J Ind Microbiol Biotechnol (2014) 41:1479–1486
DOI 10.1007/s10295-014-1490-8

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Efficient production of methionine from 2-amino-4-methylthiobutanenitrile by recombinant *Escherichia coli* harboring nitrilase

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Received: 15 March 2014 / Accepted: 9 July 2014 / Published online: 2 August 2014
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Abstract Methionine as an essential amino acid has been attracting more attention for its important applications in food and feed additives. In this study, for efficient production of methionine from 2-amino-4-methylthiobutanenitrile, a codon-optimized nitrilase gene was newly synthesized and expressed, and the catalytic conditions for methionine production were studied. The optimal temperature and pH for methionine synthesis were 40 °C and 7.5, respectively. The recombinant nitrilase was thermo-stable with half-life of 5.52 h at 40 °C. The substrate loading was optimized in given amount of catalyst and fixed substrate/catalyst ratio mode to achieve higher productivity. Methionine was produced in 100 % conversion within 120 min with a substrate loading of 300 mM. The production of methionine with the immobilized resting cells in packed-bed reactor was investigated. The immobilized nitrilase exhibited good operation stability and retained over 80 % of the initial activity after operating for 100 h. After separation, the purity and the total yield of methionine reached 99.1 and 97 %, respectively. This recombinant nitrilase could be a potential candidate for application in production of methionine.

Keywords Methionine · Nitrilase · 2-amino-4-methylthiobutanenitrile · Immobilization · Optimization

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Introduction

Methionine is one of the amino acids containing the sulfur with an unbranched and highly hydrophobic side chain. It plays an important role in food and feed additives. Besides its role in protein biosynthesis, methionine participates in transmethylation, which improves the bioavailability of selenium and zinc and also directly used to therapy disorders like allergy and rheumatic fever [7, 9].

Due to the annual growth of global market, the production of methionine becomes a hot issue. The bulk demand for methionine, about 800,000 tons annually across the globe, is met by chemical methods. The hydrolysis of nitriles to the corresponding carboxylic acids by the chemical methods typically requires strongly acidic or basic reaction conditions and high reaction temperatures, and usually produces large amounts of inorganic salts as unwanted waste [6, 10]. There are reports about the production of methionine by submerged cultivation, but it has not been adopted commercially because microorganisms, either mutated or genetically engineered, have not produced sufficiently high concentrations of methionine [1, 2, 11, 16, 18]. In contrast, enzyme-catalyzed hydrolysis of nitriles to the corresponding carboxylic acids is run at ambient temperature and does not require strongly acidic or basic reaction conditions. Nitrilases (EC 3.5.5.1) are a well-studied class of hydrolases that have been used for many industrial-scale biotransformations [4, 15, 20, 24]. The nitrilases have been reported in biotransformation to prepare methionine or methionine derivatives from substituted 4-methylthiobutyronitriles; however, it has been reported with insufficient cell-specific activity [3, 8, 17, 19]. Therefore, to screen the nitrilases with high activities has become a hot issue. With the recent development in genetic engineering and bioinformatics, it is possible to obtain novel nitrilases by

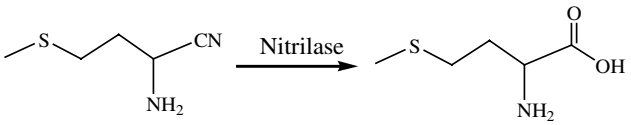


Fig. 1 Process for preparation of methionine from 2-amino-4-methylthiobutanenitrile by nitrilase

analysis of putative nitrilase sequences structures and activities information [13].

In the current study, a recombinant *Escherichia coli* BL21 (DE3) harboring nitrilase from *Acidovorax facilis* (GenBank: DQ444267) was selected for the biosynthesis of methionine from 2-amino-4-methylthiobutanenitrile (Fig. 1). The aim of this work is to demonstrate the suitability and the robustness of this catalyst toward 2-amino-4-methylthiobutanenitrile. The studies showed this recombinant nitrilase could be a potential candidate for the upscale production of methionine.

Materials and methods

Strains and chemicals

The *E. coli* strains JM109 (Tiangen biotech Co., Ltd, Beijing, China) and BL21 (DE3) (Novagen, Darmstadt, Germany) were used as hosts for cloning and expression, respectively. The plasmids pMD18-T (TaKaRa, Otsu, Japan) and pET-28b (+) (Novagen, Darmstadt, Germany) were used for the cloning and expression of nitrilase, respectively. The nitrilase gene with a length of 1121 bp was synthesized using PCR assembly method after condons optimization by software against *E. coli* as host using *Acidovorax facilis* as references [5, 13, 14, 21]. The recombinant *E. coli* BL21 (DE3) harboring the plasmid pET-28b (+)-Af-*NIT* was constructed and maintained in glycerol frozen stock at −80 °C in our lab. All the chemicals used were of analytical grade and commercially available.

