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BIOCATALYSIS - REVIEW

Efficient bioconversion of L-glutamate to γ-aminobutyric acid by *Lactobacillus brevis* resting cells

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Abstract This work investigated the efficient bioconversion process of L-glutamate to GABA by *Lactobacillus brevis* TCCC 13007 resting cells. The optimal bioconversion system was composed of 50 g/L 48 h cultivated wet resting cells, 0.1 mM pyridoxal phosphate in glutamate-containing 0.6 M citrate buffer (pH 4.5) and performed at 45 °C and 180 rpm. By 10 h bioconversion at the ratio of 80 g/L L-glutamic acid to 240 g/L monosodium glutamate, the final titer of GABA reached 201.18 g/L at the molar bioconversion ratio of 99.4 %. This process presents a potential for industrial and commercial applications and also offers a promising feasibility of continuous GABA production coupled with fermentation. Besides, the built kinetics model revealed that the optimum operating conditions were 45 °C and pH 4.5, and the bioconversion kinetics at low ranges of substrate concentration ($0 < S < 80$ g/L) was assumed to follow the classical Michaelis–Menten equation.

Keywords *Lactobacillus brevis* · Glutamate · γ-Aminobutyric acid · Bioconversion · Kinetics model

This article is dedicated to Dr. Arnold L. Demain for his 90th birthday, and his lifetime devotion and worldwide impact on industrial microbiology and biotechnology.

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Introduction

γ-Aminobutyric acid (GABA) is a natural non-proteinogenic amino acid, which is widely found among animals, plants and microorganisms [4, 11, 14, 20]. In mammalians, GABA acts as an important type of inhibitory neurotransmitter to modulate the overall excitability of the central nervous system [1, 9]. In addition, it possesses several well-known physiological functions, such as improving brain functions, antianxiety effects, tranquilizer effects, boosting fertility, diuretic effects, anti-diabetic effects and treatment of epilepsy [9, 29]. Thus, GABA has broad application prospects in food and pharmaceutical industries as a bioactive compound [4–6, 13, 14].

GABA production can be divided into chemical and biological syntheses [2, 6, 13, 20]. However, GABA by chemical synthesis cannot be applied to food industry in China due to its unsafety. Lactic acid bacteria (LAB), the well-known and generally regarded as safe (GRAS) food microorganisms [22–24, 32], are used worldwide in food industry. Many researches disclosed that LAB possess the high activity of glutamate decarboxylase (GAD, EC 4.1.1.15), which is a sole enzyme to catalyze an irreversible α-decarboxylation reaction of L-glutamate or its salts into GABA [12, 18, 26, 27]. Various LAB species demonstrated the ability to produce GABA, including *Lactobacillus paracasei* isolated from traditional fermented foods [13], *L. buchneri* from kimchi [1, 33], *L. brevis* from kimchi [27], alcohol distillery lees [28] and naturally pickled Chinese vegetables [32].

Since resting cells are no longer growing but metabolically active, bioconversion technology using resting cells is a green method for the preparation of various metabolic products in food, pharmaceutical and other industries [7,

19]. The use of resting cells in bioconversion processes can be more favorable than growing cells due to its easier and more flexible operation in the reaction system as cell factories [10, 16, 24]. In the previous studies, the bioconversion of L-glutamate to GABA by LAB resting cells is a possible approach for GABA production due to its potential of cost efficiency, safe and high catalytic efficiency, environmental-friendly compatibility and easy downstream processing [3, 5, 8, 15, 22–24, 32, 33]. However, due to the lower GABA titer or longer bioconversion period for bioconversion process, there is still much space for the enhancement of GABA production.

In this study, the process of bioconversion from L-glutamate to GABA by *L. brevis* TCCC 13007 resting cells and the influence of several factors on GABA production were investigated. The optimal composition of bioconversion system and the kinetic characterization of bioconversion by *L. brevis* TCCC 13007 resting cells were also determined to bring insight into the conversion of L-glutamate to GABA catalyzed by the GAD of GRAS LAB.

Materials and methods

Strain, chemicals and media

Lactobacillus brevis strain TCCC 13007 (the same as CGMCC No. 3414 of China General Microbiological Culture Collection Center) used in the present work was isolated from naturally pickled Chinese vegetables in our previous work and preserved in Tianjin University of Science & Technology's Center of Culture Collection [32].

GABA standard and pyridoxal 5′-phosphate (PLP) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Monosodium glutamate (MSG, purity ≥ 99 %) was bought from a local supermarket in China. All other chemicals were of analytical and biochemical grades.

