CHEMICAL ENGINEERING TRANSACTIONS
VOL. 78, 2020
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Jiří Jaromír Klemeš

ISBN 978-88-95608-76-1; **ISSN** 2283-9216
DOI: 10.3303/CET2078078

Refinery of Gamma- Aminobutyric Acid (GABA) from the Fermentation of Rice Bran Extract by Ultrafiltration

Dat Quoc Lai*, Tam Thanh Pham

Faculty of Chemical Engineering, University of Technology, Vietnam National University – Ho Chi Minh City, 268 Ly Thuong Kiet, Ward 14, District 10, Ho Chi Minh City, Vietnam
lqdat@hcmut.edu.vn

Gamma- Aminobutyric Acid (GABA) has been known as a bioactive related to the hyperglycemia, boosting the immune system, lowering blood pressure, inhabitation of development of cancer cells and assists the treatment of anxiety disorders. It has been supplied for human from germinated beans, cereal grains and tea. Recently, GABA has been also synthesized from the fermentation through the assimilation of glutamic acid by bacteria. In this paper, application of ultrafiltration (UF) for recovery of GABA from the fermentation broth of rice bran extract was investigated, with 1,000 Da of molecular weight cut off of membrane. Pretreatment of the broth by combination of centrifugation and microfiltration prior to UF improved the performance of UF process. Influence of operating pressure, in range of 2 -10 bar, was investigated and 6 bar was suitable value of operating pressure for UF of the fermentation broth. The mechanism of fouling in UF under influence of methods of pretreatment and operating pressure was also analysed, consequently, determined the flux decline model in UF. Compared to feed, content of protein in broth reduced 65 %, while, content of GABA was insignificantly changed, at 2.5 of concentration factor. Content of soluble solid, sugar and amino acid in the broth also reduced after UF process. It means that, UF process is potential technology for recovery of GABA from fermentation broth of rice bran extract.

1. Introduction

Recently, Gamma- Aminobutyric Acid (GABA), a bioactive, has been considered for human health. GABA has been reported that it affects human with some physiological functions: antihypertensive effect (Hayakawa et al., 2002), anti-stressfulness (Vaivaa et al., 2004), relation with unipolar depressive disorder (Bjork et al., 2001), regulation of muscle tone (Watanabe et al 2002). GABA source for human health has been supplied from tea (Tsushida and Murai 1987), germinated brown rice (Saikusa et al., 1994) and germinated soybean (Aoki et al., 2003). Fermentation by microorganism for transformation of glutamic acid to GABA has been considered as an effective method to produce GABA for supplement and drug. Microorganisms used for GABA production have been bacteria (Yokoyama et al., 2002), fungi (Kono and Himeno, 2000) and yeast (Hao and Schmit, 1993).

Rice bran, a byproduct of rice processing, is a rich source of nutrition for human health. Unfortunately, it has been utilized as feed with low economic efficiency. Rice bran contains 2.0 – 2.5 % of glutamic acid (Luh, 1991); thus, it is a rich source to supply subtract for fermentation to produce GABA. Utilization of rice bran extract with adding 1 wt% of yeast extract, 4 wt% of sucrose, 12 wt% of monosodium glutamate, the fermentation by L. sakei B2-16 obtained 660 nM of GABA with conversion yield being approximately 100 % (Kook et al., 2010). In previous work, fermentation of rice bran extract by Lactobateria without adding external glutamate source, GABA was obtained at 3 g/L (Lai et al., 2017). Nevertheless, in fermentation broth, concentration of GABA is low and containing impurities (bacteria, residues of non-fermented components, such as: protein, sugar etc.), which negatively influences on the further steps for purification of GABA. Thus, it is necessary to refine GABA for improvement of purity for utilization.

The aims of the present research were to investigate into the feasibility of UF for improving purity of GABA from fermentation broth of rice extract. The research also focused on the fouling analysis in UF of the fermentation broth for modelling the decline of flux.

Paper Received: 18/05/2019; Revised: 19/09/2019; Accepted: 02/10/2019
Please cite this article as: Lai D.Q., Pham T.T., 2020, Refinery of Gamma- Aminobutyric Acid (GABA) from the Fermentation of Rice Bran Extract by Ultrafiltration, Chemical Engineering Transactions, 78, 463-468 DOI:10.3303/CET2078078

2. Materials and methods

2.1 Materials

Rice bran was purchased from local rice milling factory with 10 % of milling degree and 8 wt% of moisture. It was treated with n-hexane for removing fat. The fatty content of defatted rice bran was 3 wt%. It was stored, as stock, at 4 °C for limiting activity of lipase until used for the fermentation. GABA and analytical reagents were supplied by Sigma Aldrich (USA) with analytical grade. Flavorzyme, a mixture of endoprotease and exopeptidase synthesized by *Aspergillus oryzae*, was supplied by Novozymes (Denmark). All chemicals, reagents and enzyme were stored at 4 °C.

