml formaldehyde respectively, and placed inverted in a biochemical incubator at 30°C to cultivate.
(3) Formaldehyde degrading bacteria re-screening
Numbered the different single colony strains obtained by preliminary screening, sieved in beef culture medium, formaldehyde medium respectively, placed inverted in a biochemical incubator at 30°C to cultivate.
(4) Purification of formaldehyde degrading bacteria
The single colony strains on the re-screening medium were uniformly coated on the beef extract peptone medium and the formaldehyde medium, and placed inverted in a biochemical incubator at 30°C to cultivate.
(5) Activation of formaldehyde degrading bacteria
Inoculated a ring of formaldehyde the degradation of bacteria after purification to the liquid beef extract peptone medium in the clean bench with a ring, and placed in a 30℃ shaking incubator to culture.
(6) Measurement of cell concentration
The absorbance values of the degrading bacteria were measured by the TU-1800S visible spectrophotometer at 600 nm. The culture medium was used as the contrast. And recorded the data.
(7) Selection of Optimum Formaldehyde Degradation Bacteria
Added 0.03 ml formaldehyde solution in the sterilized 150ml liquid beef paste peptone medium and shook it up. Shook with a pipette of 0.45ml in 150ml formaldehyde liquid medium. Each strain was made three parallel and placed in a shaking incubator at 30°C and 140 r/min. The absorbance values of the fermentation broth were measured by acetylacetone spectrophotometry every 12 h to screen out the best formaldehyde degradation bacteria.
(8) Drawing of formaldehyde standard curve
Took 7 clean test tubes and add 0.00ml, 0.50ml, 1.00ml, 1.50ml, 2.00ml, 2.50ml, 3.00ml formaldehyde standard solution to the labeled tube with pipettes. The water was added to the scale 10ml. Then added 2ml acetyl acetone solution to each test tube and mixed it well. Then wrapped the membrane in the test tube and heated it 10 min in the boiling water. Removed it and cooled it to room temperature. The absorbance of each tube was measured by TU-1800S visible spectrophotometer at 414 nm wavelength and pure water as contrast. Drew the standard curve with the absorbance Y as the ordinate, the standard solution content X (μg) of formaldehyde as the abscissa (Xue et al., 2011; Yao et al., 2009).
(9) Determination of Formaldehyde in Fermentation Broth by acetylacetone spectrophotometry
Transferred the corresponding 10 ml of the bacterial solution to the corresponding centrifuge tube with a pipette. Then poured the removed broth into the corresponding tube and added 2.00ml of acetylacetone solution to each tube with a pipetting gun, mixed at last. Recorded data according to the above method that selected formaldehyde-free medium as a contrast.

(10) Optimization of fermentation conditions for formaldehyde-degrading bacteria
1. Selection of the best inoculation amount
Added 100 ml of liquid medium with a sterilized pH of 7.0 to 0.02ml of formaldehyde solution and shook it well. Added 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml of bacteria in the liquid medium and shook. Each inoculation was made three parallel and placed in a shaking incubator at 30°C and 140 r/min. The absorbance values of the fermentation broth were measured by acetylacetone spectrophotometry every 12h.
2. Selection of optimal initial pH
Added 100ml of the liquid medium with the pH of 5.0, 6.0, 7.0, 8.0 and 9.0 to 0.02ml formaldehyde solution respectively and shook it well. Accessed to bacteria according to the best inoculation amount. Each inoculation was made three parallel and placed in a shaking incubator at 30°C and 140 r/min. The absorbance values of the fermentation broth were measured by acetylacetone spectrophotometry every 12 h.
3. Selection of optimum culture temperature
Added 100ml of the liquid medium at the optimum initial pH to 0.02 ml formaldehyde solution and shook well. Accessed to bacteria according to the best inoculation amount. Each bacteria was made three parallel and placed in incubator at 24°C, 27°C, 30°C, 33°C, 36°C and 140 r/min to cultivate. The absorbance values of the fermentation broth were measured by acetylacetone spectrophotometry every 12h.
4. Selection of the added optimum amount of formaldehyde
Added 100 ml of the liquid medium to 0.005ml, 0.01ml, 0.02ml, 0.03ml, 0.04ml formaldehyde solution at the optimum initial pH and shook well. Accessed to bacteria according to the best inoculation amount. Each inoculation was made three parallel and placed in the optimum temperature of the incubator at 140 r / min. The absorbance values of the fermentation broth were measured by acetylacetone spectrophotometry every 12 h.
5. The choice best speed
Added the optimum amount of 100 ml of the liquid medium to the formaldehyde solution at the initial pH and shook it well. Accessed to bacteria according to the best inoculation amount. And then placed in the optimum temperature of the incubator at a speed of 60 r/min, 100 r/min, 140 r/min, 180 r/min and 220 r/min to culture.