The glucose-yeast extract-peptone (GYP) medium [8] at natural pH was used for seed cultivation of *L. brevis* TCCC 13007. The cultivation medium consisted of (g/L): glucose 35, yeast extract 30, sodium acetate 2, $MgSO_4 \cdot 7H_2O$ 0.2, $(NH_4)_2SO_4$ 0.2, MSG 50, pH 6.0. Glucose, MSG and the other medium components were autoclaved separately at 121 °C for 20 min and mixed just prior to inoculation.

Preparation of resting cells and bioconversion process

The cells were harvested by centrifugation at 5000 rpm for 10 min at 4 °C after 48 h cultivation, and then washed twice with sterile saline. Finally, the resting cells were resuspended in 0.6 M citric acid–sodium citrate buffer (citrate buffer) containing MSG (pH 4.5) optimized from various buffers. The bioconversion was performed at 45 °C and 180 rpm shaking for 8 or 12 h [23].

Kinetics model establishment of GABA bioconversion

The kinetic parameters of the bioconversion by *L. brevis* TCCC 13007 resting cells were investigated using the Lineweaver–Burk double reciprocal plot. The bioconversion ratio equation was established under different bioconversion conditions at pH 4.5 (35, 45 and 55 °C) or 45 °C (pH 4.0, 4.5 and 5.0), which was performed at 180 rpm in 250 mL flask containing 30 mL of 0.6 M citric acid-sodium citrate buffer (pH 4.5), 0.1 mM PLP and 50 g/L wet cells. GABA concentration in the samples was analyzed by HPLC after the bioconversion. All the experiments were performed in triplicate.

Analytical methods

For determination of GABA content in bioconversion, the supernatant after the removal of *L. brevis* TCCC 13007 resting cells was mixed with 2,4-dinitro-1-fluorobenzene for derivation at 60 °C for 1 h, and then the mixture was filtered by 0.22 μm cellulose acetate membrane filter [17]. The derivative was analyzed by an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent ZORBAX StableBond C18 column (4.6 × 250 mm I.D., 5 μm particle). The operating conditions were as follows: flow rate of 0.8 mL/min, VWD UV detector at 360 nm and column temperature at 30 °C. Gradient elution with water/acetonitrile/0.05 M sodium acetate solution as mobile phase was used for quantitative GABA analysis [31].

The conversion ratio of MSG to GABA was calculated as follows [32]:

$$\text{Conversion ratio} = \frac{\text{Final GABA concentration (mM)}}{\text{Total MSG concentration (mM)}} \times 100\,\% \quad (1)$$

Results

Effect of wet cell concentration on GABA production

In the bioconversion process of GABA, cell concentration is closely connected with conversion ratio. As shown in Fig. 1, when the cell concentration is excessive, the specific conversion ratio was too low to improve the utilization ratio of cells. Although there was a high specific conversion ratio at low cell concentration, a corresponding low GABA production was observed. The result indicated that specific conversion ratio dropped fast at the concentration of 50–150 g/L wet cells with 50 g/L MSG. In view of the GABA production, cultivation cost and the specific

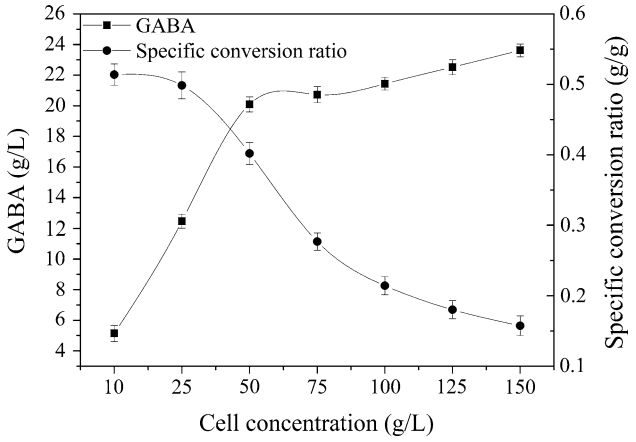

Fig. 1 Effect of cell concentration on GABA production and specific conversion ratio by *L. brevis* TCCC 13007 resting cells *filled square* GABA (g/L); *filled circle* specific conversion ratio (g/g)

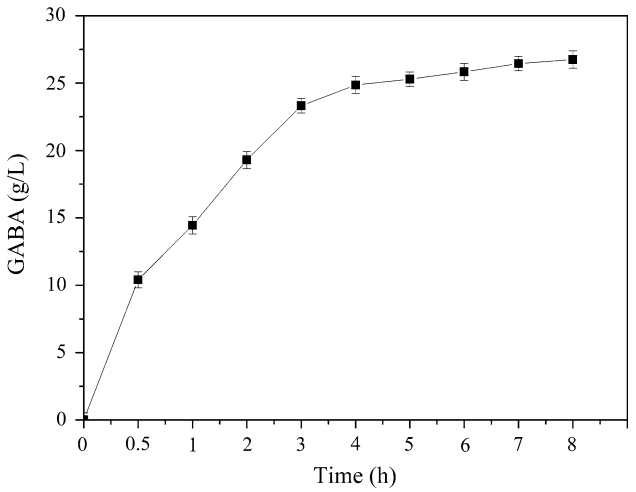

Fig. 2 Effect of bioconversion time on GABA production by *L. brevis* TCCC 13007 resting cells

conversion ratio of the cells, a 50 g/L wet cell concentration was used for further bioconversion analysis in this study.