2.2 Preparation of fermentation broth

Defatted rice bran was treated by Flavorzyme at the conditions as follows: ratio of DRB: water: 1:10 (w:w); concentration of enzyme: 2 wt%, pH: 8, temperature: 50 °C, time: 120 min. After the treatment, the mixture was sterilized at 121 °C and 10 min and filtrated by cloth to obtain the rice bran extract. Then, Lactobacillus brevis VTCC-B397 (isolated from naturally pickled vegetable) was added at 108 cfu/mL of bacterial density. The fermentation was carried out under the conditions as follows: 30 °C, pH 5.0 and 48 h. After fermentation, the broth was sterilized at 121 °C and 10 min for inactivation of bacteria (Lai et al., 2017).

2.3 Pretreatment of the broth

Influence of 4 pre-treatment methods on UF of fermentation broth prior to UF was investigated. The procedures of 4 methods were expressed as the following:

- Cloth filtration: the broth was filtrated by cloth under vacuum pressure.
- Centrifugation: the broth was centrifuged at 6,000 rpm of speed in 30 s.
- Micro filtration: the broth was filtrated by cartridge with 0.45 µm of pore size.
- Combining centrifugation with microfiltration: the broth was centrifuged at 6,000 rpm in 30 s, followed by microfiltration by cartridge with 0.45 µm of pore size.

2.4 Membranes apparatus

Membrane apparatus used for experiment was a flat and frame system (Labstak M20), manufactured by Alfa Laval, Denmark, (Figure 1a). It was equipped 5 couples of membranes with 20 cm of diameters and 0.018 m2 of active area for each membrane. Membrane was ETNA01 PP (Alfa Laval, Denmark). It was UF composite membrane made from com fluoro polymer, with 1,000 Da of molecular weight cut off. Temperature of operation was ambient. Initial volume of feed was 20 L. Feed flow rate was 1,000 L/h, supplied by high pressure pump (Hydra Cell pump, Wanner Engineering Inc., USA). The system operated with full circulation of retentate. After each experiment, the cleaning was conducted as the following procedure: cleaning by distilled water in 10 min, then, cleaning with alkali solution (pH10) in 20 min, followed by cleaning with acidic solution (pH 2) in 20 min; finally, cleaning by water until to reach pH 6-7. The completed cleaning membrane was recovered, at least, 95 % of pure water permeability, compared to that of original membrane.

2.5 Fouling analysis

The mechanisms of fouling in UF of fermentation broth was analysed based on Hermia's equation (Hermia, 1982):

$$\frac{d^2t}{dV^2} = k\left(\frac{dt}{dV}\right)^n \quad (1)$$

where, V is volume (m^3) of accumulative permeate at t (s) of operating time. k is constant of model.
Based on Eq(1), 4 mechanisms of fouling are proposed (Figure 1b). Value of n and the derivative equation of Eq(1) was stated in Table 1. Where, J and J_0 (m^3/s) are the permeate flux at t (s) of operating time and intimal time. In order to determine the equation which shows the good fit experimental data, linear regression was applied, and based on correlation coefficient (R^2) value to select the best fit model for determination of fouling mechanism in UF of fermentation broth.

