Fast Hydrolysis for Determination of Lipopeptides on Solid Surface

Shi-Zhong Yang a, Jing You a,b, Wen-Rui Kang a, Bo-Zhong Mu a *

a State Key Laboratory of Bioreactor Engineering and Institute of Applied Chemistry, East China University of Science and Technology, Shanghai 200237, China;
b Petroleum Production Engineering Research Institute of Huabei Oilfield Company, CNPC, Renqiu 062552, Hebei Province, China
Contents

1 Introduction
2 Experiment
3 Results and Discussion
4 Conclusion
1 Introduction

1.1 lipopeptides

Cyclic ones:

Linear ones:

R–CH–(CH$_2$)$_n$–CO $\rightarrow$ A$_1$ $\rightarrow$ A$_2$

\[ \text{O(NH)} - \text{Am} \leftarrow \ldots \leftarrow A_3 \]

R – C – NH – A$_1$ $\rightarrow$ A$_2$ $\rightarrow$ A$_3$

\[ \text{O} \leftarrow \ldots \]

A$_1$ $\rightarrow$ A$_2$ $\rightarrow$ $\ldots$ $\rightarrow$ cA$_1$ $\rightarrow$ cA$_2$ $\rightarrow$ cA$_3$

\[ \text{C=O} \leftarrow \ldots \leftarrow cAm \leftarrow \ldots \leftarrow \]

R$_1$OCH$_2$CHCH$_2$SCH$_2$CHCOGly-Gly-Gly-R

OR$_2$ NHR$_3$
1 Introduction

Lipopeptides

Foods

Detergents

Green production

Environmentally friendly

Comestics

Surface-surficial activities

Environment

Chemical Engineering

Low contents in samples

Mixture

No sensitive methods
1 Introduction

Sufactin, a lipopeptide was used in EOR

12000 t/a
1 Introduction

Problem:

- Mixed System
  - HPAM 0.12%
  - Lipopeptide 0.4%
  - Na₂CO₃ 0.6%
  - Petroleum sulphonate 0.4%

High Activity
Low Usage
Strong Interference

Importance

Separation and quantification
1 Introduction

Separation method was established

- Solvent Extraction
  - Distribution equilibrium
  - Hydrolysis
  - Labeling
- HPLC
- Distribution coefficient
- Separation method
- Pure lipopeptide
1 Introduction

1.2 Lipopeptide determination

Separation

- Acid precipitation
- Solvent extraction
- Chromatography

Quantification

- HPLC
  - Natural ultraviolet absorbance
  - Low sensitivity
  - Needing pure standard
- Spectrometry of Amino acid
  - Solvent separation
  - Hydrolysis
- GC-MS
  - Hydrolysis
  - Trimethylsilylation
1 Introduction

Object

1 Val
1 Asp
4 Leu
1 Glu

Amino acid could be labelled with dansyl
1 Introduction

HPLC of Labelled Amino Acids Released by Hydrolysis of Lipopeptide
1.3 Lipopeptide Hydrolysis

Tranditional method stems from protein hydrolysis

Hydrolysis Condition: in 6 M HCl at 110°C for 24 h

It has been improved to

Hydrolysis Condition: in 6M HC at 90°C for 24 h

At relative low temperature, but for long time and needing post treatment of evaporating the acid water solution
2 Experiment

Anino acids will be labeled by dansyl
Stainless Steel Reaction Kettle
lined PTFE

Sample was stained
HCl solution was filled separately
Sealed
Heated
Labled directly
Determined
3 Results and Discussion

Orthogonal experimental design for optimal condition

3.1 Effect trend of heating temperatures

Index increased sharply with temperature increase
3 Results and Discussion

3.2 Effect trend of acid concentrations

Index increased sharply then slowly with acidity increase.
3.3 Effect trend of acid quantities

Index increased sharply then decreased slowly with acid amount.
The deviations were 18, 12 and 6 for temperature, acidity and acid amount, respectively.

The acidity was 12 M, could not increase.

The acid amount should be fixed at 0.5 mL.

The hydrolysis temperature and time will be modified for good results.
3 Results and Discussion

At different temperature using 0.50 mL 12 M HC for 4 H

Index was almost stable between 110-150 celsius degree
3 Results and Discussion

For different time at 150 °C using 0.50 mL 12 M HC

Index was almost stable after 4 h hydrolysis.
3 Results and Discussion

3.4 good results at optimal condition

0.5 mL of 12 M HCl, hydrolysis at 150°C for 4 h

Good linear relationship could be obtained

Linear equation: $y = 12.15c$
Linear coefficient: $r^2 = 0.9940$
# 3 Results and Discussion

## 3.5 Results of Determining Samples

Hydrolysis was applied in determining samples.

Determination results using hydrolysis method:

<table>
<thead>
<tr>
<th>Method</th>
<th>Parallel samples</th>
<th>( n = 5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value</td>
<td>7.05</td>
<td></td>
</tr>
<tr>
<td>( S )</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>RSD(%)</td>
<td>6.63</td>
<td></td>
</tr>
<tr>
<td>Working curve</td>
<td>( y = 12.15c )</td>
<td></td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>92.1</td>
<td></td>
</tr>
</tbody>
</table>
4 Conclusion

Acidity, acid amount, temperature and time were evaluated through orthogonal experimental design to resolve the long time and post-treatment of lipopeptide hydrolysis.

The optimal condition of hydrolysis was determined: at 150°C and 0.50 mL 12 M HCl for 4 h, conducted on gas-solid surface.
The hydrolysis time of lipopeptides was shortened from 24 to 4 h.

The hydrolysis post-treatment, such as evaporating solvent, was eliminated.
Thank You!

敬请指正！