3. Results and Analysis

3.1 Screening of formaldehyde degrading bacteria

The soil supernatant liquid was applied to different formaldehyde content medium. Each plate grew bacteria with only 0.02ml formaldehyde added. A total of five strains with different colony morphology were obtained. That are Formaldehyde degradation bacteria 1, formaldehyde degradation bacteria 2, formaldehyde degradation bacteria 3, formaldehyde degradation bacteria 4 and formaldehyde degradation bacteria 5. Then the 5 strains were screened and purified.

3.2 formaldehyde standard curve drawing

According to the method in 2.2, the standard curve is drawn with formaldehyde concentration μg as abscissa and absorbance value as ordinate, as shown in Figure 1

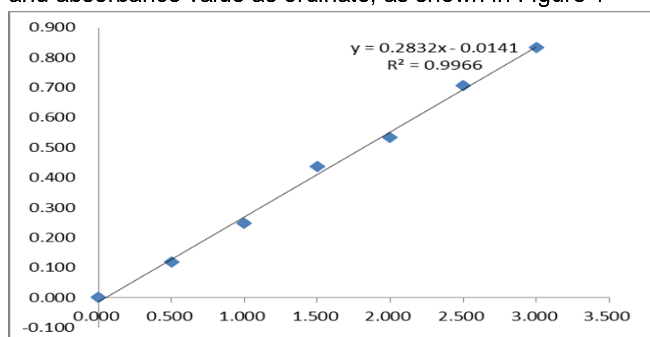

Figure 1: Standard curve of formaldehyde

The absorbance was measured at the maximum absorption wavelength 414nm, and the regression equation between absorbance y and formaldehyde content x (mu g) was obtained: $y=0.2832x-0.0141$ ($R2=0.9966$).

3.3 Screening of optimum formaldehyde degrading bacteria

According to the method of 2.2, each formaldehyde degrading bacteria was degraded by formaldehyde fermentation, and the degradation result of each formaldehyde degrading bacteria was shown as figure 2. Formaldehyde degrading bacteria 1 was inoculated in liquid medium for one week and was not grew. Formaldehyde degrading bacteria 4 inoculated in liquid medium showed granular growth, and the growth was also very slow. Therefore, the fermentation data of three strains were obtained. Compared with formaldehyde degrading bacteria 3 and 5, the degradation rate of formaldehyde degrading bacteria 2 was smaller. Although the formaldehyde degradation bacteria 5 and formaldehyde degradation bacteria 3 had the same initial capacity of decomposition formaldehyde, but the degradation of bacteria is still very obvious in No. 3 bacteria after 24 hours, and 5 bacteria did not degrade formaldehyde. So we selected No. 3 bacteria for our optimal strain.

