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Email : editorijhess@gmail.com

Enzymatic Synthesis of Amoxicillin Using Penicillin G Acylase (PGA): A Systematic Literature Review

Asyafa Nursyahada[1)], Ahmad Wibisana[2)], Ignasius Agyo Palmado[3)], Iin Hardiyati[4)],Nurvidian Khasanah[5)]
[1)] Sarjana Farmasi Fakultas Ilmu Kesehatan Universitas Medika Suherman, Bekasi, West Java, 17530, Indonesia
[2)] Research Center for Pharmaceutical Ingredients and Traditional Medicines, National Research and Innovation Agency (BRIN), Banten 15314, Indonesia
[3,4,5)] Department of Pharmacy, Faculty of Health Sciences, Universitas Medika Suherman, Bekasi, West Java, 17530, Indonesia

*Corresponding Author
E-mail: nursyahadaasyafa@gmail.com

Abstract

Amoxicillin is a β-lactam antibiotic widely used in the treatment of bacterial infections. Enzymatic synthesis of amoxicillin using Penicillin G acylase (PGA) is an alternative to conventional chemical synthesis because it has high selectivity and can proceed under milder reaction conditions. This study aims to examine the development of enzymatic synthesis of amoxicillin using PGA, strategies to increase synthesis efficiency, and obstacles encountered in the process. The method used is a Systematic Literature Review (SLR) by reviewing 15 journals that discuss the enzymatic synthesis of β-lactam antibiotics, especially the use of PGA in amoxicillin synthesis. Data were analyzed descriptively and comparatively based on the synthesis method, type of biocatalyst, reaction system, process improvement strategy, and research results. The results of the study indicate that the efficiency of amoxicillin synthesis can be improved through enzyme immobilization and engineering, optimization of reaction conditions, the use of ionic liquids, in situ product removal, and one-pot and batch reactor reaction systems. However, several challenges remain, such as low substrate concentration, suboptimal synthesis yields, side hydrolysis reactions, and crystallization and clogging of immobilized PGA. Overall, the use of PGA has great potential for the development of enzymatic amoxicillin synthesis, but optimization of the biocatalyst and reaction system is still needed to support larger-scale applications.

Keywords: Amoxicillin; Penicillin G Acylase; Enzymatic Synthesis; B-Lactam; Biocatalyst; Systematic Literature Review.

INTRODUCTION

Amoxicillin is a β-lactam antibiotic which is a derivative of aminopenicillin and is widely used in the treatment of various bacterial infections.(pour karim et al., 2022) Amoxicillin has bactericidal properties and is effective for various gram-positive and gram-negative bacteria. One of the negative bacteria that can cause various infectious diseases is Escherichia coli, which is a member of the Enterobacteriaceae family and is found in the intestines of humans and animals.(Mulchandani et al., 2025)Amoxicillin exerts its antibacterial effect by disrupting the bacterial cell wall formation process, particularly in the final stages of peptidoglycan synthesis. (Kong et al., 2010) This mechanism occurs through amoxicillin binding to Penicillin Binding Proteins, which play a role in the transpeptidation process. Inhibition of this process disrupts peptidoglycan formation, thus decreasing the integrity of the bacterial cell wall and ultimately leading to cell lysis.(pour karim et al., 2022)Although amoxicillin plays an important role in various bacterial infections, inappropriate and excessive use can increase the risk of the emergence and spread of antimicrobial-resistant bacteria (Jena et al., 2024).

Globally, it is estimated that around one-third of antibiotic use each year is still done inappropriately.(Mulchandani et al., 2025)The high use of antibiotics demonstrates the importance of developing effective and efficient amoxicillin production. In addition to its use, the presence of antibiotic residues in the environment is also a concern because it can have ecological impacts and potentially contribute to the development of bacterial research.(pour karim et al., 2022). The development of semisynthetic β-lactam antibiotic synthesis methods is currently increasingly directed towards the use of biocatalysts because they are able to increase reaction selectivity, reduce the use of hazardous chemicals, and support a more sustainable process. (lalitha & Veerappapillai, 2024). The

development of semisynthetic β-lactam antibiotic synthesis methods is currently increasingly directed towards the use of biocatalysts because they are able to increase reaction selectivity, reduce the use of hazardous chemicals, and support a more sustainable process. (lalitha & Veerappapillai, 2024). One of the biocatalysts widely used in the synthesis of semisynthetic β-lactam antibiotics is Penicillin acylase (PA). Penicillin acylase (PA) is a hydrolase enzyme that functions to break the amide bond in penicillin antibiotics. One such group is (PGA; EC3.5.1.11) which has an important role in the β-lactam antibiotic industry. Mini enzymes are used in the 6-aminopenicillanic acid 6-APA process, which is the main raw material in the manufacture of semisynthetic antibiotics such as amoxicillin and ampicillin. (Tishkov et al., 2010)