Effect of bioconversion time on GABA production

As a cytosolic enzyme in all known microorganisms, GAD could only catalyze the conversion of GABA from specific L-glutamate substrate after passing through the cytomembrane to enter into the cytoplasm, which is a gradual process. Figure 2 suggested that GABA production from 50 g/L MSG increased with the extension of bioconversion time in 8 h. The reaction ratios of GABA bioconversion were much higher at 4 h than at 4–8 h, and the increasing of GABA yield became gently after a 4 h reaction time. It might be due to the fact that the ratios of substrate depletion and product accumulation went in a relative balance and substrate depletion also affected the reaction ratio.

Table 1 Effect of different buffer systems on GABA production

Buffer system	GABA (g/L)
Citric acid–sodium citrate buffer	31.33
Citric acid–ammonium citrate buffer	30.30
Lactic acid–sodium lactate buffer	29.33
Acetic acid–ammonium acetate buffer	29.28
Acetic acid–sodium acetate buffer	29.08
Tartaric acid–NaOH buffer	26.48
Citric acid–NaOH-HCl buffer	24.46
Succinic acid–NaOH buffer	10.82
Na_2HPO_4–citric acid buffer	10.82
Potassium biphthalate–KOH buffer	1.75

Table 2 Effect of citrate buffer concentration on GABA production

Citric acid–sodium citrate buffer (M)	GABA (g/L)
0.1	20.24
0.2	26.13
0.4	28.09
0.6	32.80
0.8	32.96
1.0	32.82
1.2	32.53

Effect of different buffer systems and concentrations on GABA production

Sanders et al. [21] reported that α-decarboxylation reaction of L-glutamate occurred with the simultaneous consumption of H^+ and production of CO_2, which led to an increase of the intracellular pH, and thus beyond the optimal pH of GAD activity. Therefore, selecting a buffer system was necessary for the bioconversion process. Table 1 displayed that *L. brevis* TCCC 13007 resting cells expressed the highest bioconversion ability from 80 g/L MSG to GABA in citric acid-sodium citrate buffer system. Consequently, citric acid-sodium citrate buffer is a perfect buffer system for bioconversion of L-glutamate to GABA here.

Table 2 illustrated the bioconversion ability of the resting cells at different concentrations of citric acid-sodium citrate buffer for maximum GABA yield from 80 g/L MSG. The bioconversion ability of the prepared resting cells greatly increased with the increasing of buffer concentration at 0.1–0.6 M, and then became almost constant at 0.6–1.2 M. It is likely that the resting cell bioconversion ability

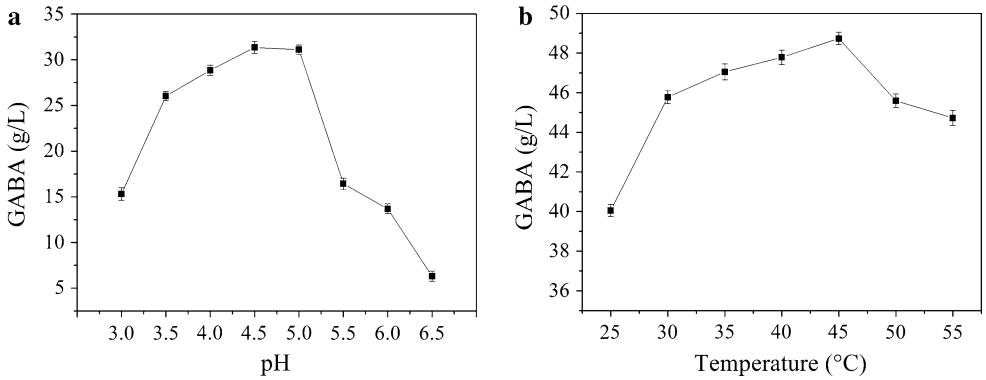

Fig. 3 Optimization of the biocatalysis conditions from 80 g/L MSG. **a** Effect of pH on GABA production; **b** effect of temperature on GABA production

was related to buffering capacity and ionic strength of the buffer solution, and high buffer concentration was helpful to maintain the pH for the enzymatic reaction system.

