Short communication

S-Adenosyl-L-methionine production by *Saccharomyces cerevisiae* SAM 0801 using DL-methionine mixture: From laboratory to pilot scale

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\section*{ABSTRACT}

This study sought to develop a cost-effective biological catalysis process for S-adenosyl-L-methionine (SAM) production. During the process, a mixture of D-methionine (D-Met) and L-methionine (L-Met), namely DL-Met, was used as substrate to replace the conventional but expensive pure L-Met. The concentration of L-Met in substrate was optimized. When 80 g/L of L-Met was added in a 5 L-scale bioreactor, 13.74 g/L of SAM was produced with an L-Met conversion rate of 32.15%. The fermentation process was then scaled up to meet the requirements of realistic industrial SAM production. In a 300 L-scale fermentation process, 10.45 g/L of SAM was achieved, with an L-Met conversion rate of 32.61%. Moreover, the proportion of D-Met remaining in broth increased from 50% at the beginning to 76.89% at the end of fermentation. The fermentation process is generally appropriate for commercial SAM production.

1. Introduction

S-Adenosyl-L-methionine (SAM) is an essential metabolite in living cells which showed important roles in many metabolic reactions [1–3]. It demonstrated that SAM serves as an activation group in biological reactions and pathways, and has also been widely used in human therapies for diseases such as Alzheimer’s disease, osteoarthritis, fibromyalgia and depression [4–8]. Unfortunately, the production of SAM is limited by the high processing cost [9].

In the past decades, a variety of studies, including chemical and biological catalysis, were attempted to establish a low-cost and efficient route for SAM production [6]. However, a number of bottlenecks, such as the rigorous conditions for SAM separation and the high cost of ATP, have economically hindered these processes [9]. Generally, in comparison to the chemical catalysis ways, SAM production by microbial catalysis is proved to be a more feasible way for industrialization [1,8]. In previous works, several microorganisms were screened, and genetic strains were used to increase the production of SAM in fermentation processes [1]. In these methods, typically, L-methionine (L-Met) and ATP were supplied in the substrate, and SAM is synthesized by cellular S-adenosylmethionine synthetase [8,10,11]. The volumetric productivity of SAM was greatly improved after process optimization [1,2]. Furthermore, valuable by-products may be also co-generated along with SAM, which will further improve the economics of SAM production [1,12].

The cost of downstream procedures for SAM separation depends on the intracellular concentration of SAM. In previous studies, a relatively high concentration of SAM, exceeding 1% (w/w), was achieved in the culture of *Saccharomyces cerevisiae* and *Pichia pastoris* (see Table 2). However, the drawbacks of using yeast as microbial catalysis are obvious: the expensive L-Met substrate needs to be added, and the SAM productivity is relatively low [13–15]. Based on our market survey in China, the cost of pure L-Met is approximately 3 times higher than that of DL-methionine (DL-Met), a mixture of D-Met and L-Met. It is because of the difficulties in chiral separation [16]. In the current work, to decrease the substrate cost of SAM production and further improve the economics, L-Met was replaced by DL-Met. *Saccharomyces cerevisiae* SAM 0801, a mutant strain with high S-adenosylmethionine synthetase activity, was employed as biocatalyst.

As the yeast cells exhibit limited ability to ingest D-Met, only L-Met from the DL-Met mixture was successful utilized and converted into intracellular SAM. With the objective of designing a commercial-scale plant, the fermentation process was gradually scaled up from 5 L to...
It indicated that a relatively high ethanol content leaded to severe inhibition of yeast cell metabolism [19], which might further negatively influence on the SAM production. To maintain a low ethanol concentration in broth, the feeding of the supplemented medium should be kept at a considerably low level.

When the concentration of the by-product ethanol was decreased, the supplemented medium gradually fed into the bioreactors. The substrates were introduced into the bioreactor when the dry cells weight reached approximately 70 g/L. The fermentation period was prolonged by 24 h after addition of the substrates. During this period, SAM was accumulated, accompanied with an increase of biomass in both of the two groups. Subsequently, the rate of SAM yields and biomass growths were decreased obviously, and the cultivations were terminated.

The average loading rates of glucose and substrate in the ν-Met group were 9.06 g/L h and 1.01 g/L h, whereas they were 8.77 g/L h and 0.44 g/L h in the control group. At the end of fermentations, the SAM concentrations in the ν-Met group and the control group reached 13.74 g/L and 14.17 g/L, respectively. Correspondingly, 32.15% and 32.05% of the L-Met in the feedstock was converted into SAM by microbial catalysis. Therefore, the key parameters of fermentation were comparable, indicating that ν-Met was a potential substrate to replace expensive ε-Met for SAM production. More importantly, the ν-Met ratio in the ν-Met mixture could be rapidly increased to 81.94% in the fermentation broth.