Penicillin G Acylase generally has a heterodimer structure consisting of α and β subunits. (Pan et al., 2022). In the enzymatic synthesis of amoxicillin, PGA can act as a biocatalyst in the formation of products through the reaction between 6-APA and a suitable acyl group donor. This reaction allows the formation of amoxicillin through a selective acylation process. Enzymatic synthesis of β-lactam antibiotics using PGA is an alternative to conventional chemical synthesis processes because it can occur under milder reaction conditions, has high selectivity, and is more environmentally friendly. (Pan et al., 2022) However, the implementation of PGA-catalyzed synthesis on a large scale still faces several obstacles, such as low substrate concentration, suboptimal yield, and limited biocatalysts with suitable characteristics. (Pan et al., 2022) Various strategies have been developed to improve the efficiency of β-lactam antibiotic synthesis using PGA, including enzyme engineering, solvent system modification, in situ product removal, and the development of a one-pot reaction system. (Pan et al., 2022). One widely studied strategy is the use of immobilized PGA. In the amoxicillin synthesis process using immobilized PGA, high enzyme activity can cause rapid amoxicillin formation in local areas, resulting in a supersaturated solution. Because amoxicillin has low solubility, this condition can cause amoxicillin to crystallize and precipitate on the surface of the enzyme matrix. (Fan et al., 2024). Enzymatic synthesis of amoxicillin can be carried out using a batch reactor system.

In a batch reactor, PGA not only plays a role in the formation of amoxicillin, but can also trigger two side reactions, namely amoxicillin hydrolysis and reactant or ester hydrolysis. (Martins Neto et al., 2025) The existence of these side reactions indicates that the reactor operating conditions need to be controlled to obtain optimal amoxicillin formation. Dynamic simulation and optimization can be used to determine optimal control policies to achieve product specification targets in a bioprocess. (Cuthbertson et al., 2019). In addition, by incorporating the effect of temperature into the model, the temperature profile of the batch reactor can be optimized according to the desired production goals. (Cuthbertson et al., 2019). The results of this optimization can be used to determine the temperature control profile during the batch process so that the amount of amoxicillin produced can be optimized while minimizing raw material consumption. (Cuthbertson et al., 2019) In addition to temperature, reaction time is also an important factor in the batch process because too long an operation time can cause the formed amoxicillin to be degraded again by enzymes. (Martins Neto et al., 2025).

RESEARCH METHODS

Research Design

This study uses the Systematic Literature Review (SLR) method to identify, review, and analyze previous studies on the enzymatic synthesis of amoxicillin using Penicillin G acylase (PGA). The SLR method in this study is used to collect and integrate various relevant previous research results so that a more comprehensive picture can be obtained regarding the development of enzymatic amoxicillin synthesis. This study focuses on the use of PGA as a biocatalyst in the formation of amoxicillin, the characteristics of the application of the enzyme used, the conditions or processes of synthesis, and various approaches that have been developed to improve the efficiency of the process. In addition, this study was conducted to compare the results of previous studies, then the results of the study are used to describe the development of the enzymatic amoxicillin synthesis approach, the advantages and

disadvantages of using PGA, and strategies that can improve the results and efficiency of the synthesis process.

Research Questions

This journal was conducted to answer several research questions, namely:

No	Question
1	How is the development of the enzymatic synthesis method of amoxicillin using PGA?
2	What factors influence the efficiency of enzymatic synthesis of amoxicillin?
3	What strategies have been developed to improve the yield and efficiency of amoxicillin synthesis using PGA?

Data Sources And search Strategy

Literature search was conducted using scientific databases with the keywords enzymatic synthesis of *amoxicillin.*,amoxicillin synthesis,Penicillin G acylase,PGA,enzymatic biosynthesis of β-lactam antibiotics,immobilized Penicillin G acylase,Andamoxicillin enzymatic production. These keywords were then used to obtain articles relevant to the enzymatic synthesis and production of amoxicillin and the use of PGA as a biocatalyst. The search results were then selected based on the suitability of the title, abstract, and content of the articles. Articles considered in the review included research on the enzymatic synthesis or production of amoxicillin, the use of PGA, and various approaches related to increasing the efficiency of the synthesis process.