Expression and purification of the nitrilase

Recombinant strain harboring the plasmid pET-28b (+)-Af-*NIT* having histidine tag at the carboxyl terminus was grown in Luria–Bertani (LB) (Yeast extract 5 g/L, Tryptone 10 g/L and NaCl 5 g/L) liquid medium containing 50 μg/ml kanamycin at 150 rpm and 37 °C. Nitrilase expression was induced with addition of isopropyl β-D-l-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM when the optical density was approximately 0.6 and then incubated at 28 °C for 10 h. After centrifugation at 9,000 rpm for 20 min, the cells were harvested and preserved for further experiments.

All purification steps were carried out at 4 °C. The wet cell was disrupted by sonication in buffer (20 mM NaH_2PO_4, pH 8.0; 300 mM NaCl). The cell debris was subsequently removed by centrifugation at 12,000 rpm for 20 min. The soluble fraction was loaded onto a Nickel-NTA superflow column (10 ml, Applied Biosystems, Foster, CA, USA) equilibrated with a binding buffer (20 mM NaH_2PO_4, pH 8.0; 300 mM NaCl). The column was eluted with two volumes of washing buffer (20 mM NaH_2PO_4, pH 8.0; 300 mM NaCl; 50 mM imidazole) and unbounded proteins were washed out from the column. Later, the expected protein was eluted from the column with an elution buffer (20 mM NaH_2PO_4, pH 8.0; 300 mM NaCl; 500 mM imidazole). The fractions containing nitrilase were pooled, dialyzed and analyzed by SDS-PAGE. Protein concentration was determined by the BCA protein assay kit (KeyGEN Biotech, China).

Effects of factors on the methionine production

The effects of pH, temperature and chemicals on the catalytic activities were investigated. The optimal pH for the reaction was determined in 10 ml reaction system at 30 °C, with a pH range of 4.0–9.0 using 50 mM citric acid-sodium citrate buffer (pH 4.0–6.0), K_2HPO_4–KH_2PO_4 buffer (pH 6.0–7.0) and Tris–HCl buffer (pH 7.0–8.5). The pH stability of nitrilase was carried out by pre-incubating the cells at 25 °C in the aforementioned buffers at pH values range from 4.0–8.5 for 6 h. The residual enzyme activities were determined under the standard assay conditions.

The optimal temperature for the reaction was determined at temperatures ranging from 25 to 50 °C. Thermal stability was investigated by incubating cells at different temperatures from 30–50 °C in water bath and the residual activities were assayed under the standard assay conditions at certain interval of time within 20 h. The half-life of nitrilase at each temperature was determined by plotting the natural logarithm of residual activity (lnRA) versus time at each test temperature. The effects of different chemicals on the biotransformation were assessed with metal ions and EDTA (final concentration of 5 mM). The optimal substrate concentration was investigated in a concentration ranging from 50 to 300 mM.

Analytical methods

The standard reaction was performed in 10 ml Tris–HCl buffer (50 mM, pH 7.5) containing 0.1 g resting cells and 50 mM 2-amino-4-methylthiobutylnitrile at 37 °C, 150 rpm for 30 min on a rotary shaker. Samples were withdrawn at regular intervals and the reaction was quenched by adding 100 μl of 6 M HCl. After centrifugation, substrate and product were determined by HPLC (LC-10AS, Shimadzu, Japan), equipped with a C18 (250 mm × 4.6 mm, 5 μm)

column and a SPD-2A prominence UV/VIS detector (Shimadzu, Japan) set at 210 nm. The mobile phase was composed of methanol and water at a ratio of 25:75 (V/V). One unit (U) of the nitrilase activity is defined as the amount of enzyme that produces 1 μmol of methionine per minute under the assay conditions. All activity measurements were performed in triplicate.

Cells immobilization

25 ml cell suspension (containing 1.5 g wet cells and 2.5 g PEG6000) was added into 75 ml of 2.5 % sodium alginate (SA) solution, and mixed thoroughly to give homogeneous cell/PEG-SA suspension. By using a peristaltic pump, cell/PEG-SA solution was extruded through silicone tubing and dripped into 500 ml of 2.5 % $CaCl_2$ solution to give the entrapped beads. The entrapped beads obtained were maintained in the 2.5 % $CaCl_2$ solution at 4 °C for 6 h. Finally, PEG-SA cell gel beads were subsequently washed three times with sterile distilled water.

