Short communication

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

Wenqiang Ren^{a,b,c,*}, Di Cai^{b,c,1}, Song Hu^{b,c}, Shasha Xia^a, Zheng Wang^{b,c}, Tianwei Tan^{b,c}, Qinghua Zhang^{a,*}

^a Research Center for Eco-Environmental Sciences, Chinese Academy of Science, Beijing 100085, PR China

^b Beijing Key Lab of Bioprocess, Beijing University of Chemical Technology, Beijing 100029, PR China

^c National Energy R & D Center for Biorefinery, Beijing University of Chemical Technology, Beijing 100029, PR China

ARTICLE INFO

Keywords:

S-Adenosyl-L-methionine

Fermentation

Saccharomyces cerevisiae

DL-Methionine

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 DL-Met in substrate was optimized. When 80 g/L of DL-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 as 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 successfully 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

* Corresponding authors at: Research Center for Eco-Environmental Sciences, Chinese Academy of Science, No.18 Shuangqing Road, Haidian District, Beijing, 100085, PR China.

E-mail addresses: wqren@rcees.ac.cn (W. Ren), qhzhang@rcees.ac.cn (Q. Zhang).

¹ Equal contributors.

300 L system. The results indicated no obvious difference in SAM production when either DL-Met or L-Met was used as substrate. In contrast, a relatively high SAM production with high conversion rate was obtained using DL-Met based medium.

2. Materials and methods

2.1. Strains and medium

Saccharomyces cerevisiae SAM0801, a mutant yeast strain with high S-adenosylmethionine synthetase activity (approximately 60 U/mL), was screened by flow cytometer following the method described in our previous study [17]. The slant medium consisted of 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 20 g/L agar. The fermentation medium contained 15 g/L $(\text{NH}_4)_2\text{SO}_4$, 6.14 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.71 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 8 g/L KH_2PO_4 , 25 g/L glucose and 10 mL/L microelements (15 g/L EDTA, 1.5 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.3 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.3 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.4 g/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 4.5 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 1 g/L KI). For SAM production, fed-batch process was performed in order to achieve high density of yeast cells. A supplemented medium contained 600 g/L glucose as the carbon source was pumped into the bioreactor periodically. In the late fermentation stage, L-Met or DL-Met (mixture of 50 wt.% L-Met and 50 wt.% D-Met) was additionally supplemented into the bioreactor for SAM synthesis.

2.2. Culture conditions

Before inoculation, the fermentation medium was autoclaved at 121 °C for 20 min. Subsequently, 10% (v/v) active cells were inoculated into the bioreactor, and the culture medium was maintained at 30 °C with a rotation rate of 600 rpm. The pH of the culture medium was kept at 5.3 using 25% ammonia. To provide suitable dissolved oxygen, 1.5 vvm of sterile air was passed through a gas distributor. Additional medium was pumped into the bioreactor when the ethanol concentration slightly decreased. During the late period of the logarithmic phase, the substrates containing L-Met or DL-Met was added into the bioreactor and converted to the target SAM product. Similar protocols were performed for the 5 L-, 30 L- and 300 L-scale fermentations.

2.3. Analytical methods

The dry cell weight of the strains was measured similar to that described in our previous study [18]. The SAM concentration was analyzed by high-performance liquid chromatography (SPD-20A, Shimadzu, Japan), and the instrument was equipped with a C_{18} silica gel column (Agilent TC-18250). Before analysis, cells were lysed with 1.5 mol/L perchloric acid. The cell suspension was centrifuged at 8000 rpm and 4 °C.

3. Results and discussions

Biosynthetic and metabolic pathways of SAM in yeast cells are shown in Fig. 1. Experiments using L-Met served as the control group in this study. The differences between DL-Met and L-Met as substrates for SAM production were examined using a 5 L bioreactor with 3 L working volume. To evaluate the fermentation kinetics, 80 g/L of DL-Met and 40 g/L of L-Met (in order to meet a similar concentration of L-Met in feeding) were fed into the bioreactors.

As shown in Fig. 2a and b, after inoculation, the yeast biomass increased along with the consumption of glucose and nutrients in both of the two groups. In general, biomass accumulation and SAM production exhibited similar patterns during cultivation when DL-Met or L-Met was used as substrate. After entering the logarithmic phase, the yeast biomass rose rapidly with dramatic increase of glucose consumption rates. It indicated that a relatively high ethanol content led 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 DL-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 DL-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 DL-Met was a potential substrate to replace expensive L-Met for SAM production. More importantly, the D-Met ratio in the DL-Met mixture could be rapidly increased to 81.94% in the fermentation broth.