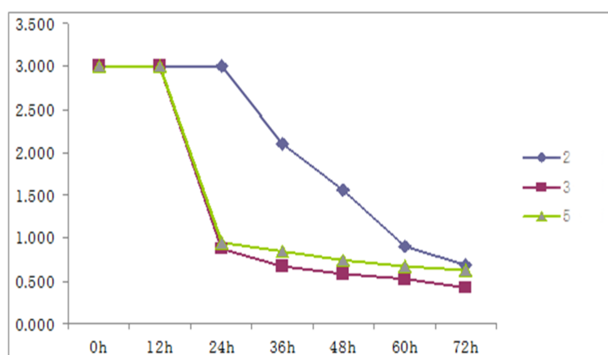

Figure 2: Average absorbance value curves of three strains of fermentation liquor of different time

3.4 Conditions optimization of for formaldehyde - degrading bacteria

(1) Amount determination of the best inoculation

The absorbance values of formaldehyde degrading bacteria 3 inoculated with different inoculation fermentation culture in 48 hours as shown in Figure 3.

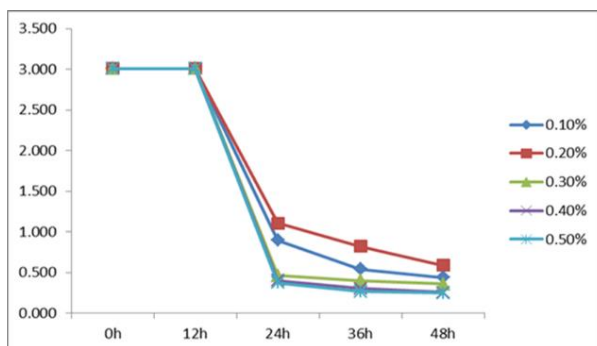

Figure 3: The average absorbance value curves of different inoculation quantity within 48 hours

It can be seen from the figure 3, the degradation rate of formaldehyde degradation bacteria increases when the inoculation amount increased, but the increase is smaller and smaller. The degradation rate is basically same when inoculation amount are 0.30%, 0.40% and 0.50%. We chose 0.30% for the best inoculation amount of the bacteria taking the cost of production into consideration.

(2) Determination of optimum pH

The absorbance values of formaldehyde degrading bacteria 3 with different initial pH fermentation culture within 48 hours as shown in Figure 4.

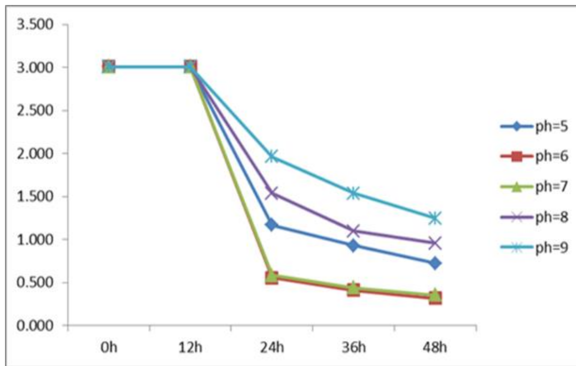

Figure 4: The average absorbance values curve of different initial pH value of the fermented liquid within 48 hours

As can be seen from the above table and graph, the degradation rate of formaldehyde-degrading bacteria increases first and then decreases when the pH value increases. When the degradation rate is basically the same when the value of pH is 7 or 8. We chose the value of 7 as the initial pH for the bacteria.
(3) Selection of optimum culture temperature
The absorbance values of formaldehyde degradation bacteria 3 at different temperatures in the fermentation culture within 48 hours as shown in Figure 5.