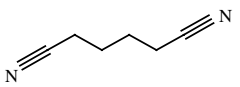
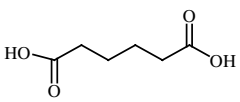
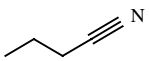
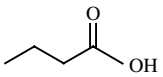
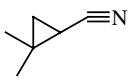
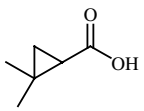
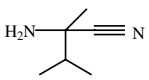
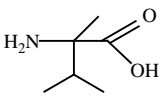
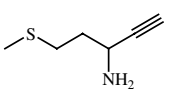
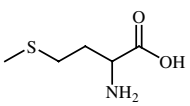
Semi-continuous production of methionine in packed-bed reactor

Immobilized cells were packed into a glass column with a water jacket for temperature control. The temperature was maintained at 40 °C using circulator. Long-term stability of the immobilized cell was evaluated for 100 h under the optimal conditions with 50 g of immobilized cells (containing 3 g wet cells), 40 mM of substrate and a feeding rate of 4 ml/min.

Isolation and identification of methionine

Reaction mixture containing methionine was centrifuged at 12,000 g for 10 min. The solution was concentrated. Methionine was crystallized from alcohol at 4 °C. The crystals were separated off on a vacuum filter, washed with water pre-cooled to 4 °C, and then dried in a vacuum drying oven. The crystals of methionine obtained were further investigated.

Table 1 Substrate specificity of the recombinant nitrilase

Substrates	Products	Activity（U/mg）
Acetonitrile	Acetic Acid	
		58.9
Hexanedinitrile	Hexanedioic acid	
		27.89
Butyronitrile	Butyric acid	
		258.9
2,2-dimethylcyclopropyl cyanide	2,2-dimethylcyclopropyl acid	
		38
2-amino-2,3-dimethylbutanenitrile	2-amino-2,3-dimethylbutane acid	
		88
2-amino-4-methylthio butylnitrile	2-amino-4-methylthiobutyric acid	
		960

The ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectra of methionine were obtained on an NMR spectrometer (AVANCE 500 MHz, Bruker, Falanden, Switzerland) with D_2O as the solvent, using 500 and 125 MHz for carbon and proton determinations, respectively. The molecular weight of the product was confirmed by ESI–MS.

Results

Purification of the recombinant nitrilase

After expression, the enzyme was purified to electrophoretic homogeneity by Ni–NTA affinity chromatography. The SDS-PAGE analysis showed a band with the apparent molecular weight of 43 kDa, which is in agreement with the molecular weight deduced from the gene sequence and previous reports [5, 14]. The purified nitrilase was found to have high activity of 980 U/mg toward 2-amino-4-methylthiobutylnitrile.

Substrate specificity demonstrated that the enzyme preferred to hydrolyzing aliphatic nitrile compounds. As shown in Table 1, the nitrilase displayed excellent activity to 2-amino-4-methylthiobutylnitrile, higher than that of acetonitrile, hexanedinitrile, butyronitrile and 2-amino-2, 3-dimethylbutanenitrile, however, no activity was observed for acrylonitrile. It also exhibited that activity toward 2, 2-dimethylcyclopropyl cyanide. For aromatic and heterocyclic nitriles such as phenylacetonitrile, mandelonitrile and 2-chloro-3-cyanopyridine, no hydrolysis was found, which is similar to the report of Liu [14]. These results further confirmed that this nitrilase belongs to the family of aliphatic nitrilases [12, 14, 23].

Effect of pH on the biosynthesis of methionine

The effect of pH on the synthesis of methionine from 2-amino-4-methylthiobutylnitrile with the recombinant nitrilase was determined. The specific pH was controlled by different buffer systems. As demonstrated in Fig. 2a, it revealed that the catalytic activity increased with pH from 4.0 to 7.5 and the maximum activity was observed under pH 7.5 with Tris–HCl buffer. The pH stability showed the recombinant nitrilase was stable between pH 7.0 and 7.5 (Fig. 2b). However, the activity decreased rapidly below 7.0 or above 7.5.