Study Selection and Eligibility Criteria

Articles selected based on the inclusion criteria included studies discussing the enzymatic synthesis of β-lactam antibiotics, particularly amoxicillin, studies using or discussing Penicillin G acylase, and studies discussing strategies for increasing synthesis efficiency such as enzyme immobilization, optimization of reaction conditions, enzyme engineering, use of solvents, in situ product removal, and other reaction systems. Articles that only discussed the clinical use of amoxicillin, the mechanism of action without discussing synthesis, or were unrelated to the focus of the study were excluded. Data from articles that met the criteria were then extracted based onauthors and year of publication, research title, research method, type of enzyme or PGA used, reaction system or reactor, synthesis strategy, and main research results.The data were then analyzed descriptively and comparatively to identify the development of the enzymatic amoxicillin synthesis method, factors that influence the synthesis process, strategies for increasing synthesis yields, and obstacles still encountered in the use of PGA as a biocatalyst.

RESULTS AND DISCUSSION

Sample Tables and Figures

No	Author & Year	Research Title	Method	Reactor	Key Results
1	Martins Neto et al. 2025	Enzymatic Synthesis of Amoxicillin in a Batch Reactor: Mathematical Modeling, Sensitivity Analysis, and Experimental Validation	Kinetic modeling, MCMC, sensitivity analysis, and experimental validation	Batch	The reaction and equilibrium constant-based models showed better predictive ability than the Michaelis–Menten model in predicting amoxicillin synthesis.
2	Cuthbertson et al. (2019)	Dynamic Modeling and Optimization of the Batch Enzymatic Synthesis of Amoxicillin	Dynamic modeling, simulation, kinetic parameter estimation, and non-isothermal optimization	Batch	Dynamic optimization yielded optimal reactor temperature and concentration profiles to improve amoxicillin synthesis performance.
3	Formigosa et al. (2026)	Mathematical Modeling of Amoxicillin Synthesis in Batch	MCMC, Genetic Algorithm, and kinetic modeling	Batch & semi-batch	MCMC shows good capability in estimating kinetic parameters and producing predictions of the amoxicillin

		and Semi-Batch Reactor: Application of Bayesian Statistics and Genetic Algorithm			synthesis process that are in accordance with experimental data.
4	(Fan et al. 2024)	Investigation of enzyme immobilization and clogging mechanisms in the enzymatic synthesis of amoxicilin	*Stirred batch reactor*with immobilized PGA and variations in substrate concentration, temperature, and stirring speed	Batch reactor with immobilized PGA	Blockage is caused by crystallization of amoxicillin in the enzyme matrix. Controlling substrate concentration and low temperature can prevent blockage and produce up to 99% conversion of 6-APA. Washing with 5% isopropanol is most effective, restoring PGA activity.
5	Pan et al. (2018)	Efficient Synthesis of β-Lactam Antibiotics with Very Low Product Hydrolysis by a Mutant Providencia rettgeri Penicillin G Acylase	Enzyme engineering (site-directed mutagenesis) on PGA from Providencia rettgeri PX04 to increase synthetic activity and reduce product hydrolysis.	Laboratory scale enzymatic reaction system using PGA and 6-APA and D-HPGME substrates.	The βF24G mutant yielded up to 95% conversion of amoxicillin, compared to 17.2% in the wild-type enzyme, with a shorter reaction time and very low product hydrolysis.
6	Pan et al (2020)	Efficient Synthesis of β-Lactam Antibiotics with In Situ Product Removal by a Newly Isolated Penicillin G Acylase	Method Penicillin G Acylase (PGA) from Achromobacter xylosoxidans PX02 was isolated, and then site-directed mutagenesis was performed at several positions to improve the ability of β-lactam antibiotic synthesis. The βF24A mutant was selected and the reaction conditions were optimized.	Using a reaction in aqueous solution with In Situ Product Removal (ISPR), the resulting amoxicillin precipitates directly from the solution, allowing the product to be separated during the process.	Amoxicillin synthesis achieved 98.7% conversion. The precipitated amoxicillin yielded 94.6% (568 mM) with 99% purity. This process also reduced byproduct formation and facilitated purification.
7	Ye et al (2023)	Enlarging the substrate binding pocket of penicillin G acylase from Achromobacter sp. for highly efficient biosynthesis of β-lactam antibiotics	Directed evolution/semi-rational engineering of Penicillin G Acylase (PGA) from Achromobacter sp. to enhance synthetic activity.	Biosynthesis reaction using engineered PGA.	The βF24G/βW154G mutant significantly increases the specific activity and synthesis/hydrolysis ratio. When used for amoxicillin synthesis, >99% conversion can be achieved in 90–135 minutes, with virtually no hydrolysis byproducts.
8	Alemzadeh et al. (2010)	Enzymatic Synthesis of Amoxicillin with Immobilized Penicillin G Acylase	Experimental; optimization of substrate concentration, PGA, pH, temperature, and reaction time	Enzymatic reaction system with immobilized PGA	The optimum conditions used PGA 5 g/L, 100 U, time 480 minutes and temperature 35 °C, with amoxicillin yield up to 50%.
9	Fathi et al. (2026)	Comparative Analytical Characterization of Amoxicillin Trihydrate Obtained by Chemical and Enzymatic Routes	HPLC, IR, NMR, MS analysis and microbiological tests	Amoxicillin products from enzymatic and chemical routes	Amoxicillin produced by the enzymatic route has a comparable concentration to the chemical route, but produces fewer impurities. Spectroscopic analysis revealed identical product structures and equivalent antibacterial activity.
10	McDonald et al. (2017)	Enzymatic Reactive Crystallization for Improving	Synthesis of ampicillin using Penicillin G Acylase (PGA) by combining enzymatic	Batch reactor with reactive crystallization, at pH 6 using saturated	The combination of reaction and crystallization increased the selectivity of ampicillin synthesis toward

