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BIODEGRADATION OF CARBAMAZEPINE USING FUNGI AND BACTERIA

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ABSTRACT

Carbamazepine is an anti-epileptic pharmaceuticalcompound which is frequently detected in wastewater. However, this compound is hardly degraded naturally due to its persistency. Thus, carbamazepine presents in water stream and household water supply as well as wastewater treatment plant. This paper focuses on various species of fungi and bacteria used in carbamazepine biodegradation and the carbamazepine degrading-enzymes involved in the degradation pathways. Selected research papers on carbamazepine biodegradation using fungi and bacteria were reviewed. The efficiency and approaches in term of methodologies and technologies used were highlighted in this paper. Such study sheds light on gaps of study and future research direction on carbamazepine biodegradation.

Keywords: biodegradation; carbamazepine; method; pharmaceuticals.

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1. INTRODUCTION

Emerging pollutants are new chemicals without regulatory status and the impacts on health and environment are poorly understood [1]. An example of emerging pollutants is pharmaceutical carbamazepine.

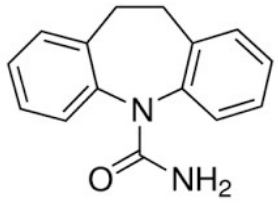

Fig.1. Carbamazepine structure

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide) is an anticonvulsant pharmaceutical commonly used in treatment of epilepsy, bipolar disorder, schizophrenia and trigeminal neuralgia [2]. Upon consumption by patients, approximately 72% of carbamazepine was absorbed, metabolized and excreted through urine. However, 28% failed to transform and discharged into water through faeces[3]. This compound eventually flows into wastewater.

Carbamazepine is frequently detected in wastewater and difficult to be degraded due to its complex structure and resistance to biodegradation [3]. Current wastewater treatment plants are not designed for removal of pharmaceuticals. Thus, this compound presents in treated wastewater at concentration ranging from ngL^{-1} to μgL^{-1}[2].

Carbamazepine in wastewater causes negative effects to environment and animals. Carbamazepine was detected in embryo of pregnant mice when the mother was exposed to environmental concentration of carbamazepine through drinking water [4]. Exposure of carbamazepine on fish Jenynsiamultidentata caused irregular cortisol activity and aggressive behaviour[5]. A study conducted on other fishes species Pimephalesnotatus and Ictaluruspunctatus showed detection of carbamazepine in brain, liver, plasma and white muscle after exposed to carbamazepine for 28 days [2]. Another study on insect, midge Chironomusriparius showed an increase in mortality of the species when carbamazepine was applied [6]. Since various studies demonstrated that carbamazepine causes adverse effects on animals, biodegradation of this compound is crucial to protect animals and the environment.

Biodegradation is an effective method for carbamazepine degradation [3]. However, complete degradation of this compound is hardly achieved. This paper focuses on efficiency of

carbamazepine biodegradation using variety of fungi and bacteria. Both fungi and bacteria have extracellular enzymes that function to cleave the complex compound bonds, hence reducing carbamazepine concentration. This paper also provides list of carbamazepine-degrading enzymes involve in biodegradation.

2. BIODEGRADATION USING FUNGI

Biodegradation of carbamazepine using fungi is conducted since fungi are known to have capabilities in degrading persistent pollutants [7]. The fungi species used in carbamazepine biodegradation include Trichodermaharzianum[8], Pleurotusostreatus (normal strain [8], AC9 [9]), immobilized Trametesversicolor[10], Trametesversicolor (ATCC [11], ATCC 42530 [12], ATCC 7731 [13] and NRRL 66313 [14]), Aspergillusniger[15], Cunninghamellaelegans ATCC 9254 [16], Umbelopsisramanniana R-56 [16] andPhanerochaetechrysosporium (mobilized strain F-1767 [17] and immobilized strain F-1767 [18]).

Summary of carbamazepine degradation by fungi according to species is listed in Table 1. Efficiency, different types of system applied, experimental condition and enzyme involved were discussed in following sections.

