Study on fermentation kinetics and extraction process of rhamnolipid production by papermaking wastewater

Keer Yu
School of Environmental Science and Engineering, Zhejiang Gongshang University, Hangzhou, 310012 China

Abstract. Paper mill wastewater (PMW) is the outlet water generated during pulp and papermaking process in the paper industry. Fermentation by wastewater can lower the cost of production as well as alleviate the pressure of wastewater treatment. Rhamnolipids find broad placations as natural surfactants. This paper studied the rhamnolipids fermentation by employing Pseudomonas aeruginosa isolated by the laboratory, and determined to use wastewater which filtered by medium speed filter paper and strain Z2, the culture conditions were optimized, based on the flask shaking fermentation. On the basis of 5L tank fermentation, batch fermentation was carried out, the yield of fermentation reached 7.067g/L and the fermentation kinetics model of cell growth, product formation and substrate consumption was established by using origin software, and the fermentation process could be simulated well. And studied on the extraction process of rhamnolipids, through fermentation dynamic equation analysis can predict the in fill material yield can be further improved. Research on the extraction process of rhamnolipid simplifies the operation of extraction, and lays the foundation for the industrial extraction.

1. Introduction
With the progress of the paper industry, papermaking wastewater has become one of the major environmental pollution sources. At present, many surfactants are petrochemical products, easily lead to environmental pollution. And more and more consumers prefer to use biological materials to produce surfactants. In this study, the biosurfactant rhamnolipid and rhamnolipid obtained by the fermentation of Pseudomonas aeruginosa were effective in removing pesticides, petroleum and heavy metals from water and soil, which also increased the effect of rhamnolipid in the environment The application of rhamnolipid can be biodegradable, low toxicity and antiviral, which is to make rhamnolipid more widely used in the medical market, and because rhamnol has a good emulsifying property, Wet and other characteristics, it is in agriculture and other industries have a great market.

In this experiment, the fermentation and fermentation of the fermentation broth and colorimetric, the screening can use the papermaking wastewater fermentation of high yield of bio-surfactant strains, optimize the culture medium and improve the production activity of the strain. On the basis of 5L fermentor, the fermentation kinetics was studied, and the fermentation kinetics model was established by using origin software, and the extraction process of rhamnolipid was optimized. The extraction process of rhamnolipid was studied, and the crude extract of rhamnolipid with high purity was obtained, which could be applied to industrialized mass production.
Currently on the market price of rhamnolipid concentrate is high, it can be said that the market prospects are good. Increased use of rhamnolipid in the field of personal care (with good wetting and foaming) and increased use in the cleaning industry as a biosurfactant (which can keep the environment safe) will drive the rhamnolipid market development. Therefore, this paper has great research and commercial value for the fermentation kinetics of rhamnolipid fermentation and its extraction process.

2. Experimental method

2.1. Treatment of Integrated Wastewater from Papermaking

Treatment 1: The papermaking wastewater will be allowed to stand for 24 hours, and the supernatant (the waste water is treated as the supernatant).

Treatment 2: Use medium-speed filter paper to filter paper integrated wastewater (this waste treatment is called filtration).

Treatment 3: direct use of papermaking wastewater (this waste water treatment called the original liquid).

Water quality analysis was performed by 5B-1 (V8) intelligent multi-parameter digestion and 5B-3BN (V8) total nitrogen analyzer. Activate the strain as a fermentative strain. A 3% (volume percent) seed culture was inoculated into the medium. The fermentation broth was shaken and sampled for analysis.

2.2. Determination of fermentation yield

After adding 20 mL of deionizer water to 100 μL of diesel oil to form a film, add 10 μL of fermentation broth to the center of the oil film and measure the diameter of the drain ring with an oil standard caliper (precision 0.02 mm). If the fermentation stock oil drainage ring exceeds the diameter of the culture dish, the fermentation broth is diluted 10 times. By spectrophotometer, spectrophotometer in the range of 300-500nm spectral scanning, take the maximum absorbance of the corresponding maximum absorption wavelength, and the determination of the concentration of absorbance, the origin of the concentration and absorbance value of the linear regression, to be Regression equation.

2.3. 5L fermentor fermentation

According to the results of single factor experiment, 3.5 L wastewater fermentation medium was packed in 5 L liquid fermentation tank. The amount of Z2 seed solution was 5%, the frying oil was 2%, the stirring speed was 200r / min, the ventilation volume was 3L / min, the fermentation temperature was 30 ℃, and the culture was carried out for 4 days.

The fermentor was bandaged with 8 layers of gauze and sterilized at 121 ℃ for 20 min. Parameter setting set on the fermentation temperature of 30 ℃, automatic; Fermentation of the stirring speed 200 r / min, automatic. Fixed electrode, thermometer, and connect the pipes. Inoculation ignites the flame ring; open the mouth, quickly into the seed liquid. Measure the drainage ring, biomass, rhamnolipid content and residual oil volume every 12h. After 4 d, the fermentation was finished.