		Ampicillin Synthesis	reactions and crystallization simultaneously	6-APA and equimolar PGME	phenylglycine by about 50%. This increase was primarily due to reduced ampicillin hydrolysis.
11	Pereira et al. (2012)	Enzymatic Synthesis of Amoxicillin by Penicillin G Acylase in the Presence of Ionic Liquids	Method Amoxicillin was synthesized using Penicillin G Acylase (PGA) with the addition of 1-butyl-3-methylimidazolium-based ionic liquids (ILs) as cosolvents. Several ILs tested were BMI·PF_6, BMI·NTf_2, and BMI·BF.	Enzymatic reaction system with water + ionic liquid as cosolvent. Optimum conditions were tested based on IL concentration and 6-APA conversion.	The use of BMI·PF_6 75% (vIL/vwater) increased the synthesis/hydrolysis (S/H) selectivity by approximately 400% compared to aqueous media. BMI·NTf_2 increased the selectivity by approximately 350% and resulted in a 6-APA conversion of more than 36% higher than that of aqueous media. No enzyme deactivation occurred and the biocatalyst particles remained intact.
12	Ye Wang Zhang et al. (2010)	ne-Pot, Two-Step Enzymatic Synthesis of Amoxicillin by Complexing with Zn^{2+}	Method Amoxicillin was synthesized using a one-pot, two-step enzymatic synthesis method using immobilized penicillin acylase (PA). Zn^{2+} was added to enhance the synthesis process.	One-pot reaction system, namely two reaction stages carried out in one container without separation of intermediate products.	After optimization, an amoxicillin yield of 76.5% was obtained. The one-pot, two-step method shows potential for simplifying the enzymatic synthesis of amoxicillin.
13	Dewi et al. (2025)	Immobilization of Penicillin-G Acylase from Bacillus thuringiensis BD1 for Enhanced Amoxicillin Production Using Na-Alginate Entrapment	Mobilization of PGA BD1 by entrapment method using Na-alginate, followed by activity test, pH and temperature stability, reusability, SEM, and synthesis of amoxicillin from 6-APA and HPGME using UPLC	Laboratory scale batch system using Erlenmeyer.	α-alginate 1.5% produced a specific activity of 41.01 U/mg; optimum conditions were pH 7 and 40°C; after 4 uses the remaining activity was approximately 20%; immobilized PGA produced a higher concentration of amoxicillin than free PGA.
14	(Suhendar et al., 2025)	Immobilization of penicillin G acylase (PGA) on cellulose nanocrystalline (CNC) produced by Escherichia coli BL21 pET28-PrPGA	Immobilization of PGA on cellulose nanocrystals (CNC) produced using E. coli BL21 pET28-PrPGA. Immobilization conditions were optimized based on stirring time and enzyme to buffer ratio using Response Surface Methodology (RSM).	Laboratory-scale reaction/immobilization system with stirring; research focuses on optimization of immobilization, not development of synthesis reactor type.	Optimum conditions yielded a relative activity of 87.322%, with experimental validation showing 87.717%. However, after three reuses, activity remained at only about 4%. CNC demonstrated potential as a PGA immobilization matrix to support more sustainable amoxicillin production.
15	Masdalifah et al. (2025	Screening for Penicillin G Acylase (PGA)-Producing Bacteria and Gene Cloning Using Degenerate Oligonucleotide Primed-PCR	Screening of PGA-producing bacteria, testing of hydrolysis and synthesis activity, isolation of the PGA gene using Degenerate Oligonucleotide Primed-PCR (DOP-PCR), then sequencing and analysis of protein structure.	laboratory scale for enzyme screening and characterization.	Of the four bacteria tested, P. rettgeri InaCC B466 showed the highest synthetic activity, while P. rettgeri InaCC B25 showed the highest hydrolysis activity. DOP-PCR successfully amplified a 2,517 bp pga gene from Pr25PGA, with 95.1% amino acid similarity to PGA of P. rettgeri PX04.