The results obtained in the ν-Met feeding group showed that ε-Met will be easily involved in the reaction of SAM production whilst ν-Met is unable to be ingested. Only trace of δ-Met was tested in the cells lysed liquor. This phenomenon could be explained by three reasons. For the first reason, even though δ-Met could be metabolized by different types of cells [20–23], ε-Met is the more favor one for cells uptake. In the current work, ε-Met was not completely converted into SAM. Hence, the utilization of δ-Met might be limited. For the second reason, although it reported that δ-Met could pass through the cells membrane by the assistance of permease proteins, there is no evidence that δ-Met could be transported into the cells. The last but not the least, the S-adenosylmethionine synthetase in yeast cells could not convert δ-Met to SAM directly. And it showed only ε-Met could be used as the substrate for SAM production based on the KEGG database.

Supplementation of the ε-Met was correlated to the overall cost of SAM production. For example, the lack of ε-Met in substrate might cause the waste of the S-adenosylmethionine synthetase activities and cofactor content. In contrast, excess ε-Met may not only raise the substrate cost but also decrease the conversion rate, which would further cause severe inhibition of the yeast cells [24]. The following experiments were performed by feeding various concentrations of ν-Met and ε-Met (the control groups) in substrates.

As illustrated in Table 1, an increase amount of Met (ε-Met or ν-Met) was accompanied with the enhancement of SAM production and yeast biomass until 80 g/L and 40 g/L of ν-Met and ε-Met in substrate, respectively. The highest dry cell weight in the ν-Met group occurred at 60 g/L ν-Met (140.35 g/L), whereas 144.6 g/L was obtained in the 40 g/L ε-Met group. Then, the SAM productivity was decreased with additional supplementation of ν-Met/ε-Met. This phenomenon could be explained by the inhibition of adenosine methionine transferase (MAT) and ATP synthase in the TCA cycle [25]. Moreover, it was interesting that the fermentation period in the ν-Met groups was always shorter than that of the control groups. Due to the shorter fermentation period, the yields of SAM in the ν-Met groups were always lower than those of the conventional ε-Met based groups. Nonetheless, the data showed that the purity of the Met, either δ-Met or ε-Met, was not sensitive on the production of SAM in the 5 L bioreactor. Therefore, ν-Met substrate showed economic possibility for SAM production based on S. cerevisiae SAM 0801.

SAM was further produced in 30 L and 300 L fermentation systems using ν-Met as substrate. Analogous to the optimized Met concentration shown in Table 1, 80 g/L of ν-Met was used. The results of 30 L
Fig. 1. Diagram of SAM biosynthesis based on α-Met substrate.

Fig. 2. Kinetic parameters of <i>Saccharomyces cerevisiae</i> SAM 0801 fermentation for SAM production. (a) Adding 80 g/L of α-Met mixture as substrate in 5 L bioreactor; (b) Adding of 40 g/L of L-Met as substrate in a 5 L bioreactor; (c) α-Met mixture as substrate in a 30 L bioreactor; (d) α-Met mixture as substrate in a 300 L bioreactor.
The results obtained in 5 L-, 30 L- and 300 L-scales fermentation system. 80 g/L ofDL-Met was supplemented into the bioreactor.

Table 1

| Substrate (g/L) | Fermentation period (h) | SAM (g/L) | Conversion of L-Met (%) | Yeast biomass (g/L) | D-Met ratio in broth (%) |
|-----------------|-------------------------|-----------|-------------------------|-------------------|-------------------------|
| L-Met           | DL-Met                  | L-Met     | DL-Met                  | L-Met             | DL-Met                  |
| 20              | 40                      | 57.3      | 56.9                    | 10.87             | 10.15                   |
| 30              | 60                      | 57.8      | 56.2                    | 12.25             | 12.14                   |
| 40              | 80                      | 58.6      | 54.1                    | 14.17             | 13.74                   |
| 50              | 100                     | 59        | 54.8                    | 11.48             | 10.23                   |
|                 |                         |           |                         |                   |                         |
| 300 L           |                         |           |                         |                   |                         |
| 8.04            |                         | 1.01      | 139.93                  | 13.74             | 32.15                   |
| 30 L            |                         | 1.00      | 140.20                  | 13.35             | 32.94                   |
| 300 L           |                         | 0.96      | 104.76                  | 10.45             | 32.61                   |

Table 2

| Scales          | Average glucose rate (g/L h) | Average D-Met substrate rate (g/L h) | Yeast biomass (g/L) | SAM (g/L) | Conversion of L-Met (%) | D-Met ratio in broth (%) |
|-----------------|------------------------------|-------------------------------------|-------------------|-----------|-------------------------|-------------------------|
| 5 L             | 9.06                         | 1.01                                | 139.93            | 13.74     | 32.15                   | 81.94                   |
| 30 L            | 8.70                         | 1.00                                | 140.20            | 13.35     | 32.94                   | 77.36                   |
| 300 L           | 8.04                         | 0.96                                | 104.76            | 10.45     | 32.61                   | 76.89                   |

a Substrate concentration refers to the Met concentration in the fed-batch medium.