Effect of temperature on the biosynthesis of methionine

To evaluate the effects of temperature on methionine formation, the reaction was carried out at various temperatures. As depicted in Fig. 3a, the enzyme activity gradually increased with the temperature from 25 to 50 °C, and the optimum temperature was around 40 °C. However, the activity

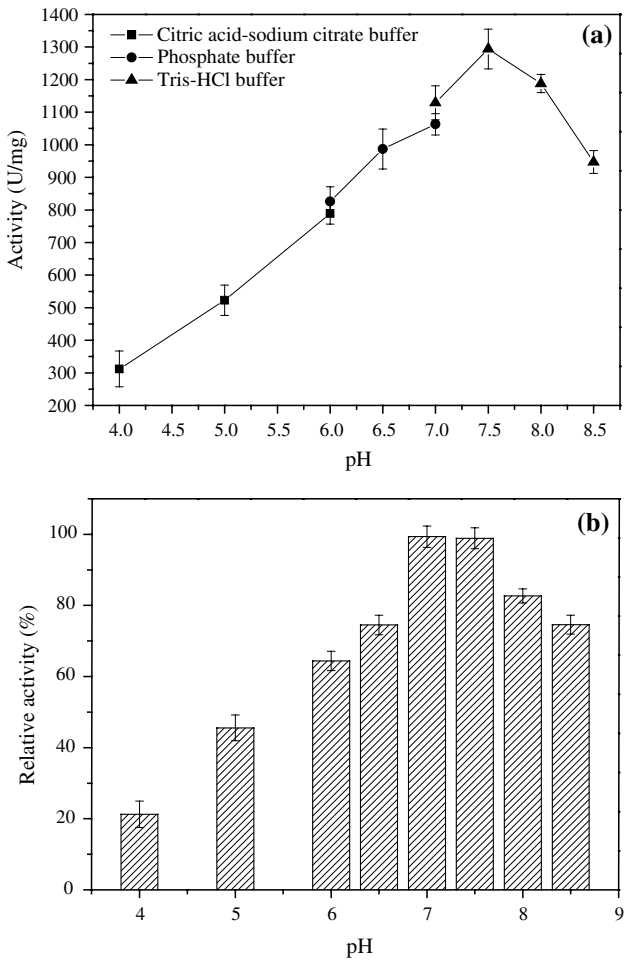


Fig. 2 Effect of pH on the biosynthesis of methionine. **a** pH dependence. The nitrilase activity was measured at different pH values (4.0–8.5). **b** pH stability. The nitrilase was preincubated at 25 °C in different pH values for 6 h and the residual activity was tested. The activity of nitrilase that assayed under the standard reaction was taken as 100 %

decreased sharply above 50 °C. The nitrilase thermostability was investigated by analyzing the residual activities after incubation at different temperatures. The enzyme was relatively stable at 30 °C with a half-life of 16.82 h (Fig. 3b). At 40 and 50 °C, the half-lives decreased to 5.52 and 0.58 h, respectively. It demonstrated that the nitrilase obtained in this study was more thermostable than that of nitrilases from *A. facilis* ZJUTB10 with half-lives of 57 min at 40 °C and 22 min at 50 °C, and *P. putida* with half-lives of 76 min at 40 °C and 9 min at 50 °C, respectively [12].

Effect of metal ions on the biosynthesis of methionine

The effects of different metal ions on the synthesis of methionine by the recombinant nitrilase were tested by incubating the resting cells in the presence of reagents at 40 °C for 30 min. The residual catalytic activities were