Based on the results of a literature review of 15 journals, several studies were obtained discussing the enzymatic synthesis of amoxicillin, a β-lactam antibiotic, using Penicillin G Acylase (PGA). The reviewed studies showed that PGA has been used through various approaches to improve synthesis efficiency, including enzyme immobilization, optimization of reaction conditions, enzyme engineering, the use of ionic liquids, in situ product removal, and the development of a one-pot

reaction system. The use of PGA in amoxicillin synthesis has been developed through various approaches, using both free and immobilized enzymes. In the early stages of development, research focused more on optimizing synthesis conditions using PGA to obtain conditions that optimally produce amoxicillin. Alemzadeh et al. (2010) showed that enzyme concentration, substrate concentration, pH, temperature, and reaction time affect the yield of amoxicillin synthesis. The optimum conditions obtained resulted in amoxicillin yields of up to 50%. Furthermore, method development was carried out through biocatalyst modifications to improve PGA performance. Pan et al. (2018) developed engineered PGA to enhance the ability of β-lactam antibiotic synthesis by suppressing the product hydrolysis reaction. Ye et al. (2023) also engineered the PGA substrate binding pocket and obtained amoxicillin conversion of more than 99% with very low hydrolysis byproduct formation. In addition to enzyme engineering, PGA immobilization is one approach developed to improve enzyme stability and reuse. Dewi et al. (2025) immobilized PGA from Bacillus thuringiensis BD1 using the entrapment method with Na-alginate. A concentration of 1.5% Na-alginate produced a specific activity of 41.01 U/mg, with optimum conditions at pH 7 and a temperature of 40°C. The immobilized enzyme could still be used up to four times, although the remaining activity was around 20%.

These results indicate that immobilization can increase the reusability of PGA and produce higher concentrations of amoxicillin compared to the free enzyme. Another approach is through modification of the reaction environment and system. Pereira et al. (2012) showed that the use of ionic liquid can increase the selectivity of amoxicillin synthesis towards hydrolysis reactions. Meanwhile, Pan et al. (2020) applied in situ product removal by removing the amoxicillin product during the synthesis process. This approach resulted in a conversion of 98.7% with a yield of 94.6% and a product purity of 99%. McDonald et al. (2017) also showed that combining enzymatic reactions with crystallization can increase the selectivity of β-lactam antibiotic synthesis by reducing product hydrolysis. Based on these various studies, the efficiency of amoxicillin synthesis is not only influenced by PGA activity, but also by reaction conditions, biocatalyst characteristics, and synthesis system design. Factors such as pH, temperature, substrate and enzyme concentrations, reaction time, enzyme stability, and the occurrence of hydrolysis and product crystallization are important aspects in determining the success of the synthesis. Therefore, various strategies such as immobilization, PGA engineering, reaction condition optimization, media modification, in situ product removal, and crystallization have been developed to improve the yield and efficiency of enzymatic amoxicillin synthesis.

CONCLUSION

Based on the results of a review of 15 journals, it can be concluded that the enzymatic synthesis of amoxicillin using penicillin G acylase (PGA) is a potential approach to produce β-lactam antibiotics more selectively and efficiently. Research developments show that PGA has been used in various forms and approaches, both as a free enzyme and immobilized, as well as through enzyme engineering and optimization of reaction conditions. The efficiency of amoxicillin synthesis is influenced by several main factors, including substrate concentration, temperature, pH, enzyme concentration, type and characteristics of PGA, and reaction conditions. In addition, product hydrolysis, enzyme deactivation, and blockage due to amoxicillin crystallization are some of the obstacles that need to be controlled in the synthesis process. Various strategies have been developed to improve synthesis yields, such as PGA immobilization, enzyme structure engineering, the use of ionic liquids, in situ product removal, reactive crystallization, and modeling and optimization of reaction conditions. Overall, these developments indicate that improving PGA performance and controlling process conditions are key to achieving higher yields and efficiency of amoxicillin synthesis, while also supporting the development of more effective and sustainable antibiotic production processes.

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