



The application of process mass spectrometer in human-like collagen production by recombinant *Escherichia coli*

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ABSTRACT

In this study, the exhaust analyzer (EA) and process mass spectrometer (PMS) were employed to investigate the influence of Human-like collagen (HLC) production. As a result, the biomass and HLC production had an obvious increase by the PMS. Using this measuring method the final optical density and HLC concentration were 152 and 8.02 g/L, respectively. Additionally, the concentration of acetate in the medium was at a lower level compared with that by the EA. The PMS was quickly and accurately to the feedback of the dissolved oxygen. Hence, the result showed that the cells were cultivated in a relative stabilized environment by the PMS, which could harvested a high level of HLC and cell density.

Key words: process mass spectrometer, *Escherichia coli*, fermentation, human-like collagen

INTRODUCTION

Collagen, mainly existing in the skin, tendon and vasculature, was an important structural protein which had the functions of supporting organs and protecting tissues [1]. Human-like collagen (HLC), expressed by the recombinant *E. coli* BL21, was a kind of polymer biological and water-soluble protein. It also had the biological activity and tri-helix structure. There was a series of biological feature in HLC, such as favorable biocompatibility, cell adhesion, acceleration of new cell and epithelial cell growing and hemostatic performance. The low immunogenicity, no risk of virus risk and high modifiability were the advantages in the HLC compared with the natural collagen protein from animals [2].

During the fermentation, there was much available information in the off-gas. As a normal analysis method, the exhaust analyzer (EA) was used to measure the oxygen and carbon dioxide in the off-gas. However, there were some deviations to regulate the cell growth and production of target protein, such as poor stability, the frequent correction and the lengthy response time. Furthermore, the gas flow and ambient humidity also were the negative influence during the detection.

However, the process mass spectrometer (PMS), employed to measure the change of the oxygen and carbon dioxide in the off-gas, had a high measuring accuracy and low excursion. The PMS not only could detect the concentration of oxygen and carbon dioxide, but also could analyze the production of volatile substance in the fermentation [3]. Some indirect parameters were also obtained by the PMS, such as oxygen uptake rate (OUR), carbon dioxide release rate (CER) and respiratory quotient (RQ). These parameters were used to regulate the fermentation in order to achieve a high yield of target protein.

In this study, the PMS was used to measure the oxygen and carbon dioxide in the off-gas. The concentration of acetate and residual glucose in the medium was detected by the two methods, PMS and EA. As the result, the excretion of acetate was at a lower level by PMA and the concentration of glucose in the medium was maintained a stabilized level. The PMA also could enhance the HLC production.

EXPERIMENTAL SECTION

Strain and medium

Recombinant *E. coli* BL21 3.7, carrying plasmid pNWCP31 and expressing HLC, was used in this study. The strain was constructed and preserved in our laboratory [4]. It contained the kanamycin resistance gene and allowed high temperature induction [5].

All solutions were prepared in the deionized water. The seed medium contained 10 g/L of glucose, 5 g/L of yeast extract, 10 g/L of peptone and 10 g/L of NaCl. The compositions of the batch medium and feeding medium were listed in Table 1.

Table 1 Medium compositions

Component	Batch medium (g/L)	Feeding medium (g/L)
Glucose	20.0	1000.0
Yeast extract	50.0	400.0
K ₂ HPO ₄	5.6	26.0
NaH ₂ PO ₄	3.4	12.0
MgSO ₄	2.5	20.0
(NH ₄) ₂ SO ₄	4.2	16.5
EDTA	0.8	3.0
Trace element	0.8	3.0
Antifoam	0.1	-

Seed culture

Primary seed cultures were inoculated from Luria-Bertani (LB) plates into the 300 mL flask containing 80 mL seed medium and cultivated for 10~12 hours at 34°C and 220 rpm. Secondary seed cultures were prepared by inoculating 15 mL of primary seed cultures into 80 mL seed medium and cultivated for 8~10 hours when the optical density at 600 nm (OD_{600 nm}) reached 4.0~5.0 [6].

Fed-batch Culture

A total of 640 mL of seed culture medium was inoculated into a 30 L fermentor (Model L1523, Bioengineering Co., Switzerland) with 16 L of sterile batch medium at 34°C. Dissolved oxygen (DO) level was maintained around 25% saturation by increasing the stirring speed and supplying air enriched with pure O₂. The pH value was maintained at 6.8 by the automatic addition of NH₄OH (25%, w/w) at the feeding stage. As soon as the initial glucose was depleted, the feeding culture was fed into the fermentor. The compositions of the batch medium and feeding medium were showed above. When the OD_{600 nm} reached around 90, the cultivation temperature was increased to 42 °C and maintained for 6 hours for induction to achieve a high-level expression of the target protein [7].