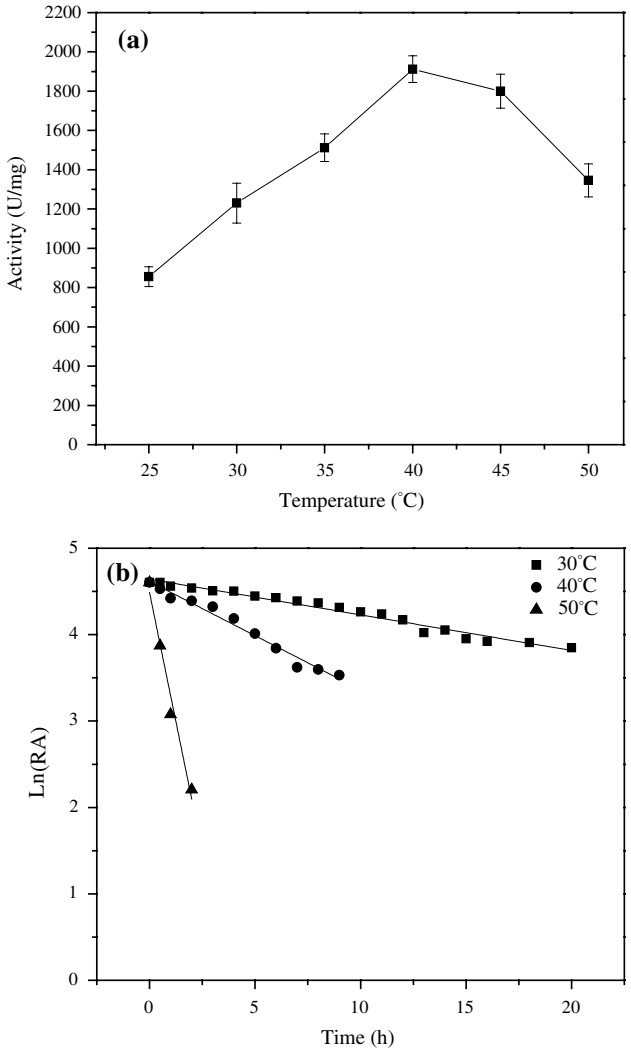


Fig. 3 Effect of temperature on the biosynthesis of methionine. **a** Temperature dependence. Reactions were performed at pH 7.5 for 30 min at various temperatures (25–50 °C). **b** Thermostability. The residual activities were assayed by incubating the enzyme at temperature 30–50 °C for the indicated time intervals

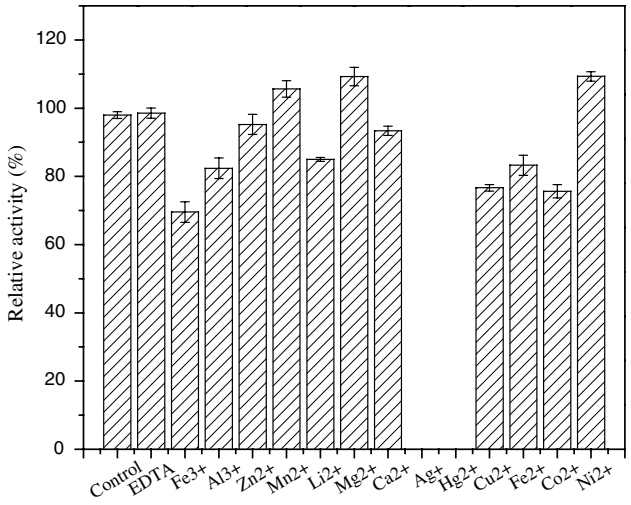


Fig. 4 Effect of metal ions on the biosynthesis of methionine. Reactions were performed with the addition of various metal ions under standard conditions. The activity of nitrilase that assayed under the standard reaction was taken as 100 %

assayed according to the standard method. The results (Fig. 4) showed that the majority of metal ions did not significantly affect the reaction except Ag^{+} and Hg^{2+}, which severely inhibited the synthesis of methionine. Fe^{3+}, Al^{3+}, Li^{2+}, Cu^{2+}, Fe^{2+} and Co^{2+} could partially inhibit the catalytic activity. The metal-chelating agent EDTA also did not inhibit the catalytic activity, indicating that this enzyme has no metal ion requirement, which is similar to the reported nitrilase from *A. facilis* ZJUTB10 [12].

Effect of substrate concentration on the synthesis of methionine

As shown in Fig. 5a, the specific activity increased from 1278 to 2892 U/mg when the concentration of substrate increased up to 200 mM. Complete conversion of 2-amino-4-methylthiobutylnitrile to methionine was attained when the concentration of substrate was below 250 mM. The study indicated that raising substrate concentration, however, decreased the conversion. To enhance the volumetric productivity of the biocatalytic process and to avoid the complicated and costly downstream process, substrate concentration was further increased at a fixed ratio of substrate to catalysts (S/C). As depicted in Fig. 5b, methionine formation was increased from 250 to 300 mM with conversion up to 100 % after reaction time of 120 min when the concentration of 2-amino-4-methylthiobutylnitrile increased from 250 to 300 mM. This is the highest productivity of enzymatic preparation of methionine ever reported [8]. However, the conversion was decreased to 92 % when the substrate concentration was further increased to 350 mM.