Table 3

| Strain                      | Scale | Substrate | Fermentation period (h) | SAM production (g/L) | SAM productivity (mg/g DCW) | L-Met conversion (%) | References |
|-----------------------------|-------|-----------|-------------------------|---------------------|-----------------------------|---------------------|------------|
| P. pastoris GS115           | 15    | L-Met     | 13.24                   | 55                  | 32.97                       |                    | [27]       |
| P. pastoris GS115           | 3.7   | L-Met     | 11.63                   | 105.73              |                             |                    |            |
| P. pastoris GS15/DS56       | 30    | L-Met     | 8.37                    | 76.99               | 42.9                        |                    | [25]       |
| P. pastoris GS15 (his 4)    | 5     | L-Met     | 13.5                    | 127.35              |                             |                    |            |
| S. cerevisiae GMCC 2842     | 10    | L-Met     | 6.06                    | 344.7               |                             |                    |            |
| S. cerevisiae ZJUS 1        | 10    | L-Met     | 8.7                     | 83.52               |                             |                    |            |
| S. cerevisiae GMCC 1225     | 15    | L-Met     | 6.1                     | 69                  |                             |                    | [30]       |
| S. cerevisiae               | 10    | L-Met     | 8.81                    | 91.00               |                             |                    |            |
| S. cerevisiae SAM 0801      | 5     | L-Met     | 13.74                   | 98.19               | 32.15                       |                    | This study |
|                             | 30    | L-Met     | 11.35                   | 80.96               | 32.94                       |                    | This study |
|                             | 300   | L-Met     | 10.45                   | 99.75               | 32.61                       |                    | This study |

a SAM productivity calculated by the author.

In recent years, numerous studies have been carried out to study the chemical and biological production of SAM [4,6]. However, there is lack of researches focused on the scale-up of SAM production by fermentation. More importantly, the researches using DL-Met as substrate in place of the expensive L-Met for SAM production remain limited. Table 3 summarizes the current advances in SAM production. Although no significant increase in SAM production was achieved, the recent researches using DL-Met as substrate in the fermentation broth was 15.37 g/L, with D-Met proportion of 77.36%.

The potential for industrial-scale production of SAM by fermentation was further evaluated based on a 300 L fermentation system. As can be observed in Fig. 2d, ethanol concentration and glucose supplement rate first increased and then gradually decreased. After 50 h of fermentation, 140.20 g/L of dry cells weight was obtained. In addition, 11.35 g/L of SAM was produced, with an L-Met conversion rate of 32.94%. This result was comparable to the control group (11.28 g/L of SAM production and a 35.57% L-Met conversion rate). Furthermore, at the end of fermentation, the residual DL-Met concentration remained in the fermentation broth was 15.37 g/L, with D-Met proportion of 77.36%.

The possibility of industrializing the process for SAM production was further evaluated based on a 300 L fermentation system. As can be observed in Fig. 2d, ethanol concentration and glucose supplement rate increased sharply to 1.52 g/L after 7 h of fermentation and then decreased to ~0.3 g/L before adding the substrate. Following the addition of DL-Met, SAM production increased while the growth rate slowed. Before the process was ended, SAM production reached 10.45 g/L with an L-Met conversion rate of 32.61%. This phenomenon was similar to the results of the control group (using L-Met as substrate, 10.76 g/L of SAM production and a 30.22% of L-Met conversion rate, data not shown). The presence of D-Met in the DL-Met mixture increased from 50% to 76.89%. The comparison of 5 L-, 30 L- and 300 L-scales of SAM fermentation was also listed in Table 2, which showed no obvious changes of SAM concentration and L-Met conversion rates. Overall, the results indicated the potential of using DL-Met as substrate in large-scale fermentative production of SAM. Simultaneously, D-Met rich broth could be also obtained.

The potential for industrial-scale production of SAM by fermentation was demonstrated using DL-Met as substrate. In 300L-scale cultivation, the use of DL-Met resulted in10.45 g/L of SAM production and a 32.61% L-Met conversion rate. The D-Met by-product could be effectively separated after yeast cells collection, with a ratio of 76.89% in the DL-Met mixture. In summary, this is the first report using DL-Met as substrate for SAM production in S. cerevisiae fermentation process. Four advantages to the approach were emphasized. First, the use of DL-Met as substrate could save approximately33% of the substrate cost when compared with the traditional L-Met based fermentation processes. In contrast to previous work, the current study was carried out at a larger scale, which more closely resembled industrialization.

The results of the current study were shown to be comparable to the control group (using L-Met as substrate, 10.76 g/L of SAM production and a 30.22% of L-Met conversion rate). Furthermore, at the end of fermentation, the residual DL-Met concentration remained in the fermentation broth was 15.37 g/L, with D-Met proportion of 77.36%.

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