2.4. Study on Extraction and Purification Methods

The pretreated fermentation broth was concentrated to 1/3 of the volume using a rotary evaporator, and the fermentation broth was adjusted to 2 or less with 6 mol / L hydrochloric acid and allowed to stand overnight at 4 ℃. After pouring into the separator funnel, add 1/3 of the volume of ethyl acetate fermentation broth, fully mixed, put it aside. Take the upper yellow clear liquid. The lower turbidity solution was concentrated at 80 ℃. Get the paste. After extraction and extraction, the supernatant was distilled to obtain crude rhamnolipid. After 3 replicates, the combined extracts were dried at 85 ℃ to further remove the ethyl acetate.
2.5. Study on Fermentation Kinetics

The kinetic model of cell growth, product synthesis and carbon source consumption was constructed by optimizing the dynamic parameters and nonlinear fitting of Logistic equation and Luedking-Piret equation. The experimental data were processed.

In this experiment, the content of rhamnolipid was determined by phenol sulfuric acid method. The fermentation broth was centrifuged at 8000rpm / min for 5min, and the biomass was measured. The biomass was measured by 5mL fermentation broth.

3. Experimental results

3.1. Determination of Nutritional Elements in Papermaking Integrated Wastewater

| Measure the project | Total phosphorus content mg/L | Ammonia nitrogen content mg/L | COD mg/L | Total phosphorus content mg/L |
|---------------------|-------------------------------|-------------------------------|----------|-------------------------------|
| Precipitation supernatant | 0.27a | 0.368a | 618b | 0.27a |
| Filter | 0.225a | 0.360b | 200c | 0.225a |
| Liquid | 0.175a | 0.521b | 783a | 0.175a |

Wastewater is containing phosphorus, the waste water after treatment of the total phosphorus content of the water is not much difference, can be intuitively seen: the supernatant in the total phosphorus content> filtered wastewater in the total phosphorus content> Total phosphorus content. Wastewater treatment options were determined by single factor experiments.

The total nitrogen content of the wastewater after the treated water is almost the same: the total nitrogen content in the supernatant of the precipitate> the total nitrogen content in the filtered wastewater> the total nitrogen content in the stock solution. The wastewater treatment options were continued by single factor experiments.

The COD value of the filtered waste water sample is significantly reduced, and it can be seen visually from Fig. 3 that the COD value in the supernatant of the precipitate is the COD value in the filtered wastewater> the COD value in the stock solution. The wastewater treatment options were continued by single factor experiments.

3.2. Single factor experiment

It can be seen from the measured value of the oil discharge ring, the row of the fermentation broth of the strain Z2 is higher than the value of the oil drainage ring of the fermentation broth 105-1, and the difference is also remarkable. But also intuitively reflects the Z2 fermentation better. The yield of rhamnolipose produced by the fermentation broth of the strain Z2 was higher than that produced by the fermentation broth 105-1, and the results of the combination of the oil drainage were more intuitively reflected. The fermentation effect of Z2 was better. Therefore, follow-up experiments were Z2 as a fermentative strain.

The measured value of the oil drainage ring of the treated wastewater was significantly higher than that of the other two treatment methods, and it was verified that the waste water was filtered after the treatment of the fermentation effect is better. And the measured value of the absorbance of the three kinds of treated wastewater combined with the measured value of the oil discharge circle shows that the measured value of the absorbency of the filtered wastewater fermentation solution is obviously higher than that measured by the other two treatment methods. Therefore, follow-up experiments using filtered wastewater.
3.3. 5L fermentor fermentation

The excretion circle, biomass, rhamnolipid content and residual oil were measured every 12 h, and the fermentation was finished after 4 days. With time, intuitively reflects the overall upward trend. Changes in 24h-60h are particularly pronounced. Oil drain ring gradually increased the size of the oil drain from 3.12mm to 32.87mm.

![Fig. 1 Determination of the fermentation broth of 5L fermentation tank](image1)

The rhamnolipid was produced by strain Z2, and the biomass, rhamnolipid yield and residual oil were measured every 12 h after fermentation for 4 days. The yield of rhamnolipid was approximately 7.067 g/L. The results are shown in Fig2.

![Fig. 2 The process of rhamnolipid fermentation](image2)

Fig. 2 shows that when the seed liquid of strain Z2 was inoculated into the fermentation medium, the growth rate of the cells was slower. 12h after the bacteria growth rate, this time rhamnolipid fat production is also growing rapidly. The yield of rhamnolipid was gradually decreased with the decrease of carbon content, and the cells were gradually used as a carbon source supplemented with some metabolites rhamnolipid period.
3.4. Study on Extraction Methods of Products
As a result of pretreatment with centrifugal operation, too cumbersome, is not conducive to industrial production, so you can use the filter method to remove the large particles in the fermentation broth, the test, after 8 layers of gauze filtered fermentation broth, large particles basically removed, The subsequent fermentation broth is acidified and washed with precipitation in the form of precipitation. After standing for 24 hours, you can precipitate the vast majority of impurities. This separation process eliminates the need for centrifugal steps, increasing the convenience of large-scale production, through the two extraction distillation that is to remove most of the impurities, saving consumption, suitable for large-scale industrial production operations.