Analysis methods

Cell density was measured at 600 nm with a spectrophotometer (UNICO Model no. 2082PCS, USA). The cell concentration was measured by the dry cell weight (DCW): 50 mL of the broth samples was centrifuged (10 min, 10000 rpm), and then washed three times with the distilled water and dried to a constant mass in a 105°C oven. The concentration of glucose and acetate was measured using a BioProfile analyzer 300A (NOVA biomedical, USA). The concentration of HLC was determined by the hydroxyproline colorimetry [8].

RESULTS AND DISCUSSION

There were four growth phases observed in the fermentation of recombinant *E. coli* BL21. Phase 1 was the lag phase that cells needed to adapt the fermentation medium environment. Phase 2 was the exponential phase of the cells at the maximum specific growth rate, which ended with the glucose exhaustion in the batch medium. The two phases were named the batch stage. Phase 3 was the feeding stage, which was supplied the glucose, yeast extract and inorganic salt at a suitable rate in order to achieve a high level of the cell density. Phase 4 was the protein synthesis phase. The cultivation temperature was raised to 42°C to induce the synthesis of the HLC protein [6].

The accurate concentration of the oxygen and carbon dioxide in the fermentation

During the phase 1 and phase 2, dissolved oxygen (DO) was maintained around 20~30% saturation by increasing

the stirring speed and supplying air enriched with pure oxygen. The cells could grow rapidly in the medium with a large number of nutrients. When the culture entered the phase 3, the cells grew at a relatively high specific growth rate. As shown in Fig. 1, the concentration of oxygen and carbon dioxide was at a stable level used the PMS. Generally, the rate of feeding was adjusted to maintain a suitable level of DO and pH value. There was no obvious change of DO increased the rate of feeding. It caused the excretion of the key by-product (acetate), which was disadvantaged to the cell growth and synthesis of target protein [9]. Fig. 1 showed that the concentration of oxygen had a significant change in the four phases. In the phase 4, the concentration of oxygen in the off-gas fell slowly, as well as the rapid rising of carbon dioxide. It was speculated that a large of oxygen was used to the synthesis of HLC.

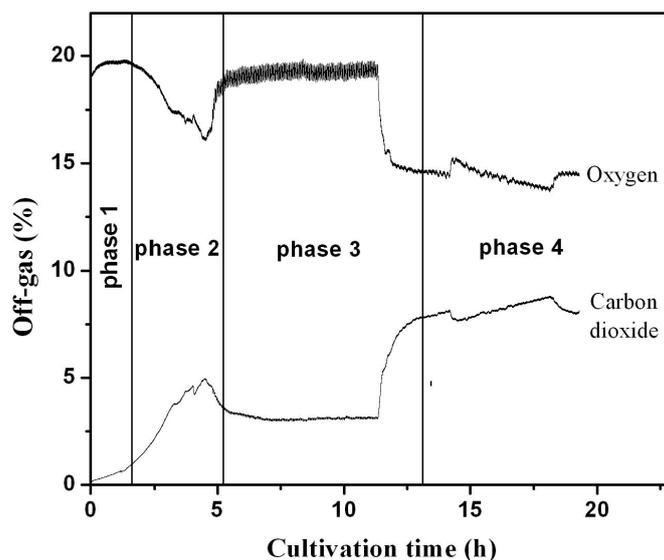


Fig. 1 The concentration of oxygen and carbon dioxide in the off-gas

Effect of the different measure apparatus on the excretion of acetate

In the batch stage, the two measure apparatus displayed similarly curve tendency (Fig. 2). However, the accumulation rate of acetate had a remarkable difference when the culture entered the phase 2. Furthermore, the concentration of acetate was obviously lower when PMS was used to regulate the fermentation than that when EA was used in the total feeding culture phases. The final concentration of acetate by the PMS and EA were 0.81 g/L and 2.1 g/L, respectively.