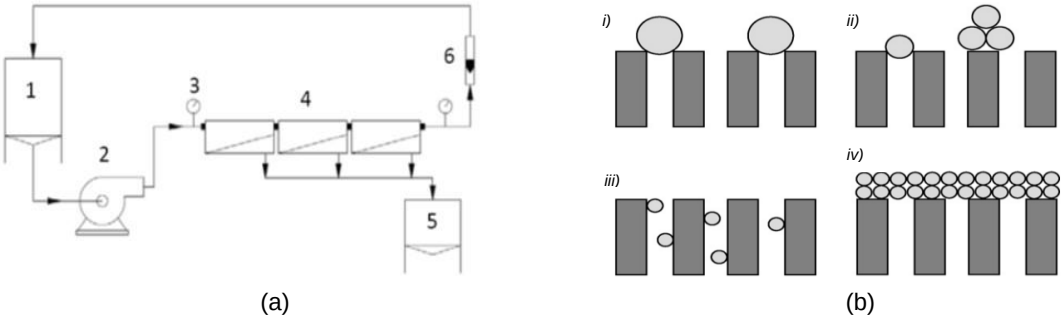
2.6 Analysis methods

Total sugar content was analysed by using sulfuric acid to form furan compounds, then adding phenol as indicator. The absorbance at 490 nm of wavelength was measured to determined content of sugar (BeMiller, 2010). Amino acid content was analysed by using ninhydrin as indicator and measuring the absorbance at 570 nm of wavelength, and glycine was used as standard (Mugg, 1983). Protein content was analysed by using Lowry method (Nielsen, 2010). GABA content was analysed by using Berthelot reagent (solution of phenol

and hypochlorite in base) and measuring absorbance at 645 nm of wavelength (Karladee and Suriyong, 2012).

Table 1: Mechanism of fouling in UF based on Hermia's equation

No.	Mechanism	Parameters	Derivative equation
1	Complete pore blocking	n = 2	$\ln(J^{-1}) = \ln({J_0}^{-1}) + kt$
2	Standard pore blocking	n = 1.5	$J^{-0,5} = {J_0}^{-0,5} + kt$
3	Intermediate pore blocking	n = 1	$J^{-1} = {J_0}^{-1} + kt$
4	Cake formation	n = 0	$J^{-2} = {J_0}^{-2} + kt$



(a) (b)

Figure 1: Schema of flat and frame pilot LabstakM20 system (a) (1: feed tank, 2: pump, 3: pressure gauge, 4: membranes, 5: permeate tank, 6: flowmeter) and mechanism of fouling proposed by Hermia (b) (i: complete pore blocking, ii: intermediate pore blocking, iii: standard pore blocking, iv: cake formation (Vela et al., 2008)).

3. Results and discussion

3.1 Influences of pretreatment methods

The UF of fermentation broth under different methods of pretreatment was conducted at 6 bar of operating pressure. Influence of pretreatment on rejection of GABA, protein, amino acids and sugar was stated in Table 2. Rejection of protein was significantly higher than one of the others due to the higher molecular weight. Rejection of GABA was lower, compared to that of protein and amino acids. The significant difference in rejection of GABA and the others means that, UF of the broth can be applied for separation of GABA from the other main components. The difference in rejections of GABA and protein was approximately 40 %. However, difference in rejections of GABA, amino acid and sugar changed with different methods of pretreatment. The change in rejection, consequently, the difference in rejections between components, was caused by the change in permeate flux. There are two mechanism of motion of solute through membrane in UF: diffusion and convection (Nakao and Kimura, 1980). When permeate flux increases, convective flow of solutes increases, lead to reduction of rejection. However, in UF, the fouling also influences on rejection due to changes in pore size and formation of selective layer on membrane surface (Saha et al., 2007). Thus, in some cases, the increase of permeate flux led to augment of rejection. Result indicates that, combining of centrifugation with microfiltration is suitable for UF of the broth for refinery of GABA from the fermentation broth of rice extract due to: low rejection of GABA (for high recovery of GABA in permeate side), significant difference in rejection of GABA and the others and higher permeate flux (for high capacity of process).
Influence of pretreatment methods on permeate flux is shown in Figure 2a. In early section, permeate flux showed a sharp decline. Due to the formation of boundary layer (concentration polarization phenomenon),

Table 2: Influence of pretreatment of broth on rejections of components in UF

No.	Pretreatement	Rejection (%)			
		GABA	Protein	Amino acids	Total sugar
1	Cloth filtration	18 ± 0	48 ± 2	24 ± 4	11 ± 0
2	Centrifugation	23 ± 1	66 ± 2	37 ± 2	13 ± 2
3	Microfiltration	28 ± 5	61 ± 0	29 ± 1	12 ± 0
4	Combining centrifugation with microfiltration	2 ± 1	43 ± 4	10 ± 2	8 ± 2