Effect of pH and temperature on the bioconversion of MSG to GABA

In GABA shunt, beside GAD, GABA transaminase (GABA-T; EC 2.6.1.19) is the second enzyme to catalyze the reversible conversion of GABA to succinic semialdehyde using either pyruvate or α-ketoglutarate as amino acceptor, and the irreversibly conversion of succinic semialdehyde to succinate is oxidized by succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.16) [14]. Thus, it is necessary to passivate the activities of the two enzymes, both of which have an optimal alkaline pH via adjusting the buffer pH. Figure 3a exhibited that the optimal pH of GABA bioconversion was 4.5.

Reaction temperature impacts significant effect on enzyme activity. The variation of the reaction temperature influences the enzyme stability and activity not only by changing interactions between enzyme and substrate, but also by altering the thermodynamic equilibrium of a reaction. Here, GABA yields increased rapidly at 25–45 °C and reached the highest at 45 °C (Fig. 3b).

Effect of PLP on GABA production

As a PLP-dependent enzyme [10], GAD could catalyze the formation of a Schiff base intermediate which is the product of the reaction between PLP and L-glutamate [25]. Hence, we assume that the yield of GABA would increase by adding PLP to the bioconversion system. In Table 3, GABA yield increased significantly when PLP were added. The results revealed that PLP was beneficial to improve the GABA yield and the GABA titer leveled off after 0.1 mM PLP was added, since an overdose of PLP inhibited the enzyme reaction and could not bind with more MSG.

Table 3 Effect of PLP concentration on GABA production

PLP (mM)	GABA (g/L)
0	35.78
0.05	40.87
0.10	48.73
0.15	48.36
0.20	48.46
0.25	48.66
0.30	48.80

Effect of MSG concentration on GABA production

Reaction ratio was greatly influenced by substrate concentrations in enzymatic reaction by double effects of either accelerating the reaction speed or inhibiting enzyme activity. In this study, the influence of initial MSG concentration on GABA yield and bioconversion ratio by *L. brevis* TCCC 13007 resting cells was determined (Fig. 4). The GABA titer gradually increased with increasing the initial MSG concentration from 40 to 130 g/L, however, the conversion ratio decreased dramatically meanwhile the MSG concentration increased over 80 g/L. These results revealed that there was no obvious substrate inhibition in GABA bioconversion at the range of 40–130 g/L MSG.

Figure 4 also indicated that the reaction was first-order kinetics at the range of 40–80 g/L MSG, mixed-order kinetics at 80–110 g/L MSG, and zero-order kinetics at over 110 g/L MSG, respectively. The result suggested that this GAD-catalyzed bioconversion well fitted the Michaelis–Menten model.

Kinetic optimization of bioconversion by *L. brevis* TCCC 13007 resting cells

Michaelis–Menten equation is generally used to describe the kinetics of enzyme-catalyzed reactions. In this study, the result demonstrated that substrate inhibition did not exist in

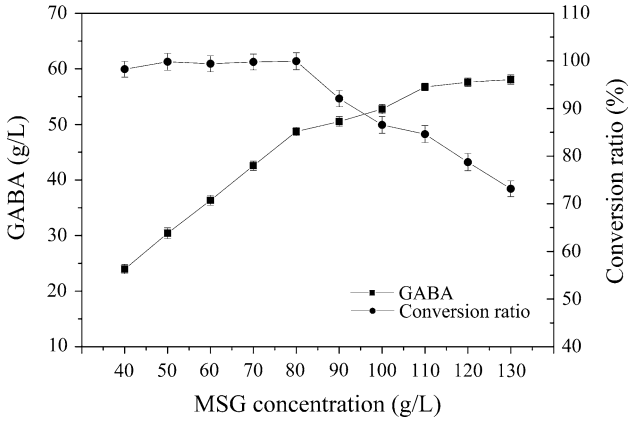

Fig. 4 Effect of MSG concentration on GABA production and conversion ratio by *L. brevis* TCCC 13007 resting cells *filled square* GABA (g/L); *filled circle* conversion ratio (%)

bioconversion process. Moreover, the kinetic characterization of biotransformation by *L. brevis* TCCC 13007 resting cells at the low range of MSG substrate concentration was assumed to follow the classical Michaelis–Menten equation: where V_{max} is the maximum ratio achieved by the system, K_m represents the Michaelis constant while [*S*] is MSG concentration.

By taking the double reciprocal of the Michaelis–Menten kinetics equation, Eq. 3 was obtained as follows:

$$\mathrm{V} = \frac{V_{\max}[S]}{K_{\mathrm{m}} + [S]} \tag{2}$$

$$\frac{1}{V} = \frac{K_{\mathrm{m}}}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}. \tag{3}$$

A linear equation $y = kx + b$ is fitted with 1/V and 1/[*S*] values by the Lineweaver–Burk double reciprocal plot. The values of the desired constant K_m and V_{max} can be determined directly from the plot. Further, K_m is not a binding constant which measures the strength of binding between the enzyme and substrate, its value takes into account the affinity of substrate for enzyme and also the ratio in which the substrate bound to the enzyme is converted to the product. Thus, the Lineweaver–Burk double reciprocal plot was employed for the kinetic characterization of biotransformation in following two conditions.