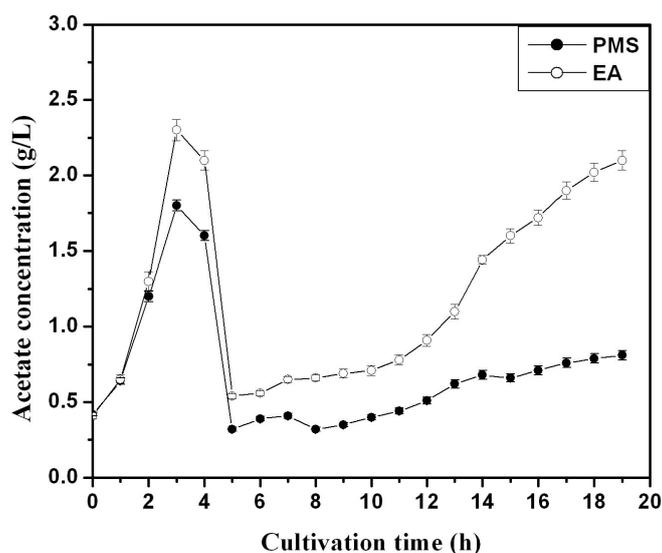


Fig. 2 The concentration of acetate used the PMS and EA

Effect of different measure apparatus on the residual glucose

Fig. 3 showed that the concentration of glucose in the medium was similar in the batch stage. When the culture entered the feeding stage, the concentration of glucose in the medium detected by the two apparatus was different. The glucose could maintain at a stable level used the PMS compared with the EA. During this phase, the high cell density was obtained that the cells need a stabilized culture environment to grow and express the target protein.

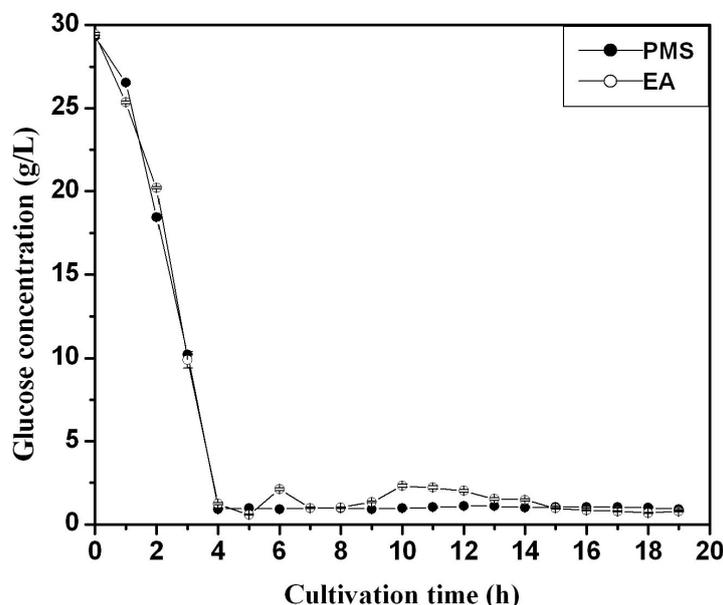


Fig. 3 The concentration of glucose in the medium

Effect of different measure apparatus on the HLC production and biomass (OD_{600})

By using the EA measure, the production of HLC and biomass only reached 7.12 g/L and 144, respectively. However, the production of HLC and biomass increased to 8.02 g/L and 152, respectively, when the PMS was used to regulate the fermentation. As the result, the PMS measure had a significant enhancement on biomass and HLC production compared with EA measure.

CONCLUSION

In this study, the concentration of oxygen and carbon dioxide was detected in the off-gas during the fermentation using the two apparatus, the exhaust analyzer and the process mass spectrometer. The results demonstrated that the production of HLC was enhanced when the PMS was applied to regulate the whole fermentation. The response was faster and more accurate to the feedback of the DO level when PMS was used than that the EA was used. Therefore, the cells were cultivated at a relative stable environment and had enough substrate to provide energy and precursor substances for the growth and synthesis of HLC when PMS was used.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (21176200, 21276210, 21106112, 21206135, 31000019 and 21106111); the Scientific Research Program of Shaanxi Provincial Department of Education, China (2013JK0696, 12JK0449, 12JS099, 12JS100, 12JS101, 2012JC23, 11JS102, 2010JC21, 2010JS107, 2010JS108, 2010JK876 and 2010JS109); China Postdoctoral Science Foundation (20110490171), China Postdoctoral Science Special Foundation (2012T50815); Shaanxi Provincial Scientific Technology Research and Development Program (2011JE003, 2011JQ4026, 2012KJXX-28, 2012JM2014 and 2010JQ2012), and Shaanxi Key Subject Program, China

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