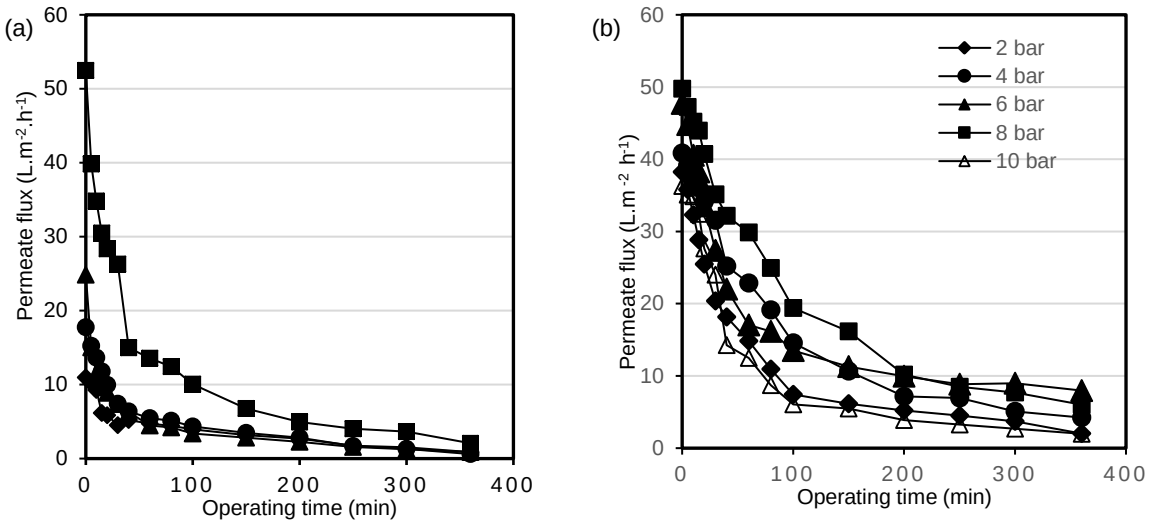


Figure 2: Influence of pretreatment on flux in UF of fermentation broth (a) (●: cloth filtration, ▲: centrifugation, ◆: microfiltration, ■: combination of centrifugation and microfiltration) and Influence of operating pressure on permeate flux in UF of fermentation broth of rice bran extract.

resistant to permeate flux increased, consequently, decreased in permeate flux. In later section, permeate flux also decrease, but with low rate of decline. The decline in later stage is contributed by fouling phenomenon: adsorption of solutes on membrane surface and on wall of pores (Bowen et al., 1995). With the pretreatment of broth by microfiltration, cloth filtration and centrifugation, permeate flux in early stage was different, but in later stage, it was approximate to each other. Pretreatment of the broth by combination of centrifugation and microfiltration led to the highest of flux, compared to the other methods of pretreatment. Under centrifugation and microfiltration, the matters causing fouling was removed more than that by the other methods of pretreatment. Thus, resistant to permeate was lower, led to higher permeate flux.

The analysis of fouling is stated in Table 3. The result indicated that, different pretreatment methods led to difference in mechanism of fouling in UF of fermentation broth. Cloth filtration removed the macro matter of broth. However, it could not reject bacteria and protein. Membrane surface was fouled by these components, caused complete pore blocking. With microfiltration, bacteria were rejected. Protein and macro molecules could reach and seal the pore, causing intermediate blocking of pore. Pore blocking occurred in initial stage of filtration, thus, permeate flux was low in early stage (Wang et al., 2012). When pretreating the broth by centrifugation and combination of centrifugation and microfiltration, the cake layer on membrane surface can be formed by boundary layer, which occurred in concentration polarization phenomenon (Bowen et al., 1995). Thus, the predominant mechanism of fouling in UF of the broth when pretreating by centrifugation and combining centrifugation and microfiltration was cake formation.

Table 3: Influence of pretreatment of broth on rejections of components in UF

No	Pretreatment	Mechanism	Modelling of flux	R^2 value
1	Cloth filtration	Complete pore blocking	$\ln(J^{-1}) = -2.4836 + 0.4837t$	0.9476
2	Centrifugation	Cake formation	$J^{-2} = -0.0339 + 0.1218t$	0.9841
3	Microfiltration	Intermediate pore blocking	$J^{-1} = 0.1156 + 0.1065t$	0.9387
4	Combining centrifugation with microfiltration	Cake formation	$J^{-2} = 0.0007 + 0.003t$	0.9635