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

Supplementation of the L-Met was correlated to the overall cost of SAM production. For example, the lack of L-Met in substrate might cause the waste of the S-adenosylmethionine synthetase activities and cofactor content. In contrast, excess L-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 DL-Met and L-Met (the control groups) in substrates.

As illustrated in Table 1, an increase amount of Met (L-Met or DL-Met) was accompanied with the enhancement of SAM production and yeast biomass until 80 g/L and 40 g/L of DL-Met and L-Met in substrate, respectively. The highest dry cell weight in the DL-Met group occurred at 60 g/L DL-Met (140.35 g/L), whereas 144.6 g/L was obtained in the 40 g/L L-Met group. Then, the SAM productivity was decreased with additional supplementation of DL-Met/L-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 DL-Met groups was always shorter than that of the control groups. Due to the shorter fermentation period, the yields of SAM in the DL-Met groups were slightly lower than those of the conventional L-Met based groups. Nonetheless, the data showed that the purity of the Met, either D-Met or L-Met, was not sensitive on the production of SAM in the 5 L bioreactor. Therefore, DL-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 DL-Met as substrate. Analogous to the optimized Met concentration shown in Table 1, 80 g/L of DL-Met was used. The results of 30 L

Fig. 1. Diagram of SAM biosynthesis based on DL-Met substrate.

Fig. 2. Kinetic parameters of *Saccharomyces cerevisiae* SAM 0801 fermentation for SAM production. (a) Adding 80 g/L of DL-Met mixture as substrate in 5 L bioreactor; (b) Adding 40 g/L of L-Met as substrate in a 5 L bioreactor; (c) DL-Met mixture as substrate in a 30 L bioreactor; (d) DL-Met mixture as substrate in a 300 L bioreactor.

Table 1

Comparison of SAM production using L-Met and DL-Met as substrates. A 5 L bioreactor with 3 L of working volume was used. The left columns show the results with L-Met substrate. The right columns show the results with DL-Met substrate.

Substrate ^a (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	L-Met	DL-Met	L-Met	DL-Met	L-Met	DL-Met
20	40	57.3	56.9	10.87	10.15	36.43	34.84	137.76	129.72	–	83.57
30	60	57.8	56.2	12.25	12.14	34.33	32.86	127.96	140.35	–	81.06
40	80	58.6	54.1	14.17	13.74	32.05	32.15	144.6	139.93	–	81.94
50	100	59	54.8	11.48	10.23	20.3	16.61	112.56	117.63	–	69.75

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

Table 2

The results obtained in 5 L-, 30 L- and 300 L-scales fermentation system. 80 g/L of DL-Met was supplemented into the bioreactor.

Scales	Average glucose supplement rate (g/L h)	Average DL-Met supplement 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	11.35	32.94	77.36
300 L	8.04	0.96	104.76	10.45	32.61	76.89

cultivation are presented in Fig. 2c. Similar to the results in the 5 L reactor, the 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 in broth 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 quickly accumulated 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.

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 process was able to realize the leap from flask- to pilot-scale cultivation. Besides, all the previous works were using L-Met as substrate, which is approximately three times more expensive than DL-Met. For the purpose of achieving a comparable supplemental ratio of L-Met, double doses of DL-Met were used as substrate. Without considering other factors, SAM production based on DL-Met could save approximately 33% 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. 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 resulted in a lower substrate cost for the SAM production. Second, the shorter fermentation period improved equipment efficiency. Third, D-Met purified in the fermentation broth, which could further promote the L-Met and D-Met mixture separation. Fourth, the larger scale process broadens the field of SAM production. Further work is needed to enhance the efficiency of the SAM and D-Met separation.

4. Conclusions

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 in 10.45 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.

Table 3

Current advances in SAM production by fermentation.

Strain	Scale	Substrate	Fermentation period (h)	SAM production (g/L)	SAM productivity (mg/g DCW)	L-Met conversion (%)	References
<i>P. pastoris</i> GS115	15	L-Met	119	13.24	55	32.97	[27]
<i>P. pastoris</i> GS115	3.7	L-Met	99	11.63	105.73 ^a	–	[28]
<i>P. pastoris</i> GS115/DS56	30	L-Met	108	8.37	76.09 ^a	42.9	[25]
<i>P. pastoris</i> GS115 (his 4)	5	L-Met	140	13.5	127.35 ^a	–	[26]
<i>S. cerevisiae</i> CGMCC 2842	10	L-Met	120	6.06	344.7	–	[10]
<i>S. cerevisiae</i> ZJUS 1	10	L-Met	60	8.7	83.52 ^a	–	[29]
<i>S. cerevisiae</i> CGMCC 1226	15	L-Met	36	6.1	69	–	[30]
<i>S. cerevisiae</i>	10	L-Met	58	8.81	91.00	–	[8]
<i>S. cerevisiae</i> SAM 0801	5	DL-Met	54.1	13.74	98.19	32.15	This study
	30	DL-Met	50.15	11.35	80.96	32.94	This study
	300	DL-Met	49.6	10.45	99.75	32.61	This study

^a SAM productivity calculated by the author.