As the pH plays an important role in GAD activity [25], the kinetic characterization of biotransformation was investigated at the pH of 4.0, 4.5 and 5.0 at 45 °C, respectively (Fig. 5a, b). The value of V_{max} at pH 4.5 (1.65 g/L/min) was the highest, and the K_m value at pH 4.5 (7.16 g/L) was apparently lower than those at pH 4.0 (8.03 g/L) and pH 5.0 (7.35 g/L). The result also exhibited that pH 4.5 was the optimal pH for bioconversion of MSG to GABA by *L. brevis* TCCC 13007 resting cells.

Since temperature is also one of the most important factors on enzyme activity, Fig. 5c, d manifested the kinetic characterization of bioconversion from 0 to 80 g/L MSG at 35 °C, 45 °C and 55 °C (pH 4.5), respectively. The V_{max} value at 45 °C (1.65 g/L/min) was apparently higher than at 35 °C (1.24 g/L/min) and 55 °C (1.42 g/L/min). In addition,

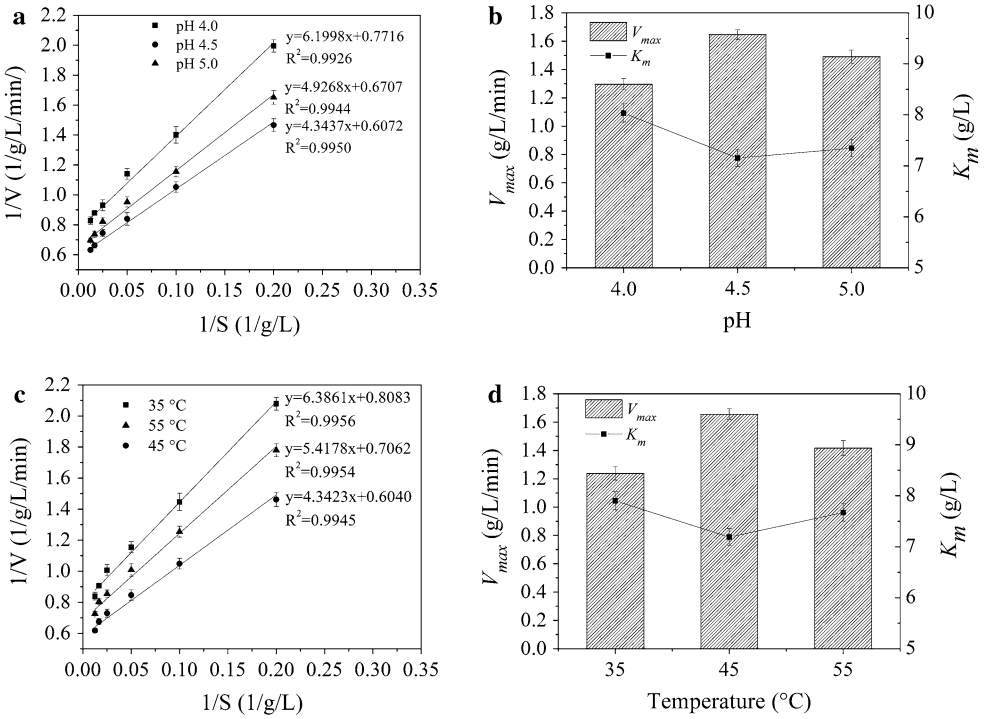

Fig. 5 Kinetic analysis of bioconversion using resting cells of *L. brevis* TCCC 13007 at **a** pH values of 4.0 (*filled square*), 4.5 (*filled circle*) and 5.0 (*filled triangle*) and **c** temperatures of 35 °C (*filled square*), 45 °C (*filled circle*) and 55 °C (*filled triangle*). The kinetic parameters were calculated using the Michaelis–Menten equation as follows: $\frac{1}{V} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$. K_m is indicated by the *filled square* (**b**) and the V_{max} is indicated by the *bar* (**d**). Data are presented as mean ± SD values from three parallel experiments

the value of K_m at 45 °C (7.19 g/L) was the smallest at the three bioconversion temperatures. Thus, 45 °C here is the optimal temperature of bioconversion for *L. brevis* TCCC 13007 resting cells.