3.2 Influences of operating pressure

Influence of operating pressure on permeate flux in UF of fermentation broth of rice bran extract is showed Figure 2b. In range of 2 – 8 bar of operating pressure, increase in operating pressure led to increase in permeate flux. Driving force in UF process is the difference of pressure between two sides of membrane. Thus, increase in operating pressure led to increase in driving force, consequently, increased in permeate flux (Nakao and Kimura, 1980). Nevertheless, at 10 bar of operating pressure, permeate flux was lower than that at a range of 2 – 8 bar. Perhaps, at 10 bar, structure of membrane was slightly compressed, led to increase in resistant to permeate flux. The fouling mechanism reinforced for this explanation: at 10 bar of operating

pressure, the mechanism of fouling was intermediate pore blocking (Table 4). It means that, solutes could not move inside the pore as standard pore blocking mechanism, the predominant mechanism of fouling at range 2 – 8 bar of operating pressure (Stoller et al., 2017). At 6 bar of operating pressure, mechanism of fouling was cake formation (Table 4). It can be explained by the formation of boundary layer, which caused to form of cake layer on membrane surface (Ng et al., 2014).

Rejection of protein increased with increase in operating pressure (Table 5). However, rejections of GABA, amino acid and total sugar were minimum at 6 bar of operating pressure. As fouling analysis result in Table 4, the fouling at 6 bar was predominantly contributed by formation of cake layer on membrane surface. Influence of this layer on permeability of GABA, amino acid and sugar through this layer was unremarkable. However, at 2, 4, 8 and 10 bar of operating pressure, mechanism of fouling was standard and intermediate pore blocking. The solute sealed the pores or adsorbed on wall of pores. Thus, permeability of GABA, amino acid and sugar through membrane possibly reduced, led to increase in rejection. Result also dictated that, 6 bar of operating pressure is suitable for refinery of GABA from fermentation broth, due to low rejection of GABA, compared to the others, and moderate permeate flux.

Table 4: Influence of pre-treatment of broth on rejections of components in UF

No	Pressure (bar)	Mechanism	Modelling of flux	R^2 value
1	2	Standard pore blocking	$J^{-0,5} = 0.1765 + 0.0801t$	0.9664
2	4	Standard pore blocking	$J^{-0,5} = 0.1564 + 0.0569t$	0.9920
3	6	Cake formation	$J^{-2} = 0.0007 + 0.003t$	0.9635
4	8	Standard pore blocking	$J^{-0,5} = 0.1431 + 0.0454t$	0.9925
5	10	Intermediate pore blocking	$J^{-1} = 0.014 + 0.0755t$	0.9880

Table 5: Influence of operating pressure on rejections of components in UF

No.	Pressure (bar)	Rejection (%)			
		GABA	Protein	Amino acids	Total sugar
1	2	11 ± 1	33 ± 2	21 ± 2	13 ± 3
2	4	13 ± 0	36 ± 0	16 ± 1	14 ± 5
3	6	2 ± 1	43 ± 4	10 ± 2	8 ± 2
4	8	12 ± 1	43 ± 5	15 ± 0	22 ± 0
5	10	14 ± 1	53 ± 3	14 ± 6	19 ± 3

3.3 UF process of fermentation broth of rice bran extract

The UF process of fermentation of rice extract was conducted and evaluated at 6 bar with combining of centrifugation and microfiltration for pretreatment. The characteristics of UF process of fermentation broth of rice extract was stated in Table 6. Result indicated that, with application of UF, protein content reduced from 20.14 g/L to 7.21 g/L, while, content of GABA insignificantly changed. Contents of sugar, amino acids and soluble solid remarkably reduced. It means that, after UF process, purity of GABA increased. Besides, the reduction of protein content in the broth can enhance the efficiency of the further steps for purification of GABA, such as ion exchange process.

Table 6: Influence of pre-treatment of broth on rejections of components in UF

Type of broth	Volume (L)	GABA (g/L)	Protein (g/L)	Amino acid (g/L)	Total sugar (g/L)	Brix (°)
Feed (after pretreatment)	20	9.51	20.14	26.24	33.73	8.68
Permeate	12	9.34	7.21	23.17	28.13	7.15

4. Conclusions

The results showed that GABA could separate from fermentation broth of rice bran extract by application of UF process with 1,000 Da of membrane pore size, 6 bar of operating pressure and pretreatment of the broth by combining centrifugation and microfiltration. The mechanism of fouling in UF under effects of pretreatment methods and operating pressure was also analysed and model of flux decline showed the good fitness with experimental data. Overall, UF showed the potentials for application to recover GABA from fermentation broth of rice bran.

Acknowledgments

This research was funded by VNU-HCM under grant number KHCN-TNB.ĐT/14-19/C06.