Production of methionine in packed-bed reactor with immobilized cells

Immobilized cells are preferred since they could be recycled and potentially reduce production costs. Among the conventional approaches of microcapsule, crosslinking, adsorption and entrapment for cell immobilization, entrapment seemed to be a better choice because it is cost-effective, easy to handle and manipulate. Here the *E. coli* BL21 (DE3)/pET-28b (+)-Af-*NIT* cells were immobilized in PEG-SA beads for the hydrolysis of 2-amino-4-methylthiobutylnitrile to methionine. Immobilized cells were packed into a glass column and the H/D ratio of the packed-bed reactor was 11.4. The economics of an immobilized cell process depends largely

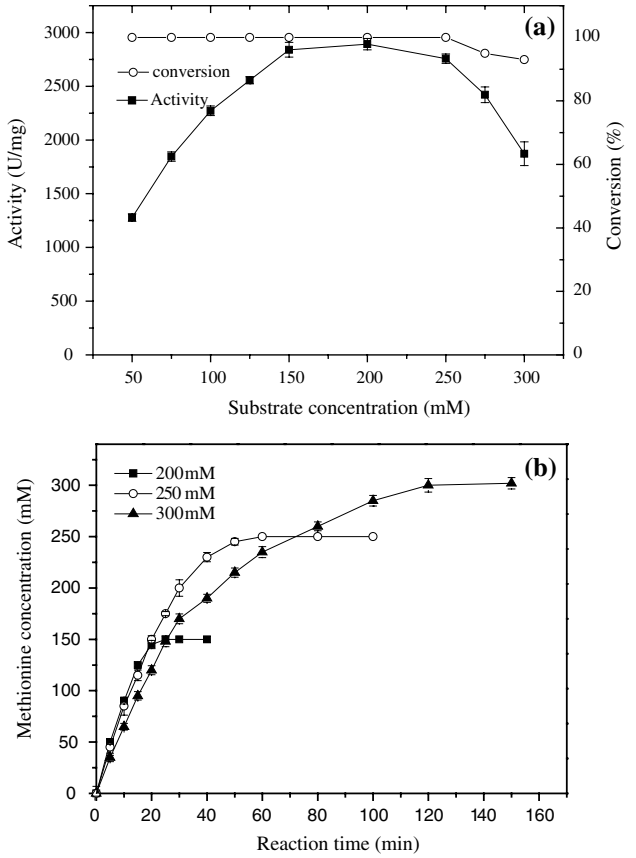


Fig. 5 Time course of methionine synthesis from 2-amino-4-methylthiobutylnitrile. **a** The concentration of methionine formed at different substrate concentrations. **b** Time courses of methionine formation at different substrate concentrations. Reaction was carried out at pH 7.5 and 40 °C

on the lifetime of the enzyme. The operation stability of an immobilized biocatalyst is an important issue for practical application.

Reactions were carried out at 40 °C for 100 h with a feeding rate of 4 ml/min at an initial substrate concentration of 40 mM. Fig. 6 shows the plot of conversion at different times. The conversion still remained above 80 % after operating for 100 h in packed-bed reactor, demonstrating a good reusability of the immobilized biocatalyst for methionine production (Fig. 6). The average productivity of the methionine in packed-bed reactor was calculated to be 1.7 g/(h gDCW), which was much higher than the reported yield of 0.09 g/(h gDCW) [16, 18]. Therefore, it showed that the immobilized cells have better operational stability. These results demonstrated that both the frequency of catalyst replacement and the operation cost of enzymatic synthesis of methionine from 2-amino-4-methylthiobutylnitrile could be reduced in practical application.

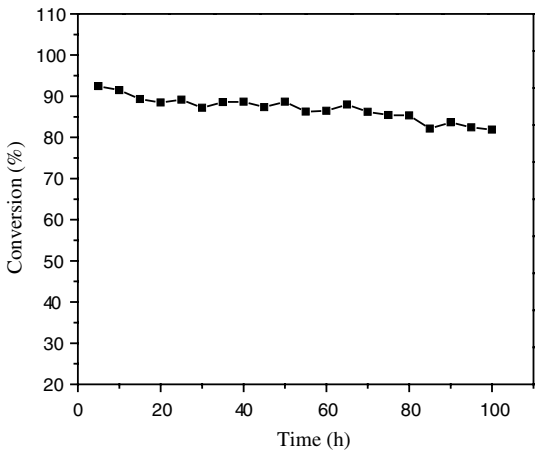


Fig. 6 Operational stability of immobilized cell in packed-bed reactor for methionine production

Isolation and identification of methionine

After isolation and purification according to the method described in methods, methionine with 99.1 % of purity and 97 % of total yield was obtained. The product was further identified by ^{13}C-NMR [δ_C (100 MHz; D_2O) 13.97 (CH_3), 29.70 (CH_2), 28.87 (CH_2), 53.94 (CH), 174.19 (CO)], ^{1}H-NMR [δ_H (500 MHz; D_2O) 3.77–3.74 (m, 1H), 2.55–2.52 (m, 2H), 2.10–1.99 (m, 2H), 2.03 (s, 3H)]. The molecular weight of the product was confirmed by ESI–MS, which revealed the following result: 172 (m/z) $(M + Na)^+$, calculated value for $C_5H_{11}O_2NS$, 149. All of these results were identical to the spectra obtained by standard sample of methionine, which indicated that the isolated product was methionine. It is revealed that the biosynthesized methionine could be easily separated with good purity and yield.