Table 1. Fungal degradation of carbamazepine

Fungi	Efficiency	System Applied	Condition	Enzyme Involved	References
Trichoder maharzia num	72%	Culture: Batch flask Extraction: SPE HLB cartridge Analysis: Liquid chromatography-high resolution tandem mass spectrometry	Initial concentration: 4µgL^{-1} Period: 15 days Temperature: 25 °C pH: 7.6 Medium: Murashige and Skoog media (liquid)	Cytochrom e P450 enzymes	[8]
Pleurotus ostreatus	68%	Culture: Batch flask Extraction: SPE HLB	Initial concentration: 4µgL^{-1}	Cytochrom e P450	[8]

		cartridge Analysis: Liquid chromatography-high resolution tandem mass spectrometry	Period: 15 days Temperature: 25 °C pH: 7.6 Medium: Murashige and Skoog media (liquid)	enzymes	
Pleurotus ostreatus PC9	GP: 99% Solid state fermentation (SSF) using cotton stalk: More than 80%	Culture: Glucose peptone (GP), solid state fermentation using cotton stalk Extraction: Not stated Analysis: Liquid chromatography-high resolution mass spectrometry	GP: Initial concentration: 37 $mmolg^{-1}$ Period: 25 days Temperature: 28 °C pH: Not stated Medium: Glucose peptone media (liquid) SSF on cotton stalk Initial concentration: 110 $mmolg^{-1}$ Period: 60 days Temperature: 28 °C pH: Not stated Medium: Cotton stalk (solid)	Cytochrome P450 enzymes, epoxide hydrolase	[9]
Pleurotus ostreatus PC9	No inhibitor: 99% CYP450 inhibitor: 30%	Culture: Flask culture of cytochrome P450 (CYP450) enzyme inhibitor, flask culture of manganese peroxidase inhibitor Extraction: Not stated	Initial concentration: 10 mgL^{-1}, 1 μgL^{-1} Period: 32 days Temperature: 28 °C pH: 4.5 Medium: Glucose peptone media (liquid)	Manganese peroxidase, versatile peroxidase	[19]

	Manganese peroxidase inhibitor: 99.7%	Analysis: Liquid chromatography-mass spectrometry			
Trametes versicolor ATCC 42530	57%	Culture: Batch flask Extraction: Not stated Analysis: High performance liquid chromatography	Initial concentration: 0.067 mgg^{-1} Period: 72 hours Temperature: 25 °C pH: 4.5 Medium: Bioslurry (solid) Carbon source: Glucose	Laccase	[12]
Trametes versicolor ATCC	Batch flask: 94% for 9 mgL^{-1} and 61% for 50 μgL^{-1} Bioreactor: 54%	Culture: Batch flask and bioreactor Extraction: Solid phase extraction (SPE) Analysis: High performance liquid chromatography-UV	Batch flask: Initial concentration: 9 mgL^{-1}, 50 μgL^{-1} Period: 6 days, 7 days Temperature: 25 °C pH: 4.5 Medium: Kirk medium (liquid) Bioreactor: Initial concentration: 200 μgL^{-1} Period: 15 days Temperature: 25 °C pH: 4.5	Laccase	[11]

			Medium: Kirk medium (liquid) Carbon source: Glucose		
Trametes versicolor (immobilized laccase on TiO_2 nanoparticles)	Biocatalytic membrane reactor: 40% Membrane hybrid reactor: 68%	Culture: Biocatalytic membrane reactor, membrane hybrid reactor Extraction: Not stated Analysis: High performance liquid chromatography	Biocatalytic membrane reactor Initial concentration: 20µM Period: 96 hours pH: 7 Medium: Permeate media (liquid) Membrane hybrid reactor: Initial concentration: 20 µM Period: 96 hours pH: 7 Medium: Permeate media (liquid) *temperature not stated	Laccase	[10]
Trametes versicolor (immobilized laccaseon chitosan nanoparticles)	0%	Culture: Magnetic biocatalyst Extraction: Not stated Analysis: Not stated	Initial concentration: 100 μgL^{-1} Period: 12 hours Temperature: 20 °C pH: 7 Medium: Sodium acetate buffer (liquid)	Laccase	[20]

Trametes versicolor NRRL 66313	Less than 60%	Culture: Aerated batch reactor Extraction: Liquid-liquid extraction Analysis: High performance liquid chromatography-photodiode array detection, gas chromatography-time-of-flight mass spectrometry	Initial concentration: 350 μgL^{-1} Period: 8 days Temperature: 25 ± 2 °C pH: 4.5 Medium: Kirk media (liquid) Carbon source: glucose (5 gL^{-1})	Laccase, lignin peroxidase and manganese peroxidase	[14]
Trametes versicolor ATCC 7731	10%	Culture: Batch flask Extraction: Not stated Analysis: High performance liquid chromatography-UV	Initial concentration: 930 μgL^{-1} Period: 22 hours Temperature: 25 °C pH: 4.5 Medium: Liquid	Laccase	[13]
Cunninghamellaelegans ATCC 9254	43%	Culture: Batch flask Extraction: Ethyl acetate and sodium sulphate Analysis: High performance liquid chromatography, liquid chromatography-mass spectrometry	Initial concentration: 1 µM Period: 25 days Temperature: 28 °C pH: Not stated Medium: Potato dextrose broth (liquid)	Not stated	[16]