Verification of the kinetics model of GABA bioconversion

Based on the above results, the optimal composition of bioconversion system of 80 g/L MSG concentration, 50 g/L wet resting cells in 0.6 M citrate buffer (pH 4.5) for 4 h incubation at 45 °C was determined for the verification of the supposed kinetics model. A kinetics model of GABA bioconversion by *L. brevis* TCCC 13007 resting cells was built using the Lineweaver–Burk double reciprocal plot, and a linear equation $y = 4.3437x + 0.6072$ was obtained. The result indicated that values of K_m and V_{max} were 7.16 g/L and 1.65 g/L/min, respectively, which were coincident with those obtained in Fig. 5. Therefore, the bioconversion of MSG to GABA by *L. brevis* TCCC 13007 resting cells can be described by Michaelis–Menten equation at the low range of MSG substrate concentration as follows:$y = \frac{7.16[S]}{1.65+[S]}$ $(0 < S < 80$ g/L).

Bioconversion ability of resting cells

The pH of reaction system rises during the process of the decarboxylation reaction of L-glutamic acid (L-Glu) to GABA. In general, to control the pH under a certain range, the pH of the bioconversion system must be controlled or adjusted using buffer or acid/base solution, which resulted large amounts of buffer salts or acid/base solution will be consumed. Also, adding plenty of chemicals could present a difficulty for purification of GABA. The pH value varied in the process of L-Glu consumption during GABA formation. L-Glu acts as a weak acid and its solubility in water was just 0.864 % at 25 °C [30], thus the mixture of L-Glu and MSG has a certain pH buffering capacity. Also, the excessive L-Glu precipitation in the reaction system was gradually dissolved and converted to GABA during the process of substrate consumption, and thereby maintained the pH of reaction system. Table 4 presented the initial pH of the reaction mixture at various ratios of L-Glu to MSG in 0.6 M citrate buffer at 45 °C. When the initial 80 g/L L-Glu was mixed with 240 g/L MSG, the pH of the reaction system was 4.48 and consistent with the optimal pH of GAD.

Moreover, with the extension of reaction time, the GABA yield increased rapidly at 0–7 h, but became gently after 7 h. Likewise, the pH of reaction system increased with prolonging the reaction time (0–7 h) and then went to a constant gradually after 7 h. When the bioconversion was performed a 10 h, the GABA titer came up to a maximum of 201.18 g/L and the molar bioconversion ratio arrived at 99.4 % (Fig. 6).

Table 4 The pH of the mixture at various ratios of L-Glu to MSG in 0.6 M citric acid-sodium citrate buffer at 45 °C

L-Glu (g/L)	MSG (g/L)	pH
80	0	2.06
80	20	2.40
80	40	2.64
80	60	2.88
80	80	3.11
80	100	3.31
80	120	3.50
80	140	3.72
80	160	3.93
80	180	4.06
80	200	4.20
80	220	4.37
80	240	4.48
80	260	4.62
80	280	4.70
80	300	4.81
80	320	4.92

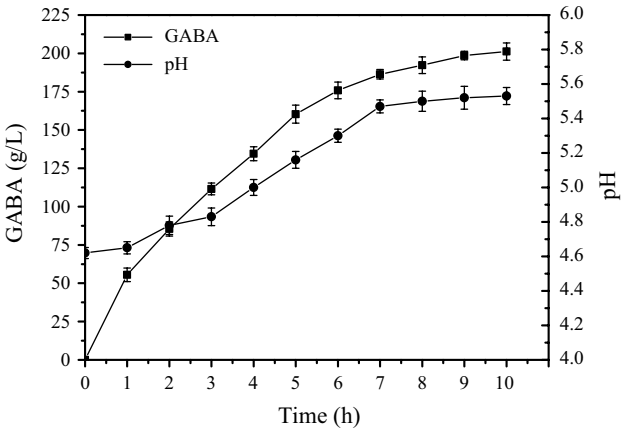

Fig. 6 Bioconversion ability of resting cells of *L. brevis* TCCC 13007 *filled square* GABA (g/L); *filled circle* pH

Discussion

Recently, fermentation method and immobilized cell reaction are the most commonly used in biological process, and resting cell bioconversion is not as the main method for GABA production. Huang et al. [8] used immobilized whole cells of *L. brevis* CGMCC 1306 with high GAD activity for GABA production, and the GABA

concentration of about 55 mM was obtained during the 10 h of operation. Choi et al. [3] optimized a semicontinuous cell entrapment system using *L. brevis* GABA 057, and 223 mM GABA was produced after 48 h of reaction. Moreover, Li et al. [34] developed a simple process for efficient GABA production by fed-batch fermentation using *L. brevis* NCL912, and high yield of GABA (1005.81 ± 47.88 mM) was achieved at 48 h. Kim and Lee [35] applied GAD from recombinant *E. coli* to bubble column reactors for GABA production, and 1.2 M of GABA was produced after 6 h with a conversion ratio of 80 %.