Discussion

Biotransformation of nitriles has attracted much attention due to the powerful function of nitrile-converting enzymes, which has been extensively explored for the industrial production of some valuable compounds. As far as methionine is concerned, it is found that only very few biocatalysts were described to produce, and usually with low enzyme activity [17, 18]. So, obtaining of a new nitrilase with high activity is an important prerequisite for its practical utilization. In this study, a nitrilase gene from *Acidovorax facilis* was synthesized and expressed in *E. coli*. Characterization of the recombinant enzyme showed that this nitrilase has excellent activity toward 2-amino-4-methlthiobutanenitrile. Although the *Acidovorax facilis* nitrilase had been reported to hydrolyze a wide variety of nitriles, the protein

engineering was used to increase this nitrilase activity in *E. coli* [5, 22]. As far as we know, this is the first time to report the nitrilase gene from *Acidovorax facilis* was overexpressed in *E. coli* to convert 2-amino-4-methlthiobutanenitrile to methionine with such a high yield.

There are several factors that influence the function of the recombinant enzyme. From this study, we found that this nitrilase was more stable to heat compared to other nitrilases, and pH 7.5 favored its activity [12]. Just like the reported nitrilase from *A. facilis* ZJUTB10 [12], this nitrilase had no metal ion requirements. The specific activity increased as the substrate concentration increased to 200 mM, however, the enzyme of nitrilase was inhibited by higher substrate concentration. The time course showed that it is effective to synthesis methionine in 100 % conversion with the substrate loading below 250 mM. At a fixed ratio of substrate to catalyst, 300 mM methionine was produced in a conversion of 100 % after reaction time of 120 min. Moreover, methionine with the purity of 99.1 % and total yield of 97 % was obtained, higher than the reported literatures [17].

Due to the low solubility of methionine, it is particularly important to timely separate the methionine in the reaction system to improve the methionine production. Packed-bed reactor is a kind of practical and efficient reactor, showing high conversion efficiency and long reaction time [24]. The major advantage of a packed-bed reactor is the reduction of liquid shearing on the immobilized biocatalyst compared to a stirred tank reactor. In this work, the immobilized nitrilase-catalyzed production of methionine in packed-bed reactor was investigated for the first time. The immobilized enzyme exhibited good operation stability and retained over 80 % of the initial activity after operating for 100 h in reactor.

In summary, it is shown that the novely synthesized nitrilase from *A. faecalis* is a useful catalyst for the preparation of methionine under mild conditions. The catalytic conditions for methionine production were studied. To our knowledge, the biotransformation preparation of methionine achieved in this study is the highest level ever reported. The biocatalytic route for the production of methionine is greener and more atom-efficient.

Acknowledgments The authors gratefully acknowledge the National Natural Science Foundation of China (No. 21202150), the Research Program of Science and Technology Department of Zhejiang Province (No. 2011R09043-07) and Natural Science Foundation of Zhejiang Province (No. R3110155).