References

Aoki H., Uda I., Tagami K., Furuya Y., Endo Y., Fujimoto K., 2003, The production of a new tempeh-like fermented soybean containing a high level of g -aminobutyric acid by anaerobic incubation with rhizopus, Bioscience, Biotechnology, and Biochemistry, 67, 1018–1023.

BeMiller J.N., 2010, Carbohydrate Analysis, Chapter In: S.S. Nielsen (Ed), Food Analysis, Springer Publishing, New York, USA, 147–177

Bowen W.R., Calvo J.I., Hernández A., 1995, Steps of membrane blocking in flux decline during protein microfiltration, Journal of Membrane Science, 101(1–2), 153-165.

Hayakawa K., Kimura M., Kamata K., 2002, Mechanism underlying γ-aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats, European Journal of Pharmacology, 438(1–2), 107-113.

Hermia J., 1982, Constant pressure blocking filtration law application to powder-law non-newtonian fluid, Transactions of the Institution of Chemical Engineers, 60, 183-187

Karladee D., Suriyong S., 2012, γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination, ScienceAsia, 38, 13–17.

Kook M., Seo M., Cheigh C., Pyun Y., Cho S., Park H., 2010, Enhanced production of γ-aminobutyric acid using rice bran extracts by Lactobacillus sakei B2-16, Journal of Microbiology and Biotechnology, 20(4), 763–766.

Lai D.Q., Tran N. T.K, Nguyen T.X.N, 2017, Gamma-amino butyric acid (GABA) synthesis of Lactobacillus in fermentation of defatted rice bran extract, AIP Conference Proceedings 1878, 020045

Luh S. B. (1991): Rice: Production and Utilization. Second Edition. AVI Publishing Corp, pp. 925

Mugg P., 1983, Technical note. A rapid hippurate hydrolysis test for the presumptive identification of group B streptococci, Pathology, 15, (3), 251–252

Nakao S., Kimura S., 1980, Analysis of solutes rejection in UF, Journal of Chemical Engineering of Japan, 14 (1), 32 – 37.

Ng C.Y., Mohammad A.W., Ng L.Y, Jahim J.M., 2014 Membrane fouling mechanisms during UF of skimmed coconut milk, Journal of Food Engineering, 142, 190-200

Nielsen S.S., 2010, Protein Nitrogen Determination, Chapter In: S.S. Nielsen (Ed), Food Analysis Laboratory Manual, 2nd Edition, Springer Publishing, New York, USA, 39 – 45

Saha N.K., Balakrishnan M., Ulbricht M., 2007, Sugarcane juice UF: FTIR and SEM analysis of polysaccharide fouling, Journal of Membrane Science, 306(1-2), 287-297

Saikusa T., Horino T., Mori Y., 1994, Accumulation of γ-Aminobutyric Acid (GABA) in the rice germ during water soaking. Bioscience, Biotechnology, and Biochemistry, 58(12), 2291-2292.

Stoller M., Pulido M.J.O, Palma L., 2017, Study on fouling behaviour of ultrafiltration and nanofiltration during purification of different organic matter polluted wastewaters, Chemical Engineering Transactions, 60, 295–300.

Tsushida T., Murai T., 1987, Conversion of Glutamic Acid to γ-Aminobutyric Acid in Tea Leaves under Anaerobic Conditions, Agricultural and Biological Chemistry, 51(11), 2865-2871.

Vaivaa G., Thomasa P., Ducrocqa F., Fontaineb M., Bossa V., Devosc P., Rasclea C., Cottencina O., Brunetf A., Laffargued P., Goudemand M., 2004, Low posttrauma GABA plasma levels as a predictive factor in the development of acute posttraumatic stress disorder. Biological Psychiatry, 55(3), 250-254.

Vela M.C.V., Blanco S.A., Garcıa J.L., Rodríguez E.B., 2008, Analysis of membrane pore blocking models applied to the UF of PEG, Separation and Purification Technology, 62,489–498

Watanabe M., Maemura K., Kanbara K., Tamayama T., Hayasaki H., 2002, GABA and GABA receptors in the central nervous system and other organs. International Review of Cytolog, 213, 1–47.

Yokoyama S., Hiramatsu J., Hayakawa K., 2002, Production of γ-aminobutyric acid from alcohol distillery lees by Lactobacillus brevis IFO-12005. Journal of Bioscience and Bioengineering, 93(1), 95-97.

Wang C., Li Q., Tang H., Yan D., Zhou W., Xing J., Wan Y., 2012, Membrane fouling mechanism in UF of succinic acid fermentation broth, Bioresource Technology, 116, 366–371.