The research of this paper introduced an efficient bioconversion method using *L. brevis* TCCC 13007 to produce GABA. Several culture conditions have been optimized and optimal results were obtained. Besides, kinetic analysis of pH and temperature, which were considered as the important factors for GABA production, were studied. Therefore, resting cells bioconversion could be used as a novel approach for GABA production when the optimal bioconversion conditions were under control. At last, due to its pH buffering capacity, a mixture of 80 g/L Glu and 240 g/L MSG was added into the buffer system to verify the bioconversion ability of resting cells. The yield of 201.18 g/L GABA was obtained after 10 h reaction with a high molar bioconversion ratio of 99.4 %. Overall, this study provided a strong groundwork for green GABA production by resting cells.

Conclusion

This study revealed that the efficient bioconversion by *L. brevis* TCCC 13007 resting cells was a promising process for GABA production. By 10 h bioconversion, the GABA titer of 201.18 g/L together with a high molar bioconversion ratio of 99.4 % was obtained. Furthermore, the bioconversion of MSG to GABA by *L. brevis* TCCC 13007 resting cells followed the classical Michaelis–Menten equation at the low substrate concentration ($0 < S < 80$ g/L). Therefore, this process presents a hopeful potential for industrial and commercial applications and also offers a promising feasibility of continuous GABA production coupled with fermentation.

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References

1. Cho YR, Chang JY, Chang HC (2007) Production of gamma-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuronal cells. J Microbiol Biotechnol 17(1):104–109
2. Choi J, Yim S, Lee S, Kang T, Park S, Jeong K (2015) Enhanced production of γ-aminobutyrate (GABA) in recombinant by expressing glutamate decarboxylase active in expanded pH range. Microb Cell Fact 1:21
3. Choi SI, Lee JW, Park SM, Lee MY, Ji GE, Park MS, Heo TR (2006) Improvement of gamma-aminobutyric acid (GABA) production using cell entrapment of *Lactobacillus brevis* GABA 057. J Microbiol Biotechnol 16:562–568
4. Dhakal R, Bajpai VK, Baek KH (2012) Production of GABA (γ-aminobutyric acid) by microorganisms: a review. Braz J Microbiol 43:1230–1241
5. Gangaraju DS, Murty VR, Prapulla SG (2014) Probiotic-mediated biotransformation of monosodium glutamate to γ-aminobutyric acid: differential production in complex and minimal media and kinetic modelling. Ann Microbiol 64:229–237
6. Gao Q, Duan Q, Wang D, Zhang Y, Zheng C (2013) Separation and purification of γ-aminobutyric acid from fermentation broth by flocculation and chromatographic methodologies. J Agr Food Chem 618:1914–1919
7. He Y, Gong J, Yu HY, Tao Y, Zhang S, Dong ZY (2015) High production of ectoine from aspartate and glycerol by use of whole-cell biocatalysis in recombinant *Escherichia coli*. Microb Cell Fact 14:55
8. Huang J, Mei LH, Wu H, Lin DQ (2007) Biosynthesis of γ-aminobutyric acid (GABA) using immobilized whole cells of *Lactobacillus brevis*. World J Microbiol Biotechnol 23:865–871
9. Jones EA (2002) Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy. Metab Brain Dis 17:275–281
10. Kang TJ, Ho NAT, Pack SP (2013) Buffer-free production of gamma-aminobutyric acid using an engineered glutamate decarboxylase from *Escherichia coli*. Enzyme Microb Technol 53:200–205
11. Kinnersley AM, Turano FJ (2000) Gamma aminobutyric acid (GABA) and plant responses to stress. Crit Rev Plant Sci 19:479–509
12. Komatsuzaki N, Nakamura T, Kimura T, Shima J (2008) Characterization of glutamate decarboxylase from a high γ-aminobutyric acid (GABA)-producer, *Lactobacillus paracasei*. Biosci Biotechnol Biochem 72:278–285
13. Komatsuzaki N, Shima J, Kawamoto S, Momose H, Kimura T (2005) Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. Food Microbiol 22:497–504
14. Kumar S, Punekar NS, SatyaNarayan V, Venkatesh KV (2000) Metabolic fate of glutamate and evaluation of flux through the 4-aminobutyrate (GABA) shunt in *Aspergillus niger*. Biotechnol Bioeng 67:575–584
15. Lei Z, Tian J, Qiu P, Wang L, Long X, Zhang S, Zeng Z, Tian Y (2014) Biosynthesis of gama-aminobutyric acid by induced resting cells of *Lactobacillus brevis* SIIA11021. J Chem Pharma Res 6(12):342–348
16. Li J, Yang J, Men Y, Zeng Y, Zhu Y, Dong C, Sun Y, Ma Y (2015) Biosynthesis of 2-deoxysugars using whole-cell catalyst expressing 2-deoxy-d-ribose 5-phosphate aldolase. Appl Microbiol Biotechnol 99(19):7963–7972
17. Márquez FJ, Quesada AR, Sánchez-Jiménez F, Núñez De Castro I (1986) Determination of 27 dansyl amino acid derivatives in biological fluids by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 380:275–283
18. Park KB, Oh SH (2007) Cloning, sequencing and expression of a novel glutamate decarboxylase gene from a newly isolated lactic acid bacterium, *Lactobacillus brevis* OPK-3. Bioresour Technol 98:312–319
19. Perkins C, Siddiqui S, Puri M, Demain AL (2015) Biotechnological applications of microbial bioconversions. Crit Rev Biotechnol. doi:10.3109/07388551.2015.1083943