References

1. Ali NM, Shakoori FR, Shakoore AR (2011) Improvement in methionine production by local bacterial isolates. Pak J Zool 43:611–614
2. Amalendu PR, Gomes J (2009) Simultaneous dissolved oxygen and glucose regulation in fed-batch methionine production using decoupled input-output linearizing control. J Process Contr 19:664–677
3. Ariagno A, Bontoux MC, Olivier FB, Largeau D (1998) Enzymatic hydrolysis of racemic a-substituted 4-methylthiobutyronitriles using a nitrilase from Alcaligenes faecalis, Gordona terrae or Rhodococcus sp. US Patent 5814497
4. Banerjee A, Sharma R, Banerjee UC (2002) The nitrile-degrading enzymes: current status and future prospects. Appl Microbiol Biotechnol 60:33–44
5. Chauhan S, Wu S, Blumerman S, Fallon RD, Gavagan JE, DiCosimo R, Payne MS (2003) Purification, cloning, sequencing and over-expression in *Escherichia coli* of a regioselective aliphatic nitrilase from *Acidovorax facilis* 72 W. Appl Microbiol Biotechnol 61:118–122
6. Gomes J, Kumar D (2005) Production of L-methionine by submerged fermentation: a review. Enzyme Microb Technol 37: 3–18
7. Kalhan SC, Marczewski SE (2012) Methionine, homocysteine one carbon metabolism and fetal growth. Rev Endocr Metab Dis 13:109–119
8. Kobayashi Y, Ono I, Hayakawa K, Mizul R, Ishikawa T (2003) Process for the production of methionine. US Patent 20050176115
9. Korendyaseva TK, Martinov MV, Dudchenko AM, Vitvitsky VM (2010) Distribution of methionine between cells and incubation medium in suspension of rat hepatocytes. Amino Acids 39(5):1281–1289
10. Kromer JO, Wittmann C, Schroder H, Heinzle E (2006) Metabolic pathway analysis for rational design of L-methionine production by *Escherichia coli* and *Corynebacterium glutamicum*. Metab Eng 8:353–369
11. Kumar D, Gomes J (2005) Methionine production by fermentation. Biotechnol Adv 23:41–61
12. Liu ZQ, Dong LZ, Cheng F, Xue YP, Wang YS, Ding JN, Zheng YG, Shen YC (2011) Gene cloning, expression, and characterization of a nitrilase from Alcaligenes faecalis ZJUTB10. J Agr Food Chem 59:11560–11570
13. Liu ZQ, Gosser Y, Baker PJ, Ravee Y, Lu Z, Alemu G, Li H, Butterfoss GL, Kong XP, Gross R, Montclare JK (2009) Structural and functional studies of *Aspergillus oryzae* Cutinase: enhanced thermostability and hydrolytic activity of synthetic ester and polyester degradation. J Am Chem Soc 131:15711–157116
14. Liu ZQ, Zhou M, Zhang XH, Xu JM, Xue YP, Zheng YG (2012) Biosynthesis of iminodiacetic acid from iminodiacetonitrile by immobilized recombinant *Escherichia coli* harboring nitrilase. J Mol Microbiol Biotechnol 22:35–47
15. Martinkova L, Mylerova V (2003) Synthetic applications of nitrile-converting enzymes. Curr Org Chem 7:1279–1295
16. Mondal S, Das YB, Chatterjee SP (1996) Methionine production by microorganisms. Folia Microbiol 41:465–472
17. Olivier FB, Pierrard J, David C, Moral P, Horbez D (2001) Industrial scale process for the preparation of 2-hydroxy-4-methylbutyric acid using a nitrilase. US Patent 6180359
18. Park SD, Lee JY, Sim SY, Kim Y, Lee HS (2007) Characteristics of methionine production by an engineered *Corynebacterium glutamicum* strain. Metab Eng 9(4):327–336
19. Rey P, Rossi JC, Taillades J, Gros G, Nore O (2004) Hydrolysis of nitriles using an immobilized nitrilase: applications to the synthesis of methionine hydroxy analogue derivatives. J Agric Food Chem 52:8155–8162
20. Shama NN, Sharma M, Bhalla TC (2011) An improved nitrilase-mediated bioprocess for synthesis of nicotinic acid from 3-cyanopyridine with hyperinduced *Nocardia globerula* NHB-2. J Ind Microbiol Biotechnol 38:1235–1243

21. Villalobos A, Ness JE, Gustafsson C, Minshull J, Govindarajan S (2006) Gene designer: a synthetic biology tool for constructing artificial DNA segments. BMC Bioinformatics 7:285
22. Wu S, Fogiel AJ, Petrillo KL, Hann EC, Mersinger LJ, DiCosimo R, O'Keefe DP, Ben-Bassat A, Payne MS (2007) Protein engineering of *Acidovorax facilis* 72 W nitrilase for bioprocess development. Biotechnol Bioeng 97:689–693
23. Xue YP, Jiang T, Liu X, Zheng YG (2013) Efficient production of *S*-(+)-2-chlorophenylglycine by immobilized penicillin G acylase in a recirculating packed bed reactor. Biochem Eng J 74:88–94
24. Xue YP, Xu SZ, Liu ZQ, Zheng YG, Shen YC (2011) Enantioselective biocatalytic hydrolysis of (R, S)-mandelonitrile for production of (R)-(-)-mandelic acid by a newly isolated mutant strain. J Ind Microbiol Biotechnol 38:337–345