Acknowledgements

This work was supported in part by the National Basic Research Program of China (Grant Nos. 2013CB733600), the Fundamental Research Funds for the Central Universities (Grant No. YS1407), and the long-term subsidy mechanism from the Ministry of Finance and the Ministry of Education of PRC. We would also like to thank Qingquan He from Beijing Key Lab of Bioprocess for help with the 300 L-scale fermentation.

References

- [1] H. Chen, Z. Wang, H. Cai, C. Zhou, Progress in the microbial production of S-adenosyl-L-methionine, *World J. Microbiol. Biotechnol.* 32 (9) (2016) 153.
- [2] H.R. Kant, M. Balamurali, S. Meenakshisundaram, Enhancing substrates availability in *Pichia pastoris* for the overproduction of S-adenosyl-L-methionine employing molecular strategies with process tuning, *J. Biotechnol.* 188 (2014) 112–121.
- [3] P. Laurino, D.S. Tawfik, Spontaneous emergence of S-adenosylmethionine and the evolution of methylation, *Angew. Chem. Int. Ed.* 56 (1) (2017) 343–345.
- [4] M. Joncquel-Chevalier, P.M. Voicu, M. Fontaine, A.F. Dessein, N. Porchet, K. Mention-Mulliez, D. Dobbelaere, G. Soto-Ares, D. Cheillan, J. Vamecq, Creatine biosynthesis and transport in health and disease, *Biochimie* 119 (2015) 146–165.
- [5] N.A. Osna, Hepatoprotective effects of S-adenosyl-L-methionine against alcohol- and cytochrome P450 2E1-induced liver injury, *World J. Gastroenterol.* 16 (11) (2010) 1366–1376.
- [6] J. Chu, J. Qian, Y. Zhuang, S. Zhang, Y. Li, Progress in the research of S-adenosyl-L-methionine production, *Appl. Microbiol. Biotechnol.* 97 (1) (2013) 41–49.
- [7] S.C. Lu, J.M. Mato, S-adenosylmethionine in liver health, injury, and cancer, *Physiol. Rev.* 92 (4) (2012) 1515–1542.
- [8] W. Zhao, F. Shi, B. Hang, L. Huang, J. Cai, Z. Xu, The improvement of SAM accumulation by integrating the endogenous methionine adenosyltransferase gene SAM2 in genome of the industrial *Saccharomyces cerevisiae* strain, *Appl. Biochem. Biotechnol.* 178 (6) (2016) 1263–1272.
- [9] X.-N. Wei, M.-J. Cao, J. Li, H. Li, Y. Song, C.-H. Du, Synthesis of S-adenosyl-L-methionine in *Escherichia coli*, *Biotechnol. Bioprocess Eng.* 19 (6) (2014) 958–964.
- [10] H. Chen, Y. Yang, Z. Wang, J. Dou, H. Wang, C. Zhou, Elevated intracellular acetyl-CoA availability by *acs2* overexpression and *mls1* deletion combined with *metK1* introduction enhanced SAM accumulation in *Saccharomyces cerevisiae*, *Biochem. Eng. J.* 107 (2016) 26–34.
- [11] G. Han, X. Hu, X. Wang, Overexpression of methionine adenosyltransferase in *Corynebacterium glutamicum* for production of S-adenosyl-L-methionine, *Biotechnol. Appl. Biochem.* 63 (5) (2015) 679–689.
- [12] G. Han, X. Hu, X. Wang, Co-production of S-adenosyl-L-methionine and L-isoleucine in *Corynebacterium glutamicum*, *Enzyme Microb. Technol.* 78 (2015) 27–33.
- [13] Y. Huang, X. Gou, H. Hu, Q. Xu, Y. Lu, J. Cheng, Enhanced S-adenosyl-L-methionine production in *Saccharomyces cerevisiae* by spaceflight culture, overexpressing methionine adenosyltransferase and optimizing cultivation, *J. Appl. Microbiol.* 112 (4) (2012) 683–694.
- [14] V. Kamarthapu, S. Ragampeta, K.V. Rao, V.D. Reddy, Engineered *Pichia pastoris* for enhanced production of S-adenosylmethionine, *AMB Express* 3 (1) (2013) 40.
- [15] J. Zhang, X. Wang, E. Su, G. Fang, Y. Ren, D. Wei, A new fermentation strategy for S-adenosylmethionine production in recombinant *Pichia pastoris*, *Biochem. Eng. J.* 41 (1) (2008) 74–78.