20. Pham VD, Somasundaram S, Lee SH, Park SJ, Hong SH (2016) Efficient production of gamma-aminobutyric acid using *Escherichia coli* by co-localization of glutamate synthase, glutamate decarboxylase, and GABA transporter. J Ind Microbiol Biotechnol 43(1):79–86
21. Sanders JW, Leenhouts K, Burghoorn J, Brands JR, Venema G, Kok J (1998) A chloride-inducible acid resistance mechanism in *Lactococcus lactis* and its regulation. Mol Microbiol 27:299–310
22. Shi F, Jiang J, Li Y, Li Y, Xie Y (2013) Enhancement of γ-aminobutyric acid production in recombinant *Corynebacterium glutamicum* by co-expressing two glutamate decarboxylase genes from *Lactobacillus brevis*. J Ind Microbiol Biotechnol 40(11):1285–1296
23. Shi XF, Zheng B, Chang CY, Cao P, Yang HJ, Gao Q (2015) Enzymatic bioconversion for γ-aminobutyric acid by *Lactobacillus brevis* CGMCC No. 3414 resting cells. Lect Notes Electr Eng 333:609–617
24. Yang T, Rao Z, Kimani BG, Xu M, Zhang X, Yang ST (2015) Two-step production of gamma-aminobutyric acid from cassava powder using *Corynebacterium glutamicum* and *Lactobacillus plantarum*. J Ind Microbiol Biotechnol 42(8):1157–1165
25. Toney MD (2005) Reaction specificity in pyridoxal phosphate enzymes. Arch Biochem Biophys 433:279–287
26. Ueno H (2000) Enzymatic and structural aspects on glutamate decarboxylase. J Mol Catal B Enzym 10:67–79
27. Ueno Y, Hayakawa K, Takahashi S, Oda K (1997) Purification and characterization of glutamate decarboxylase from *Lactobacillus brevis* IFO 12005. Biosci Biotechnol Biochem 61:1168–1171
28. Yokoyama S, Hiramatsu J, Hayakawa K (2002) Production of γ-aminobutyric acid from alcohol distillery lees by *Lactobacillus brevis* IFO-12005. J Biosci Bioeng 93:95–97
29. Yoshimura M, Toyoshi T, Sano A, Izumi T, Fujii T, Konishi C, Obata A (2010) Antihypertensive effect of a γ-aminobutyric acid rich tomato Cultivar 'DG03-9' in spontaneously hypertensive rats. J Agr Food Chem 58:615–619
30. Yu X (2009) Handbook of MSG Industry. China Light Industry Press, Beijing, China **(in Chinese)**
31. Zhang Y, Gao NF, Feng Y, Song L, Gao Q (2010) Biotransformation of sodium L-glutamate to γ-aminobutyric acid by *L. brevis* TCCC 13007 with two glutamate decarboxylase genes. In: 2010 4th international conference on bioinformatics and biomedical engineering (iCBBE 2010), vol. 2, p 1–4
32. Zhang Y, Song L, Gao Q, Yu SM, Li L, Gao NF (2011) The two-step biotransformation of monosodium glutamate to GABA by *Lactobacillus brevis* growing and resting cells. Appl Microbiol Biotechnol 94:1619–1627
33. Zhao A, Hu X, Pan L, Wang X (2015) Isolation and characterization of a gamma-aminobutyric acid producing strain *Lactobacillus buchneri* WPZ001 that could efficiently utilize xylose and corncob hydrolysate. Appl Microbiol Biotechnol 99(7):3191–3200
34. Li H, Qiu T, Huang G, Cao Y (2010) Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. Microb Cell Fact 9:85
35. Kim EJ, Lee J-H (2014) Effects and optimization of gamma-amino butyric acid (GABA) production process using glutamate decarboxylase (GAD). Korean Soc Biotechnol Bioeng J. 29(6):426–431. doi:10.7841/ksbbj.2014.29.6.426 **(in Korean)**