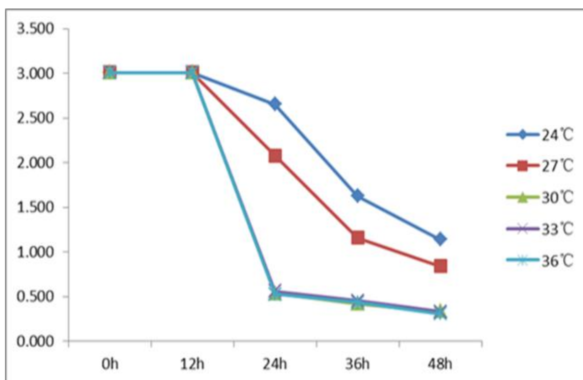

Figure 5: The average absorbance value curves of different raises the temperature of the fermented liquid within 48 hours of

It can be seen from the figure, the degradation rate of formaldehyde-degrading bacteria increased with the increase of culture temperature. But the degradation rate is basically unchanged when the incubation temperature is more than 30 degrees. So we choose 30 degrees for the best culture temperature of the bacteria.
(4) Determination of the best formaldehyde content
The absorbance average values of the formaldehyde degrading bacteria 3 with different formaldehyde addition amount of fermented liquid within 48 hours of as shown in Figure 6.
It can be seen from the figure, the degradation rate of formaldehyde degradation bacteria with 100ml beef extract peptone culture medium increased first and then reduced with the increase amount of formaldehyde. Due to the beginning of formaldehyde content is too low, the cell growth of course will become faster. The degradation rate reach maximum when added the amount of 0.01ml formaldehyde. However, we choose 100ml beef extract peptone culture medium by adding formaldehyde 0.02ml as our best formaldehyde taking the actual needs of production into account.
(5) The choice of the best speed
The absorbance values of formaldehyde degradation bacteria 3 in different speed of the incubator incubation within 48 hours as is shown in Figure 7.

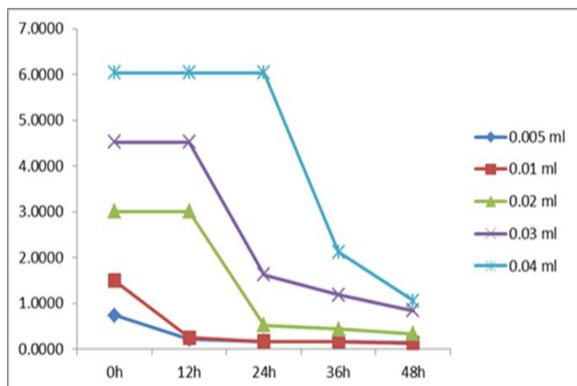

Figure 6: The average value curves of the different formaldehyde addition amount of fermented liquid within 48 hours of absorbance

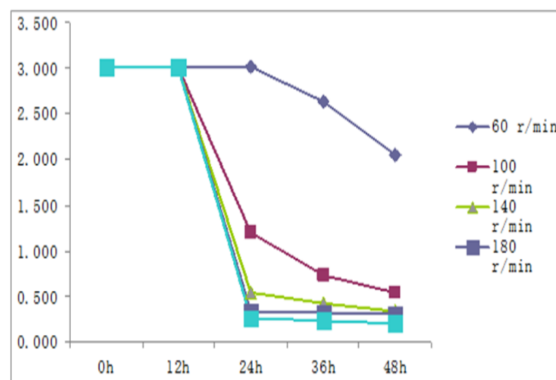

Figure 7: The average absorbance value curves of different speed of fermented liquid within 48 hours

It can be seen from the figure, the degradation rate of formaldehyde degradation bacteria increased with the increase of incubator speed. But the increase in the rate of degradation is getting smaller. And when the speed is 140r/min or more the degradation rate of formaldehyde degradation bacteria is basically the same. So we choose 140r/min as the best speed of the bacteria.

4. Conclusions

(1) Five strains of bacteria which have the ability of degrading formaldehyde were isolated, and one of them was stronger than the other four strains; (2) The optimum conditions for the degradation of the bacteria were: the inoculation amount: 0.20%, the culture temperature: 30°C, the initial pH: 7.0, the amount of formaldehyde: 0.020ml formaldehyde per 100ml medium, the speed: 140r/min.

The experimental study of formaldehyde degradation bacteria has a certain reference significance to the related research.

Acknowledgments

Supported by the National Natural Science Foundation of China (No.31460409).

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