- [16] L. Zhang, K. Gedicke, M. Kuznetsov, S. Staroverov, A. Seidel-Morgenstern, Application of an eremomycin-chiral stationary phase for the separation of dl-methionine using simulated moving bed technology, *J. Chromatogr. A* 1162 (1) (2007) 90–96.
- [17] Z. Wang, L. Zhang, T. Tan, Efficient screening a high glutathione-content mutant of *Saccharomyces cerevisiae* by flow cytometry, *Process Biochem.* 45 (7) (2010) 1168–1171.
- [18] F. Xue, B. Gao, Y. Zhu, X. Zhang, W. Feng, T. Tan, Pilot-scale production of microbial lipid using starch wastewater as raw material, *Bioresour. Technol.* 101 (15) (2010) 6092–6095.
- [19] D. Cai, S. Hu, C. Chen, Y. Wang, C. Zhang, Q. Miao, P. Qin, T. Tan, Immobilized ethanol fermentation coupled to pervaporation with silicalite-1/polydimethylsiloxane/polyvinylidene fluoride composite membrane, *Bioresour. Technol.* 220 (2016) 124–131.
- [20] R.J. Kadner, Transport and utilization of D-methionine and other methionine sources in *Escherichia coli*, *J. Bacteriol.* 129 (1) (1977) 207–216.
- [21] M. Kobayashi, A. Mizutani, K. Nishi, S. Nakajima, N. Shikano, R. Nishii, K. Fukuchi, K. Kawai, Differences in accumulation and the transport mechanism of l- and d-methionine in high- and low-grade human glioma cells, *Nucl. Med. Biol.* 44 (2017) 78–82.
- [22] C. Kong, C. Park, J. Ahn, B. Kim, Relative bioavailability of DL-methionine compared with L-methionine fed to nursery pigs, *Anim. Feed Sci. Technol.* 215 (2016) 181–185.
- [23] J.J.D.A.D. Knight, J. Dibner, Conversion of 2-hydroxy-4-(methylthio) butanoic acid to L-methionine in the chick: a stereospecific pathway, *J. Nutr.* 114 (1984) 1716–1723.
- [24] S.Y. Chan, D.R. Appling, Regulation of S-adenosylmethionine levels in *Saccharomyces cerevisiae*, *J. Biol. Chem.* 278 (44) (2003) 43051–43059.
- [25] H. Hu, J. Qian, J. Chu, Y. Wang, Y. Zhuang, S. Zhang, Optimization of L-methionine feeding strategy for improving S-adenosyl-L-methionine production by methionine adenosyltransferase overexpressed *Pichia pastoris*, *Appl. Microbiol. Biotechnol.* 83 (6) (2009) 1105.
- [26] J. He, J. Deng, Y. Zheng, J. Gu, A synergistic effect on the production of S-adenosyl-L-methionine in *Pichia pastoris* by knocking in of S-adenosyl-L-methionine synthase and knocking out of cystathionine- β synthase, *J. Biotechnol.* 126 (4) (2006) 519–527.
- [27] X.-Q. Hu, J. Chu, S.-L. Zhang, Y.-P. Zhuang, Y.-H. Wang, S. Zhu, Z.-G. Zhu, Z.-Y. Yuan, A novel feeding strategy during the production phase for enhancing the enzymatic synthesis of S-adenosyl-L-methionine by methylotrophic *Pichia pastoris*, *Enzyme Microb. Technol.* 40 (4) (2007) 669–674.
- [28] J. Zhang, X. Wang, Y. Zheng, G. Fang, D. Wei, Enhancing yield of S-adenosylmethionine in *Pichia pastoris* by controlling NH_4^+ concentration, *Bioprocess Biosyst. Eng.* 31 (2) (2008) 63–67.
- [29] J.-P. Lin, J. Tian, J.-F. You, Z.-H. Jin, Z.-N. Xu, P.-L. Cen, An effective strategy for the co-production of S-adenosyl-L-methionine and glutathione by fed-batch fermentation, *Biochem. Eng. J.* 21 (1) (2004) 19–25.
- [30] M.Y.X. Cao, Y. Xia, J. Dou, K. Chen, H. Wang, T. Xi, C. Zhou, Strain improvement for enhanced production of S-adenosyl-L-methionine in *Saccharomyces cerevisiae* based on ethionine-resistance and SAM synthetase activity, *Ann. Microbiol.* 62 (2012) 1395–1402.