3.5. Establishment of Fermentation Kinetics Model

3.5.1. Cell growth kinetics. According to the characteristics of cell growth under batch fermentation conditions, the growth curve of bacteria showed S-curve. Logistic function is a non-linear model S-shaped function. It is suitable for the process of cell growth.

The commonly used S equation describes the bacterial growth process during the fermentation process:

\[
\frac{dX}{dt} = rX \left(1 - \frac{X}{X_m}\right)
\]

Points to get,

\[
X = \frac{X_0 + X_m e^{rt}}{X_0 + X_m - X_0 e^{rt}}
\]

\(X_0\)-initial cell concentration, \(X_m\) - maximum cell concentration

Using the origin software to regression the equation, we get the equation parameters as follows: \(X_0=24.44\text{g/L}, X_m=57.05\text{g/L}, r=0.1817\). The parameters are taken into equation (2) to obtain the curve equation of biomass change with time:

\[
X = \frac{1394.302 e^{0.1817t}}{23.61 + 24.44 e^{0.1817t}}
\]

Figure 3 is the process of simulating the concentration of cells with time. The regression equation \(R^2 = 0.99639\) shows that the equation can well describe the growth of the cells.

![Fig. 3 logistic model to fit the growth curve of bacteria](image-url)
3.5.2. Synthesis of Rhamnolipid. The Luedeking-Piret equation, L-P model, was used to describe the production process of rhamnolipid.

\[
\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x
\]  

(4)

In the formula: \( P \) is the yield of rhamnolipid (g / L), \( \alpha, \beta \) is the parameter to be determined, and \( X \) is the cell concentration (g / L).

Equation (1) and (2) into equation (4), the integral operation is carried out to obtain:

\[
P = P_0 \frac{X_m \beta}{r} \ln \frac{X_m - X + X e^{\alpha t}}{X_0} - \frac{\alpha X_0 (X_m - X)(e^{\alpha t} - 1)}{X_m - X_0 + X e^{\alpha t}}
\]  

(5)

In the formula: \( P \) is the yield of rhamnolipid (g / L), \( P_0 \) is the initial amount of rhamnolipid (g / L), \( X \) is the amount of bacteria, \( X_m \) is the maximum cell volume (g / L), \( X_0 \) is the initial cell volume (g / L), \( r \) is the maximum specific growth rate (h-1), and \( t \) is time (h). The regression analysis of formula (5) was carried out by using the data of rhamnolipid production. The parameters were as follows: \( P_0 = -0.492, \alpha = 0.06993, \beta = 0.04411 \)

Rhamnolipid synthesis kinetics model is:

\[
P = 13.85 \ln \left( \frac{19.94 + 14.97 e^{0.157t}}{57.05} \right) - \frac{55.734(e^{0.157t} - 1)}{32.61 + 24.44 e^{0.157t}}
\]  

(6)

The experimental data and the fitting degree of the model \( R^2 = 0.99335 \), indicating that the model can better describe the strain Z2 fermentation production of rhamnolipid situation, as shown in Figure 4.

![Fig.4 The model fitting of rhamnolipid synthesis process of L-P curve](image)

4. Summary
In this paper, the technological conditions for the production of rhamnolipid were studied, and the fermentation kinetics and extraction and separation methods were studied. The following conclusions were drawn: the single factor experiment was used to select the method of treatment of bacteria and wastewater. The yield of Z2 rhamnolipid was higher, and the fermentation effect of the waste water after filtering by medium speed filter paper was better.

The fermentation kinetics of rhamnolipid was about 7.067 g / L by 5L fermentor, and the fermentation kinetics model of cell growth, product formation and substrate consumption was established. The model was able to simulate well Fermentation process, by analyzing the equation of
fermentation power, can be used to predict whether the feed yield can be further improved. And optimize the extraction process of rhamnolipid. The extraction process of rhamnolipid was studied by filtration and the crude extract of rhamnolipid was extracted by low-toxicity ethyl acetate. The purification step was simplified and applied to large-scale production, which had great application prospect.

In this study, we used Logistic and Luedeking-Piret equations to establish the kinetic model of cell growth, rhamnolipid formation and carbon source consumption during the fermentation process. And can be a good simulation of the fermentation process, to a certain extent, reveals its fermentation kinetics, can be used to predict the fermentation process of bacteria, products, substrate concentration, so as to optimize the fermentation process conditions for the factory scale production Theoretical reference.

This separation process eliminates the need for separation of the commonly used centrifugal operation, which for the factory to provide a large-scale production convenience, through multiple extractions can remove most of the impurities, eliminating the need for complicated steps for large-scale industrial production operations.

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