Umbelopsisramanniana R-56	26%	Culture: Batch flask Extraction: Ethyl acetate and sodium sulphate Analysis: High performance liquid chromatography, liquid chromatography-mass spectrometry	Initial concentration: 1 µM Period: 25 days Temperature: 28 °C pH: Not stated Medium: Potato dextrose broth (liquid)	Not stated	[16]
Phaneorochaetechrysosporium BKM F-1767	60-80%	Culture: Sequence plate bioreactor Extraction: 0.25 µm membrane Analysis: High performance liquid chromatography	Initial concentration: 5 mgL^{-1} and 1 mgL^{-1} Period: 100 days Temperature: 34-37 °C pH: Not stated Medium: Kirk media (liquid)	Lignin peroxidase, manganese peroxidase	[17]
Phaneorochaetechrysosporium BKM F-1767 (immobilized)	80%	Culture: Countercurrent seepage bioreactor Extraction: Not stated Analysis: High performance liquid chromatography	Initial concentration: 1000 μgL^{-1} Period: 165 days Temperature: 30 °C pH: Not stated Medium: Modified Kirk media (liquid)	Manganese peroxidase	[21]
Phaneorochaetechrysosporium BKM F-1767	More than 90%	Culture: Rotating suspension cartridge reactor Extraction: Not stated Analysis: High	Initial concentration: 1000 μgL^{-1} Period: 160 days Temperature: 25 °C pH: Not stated	Lignin peroxidase, manganese peroxidase	[18]

(immobilized) Aspergillusniger	9%	performance liquid chromatography Culture: Batch flask culture Extraction: Centrifugation (retain supernatant) Analysis: High performance liquid chromatography-diode array detector	Medium: Modified Kirk media (liquid) Initial concentration: 11.4 mgL^{-1} Period: 7 days Temperature: 26 °C pH: Not stated Medium: Minimum mineral salt media (liquid) Additional carbon: 3 gL^{-1} glucose	Not stated	[15]

2.1. Efficiency of Fungi Degradation According to Methods

Different types of methods used in carbamazepine biodegradation include batch culture, bioreactor, biocatalyst and solid state fermentation.

2.1.1. Batch Culture

Fungi Trichodermaharzianum degraded 72% of carbamazepine within 15 days, meanwhile Pleurotusostreatus degraded 68% of the compound within 15 days at similar condition in Murashige and Skoog media [8]. Another study conducted by [9] showed Pleurotusostreatus of strain PC9 managed to degrade 99% of carbamazepine in 25 days when glucose peptone (GP) media was used for degradation experiment. Another study also showed consistent result where Trametesversicolor strain ATCC 42530 degraded 57% of carbamazepine within 72 hours in bioslurry medium with glucose as additional carbon source [12].

Different types of media affect degradation efficiency. Since Murashige and Skoog media contains limited minerals, fungi utilize carbamazepine as sole carbon source immediately for growth and result in fast degradation. Glucose peptone contains carbon source for fungi growth. Thus, fungi in this media utilized both carbon source from glucose peptone and carbamazepine slowly, resulted in slower but higher degradation percentage.Fungi Aspergillusniger reported only managed to degrade 9% of carbamazepine after 7 days eventhough additional carbon of 3 gL^{-1} glucose was added [15]. This reveals that different

types of fungi species require different types of carbon source.

For effects of enzymes on carbamazepine degradation, fungi Pleurotusostreatus PC9 demonstrated 99% carbamazepine degradation within 32 days when enzyme inhibitor was absence in the culture. When enzyme inhibitors were added, degradation efficiency changed. The culture with cytochrome P450 enzyme inhibitor experienced reduction in degradation efficiency resulted in only 30% degradation. Favourably, another system with manganese peroxidase inhibitor resulted in a slight increment with degradation efficiency of 99.7% [19]. This suggests that addition of enzyme inhibitor has positive or negative effect on fungi carbamazepine degradation as it depends on fungi metabolism system.

Next, there are also studies that investigated the effect of different initial concentration on degradation efficiency. FungiTrametesversicolor of strain ATCC resulted in 94% of 9 mgL^{-1} carbamazepine degradation within 6 days. However, this fungi species only degraded 61% of 50 μgL^{-1} carbamazepine at similar condition [11]. It can be hypothesized that high concentration of carbamazepine provided more carbon source, hence the degradation was more efficient. Another study also showed relatively the similar findings where Trametesversicolor of strain ATCC 7731 managed to degrade only 10% of low concentration 930 μgL^{-1} carbamazepine within 22 hours [13].

There is also study that investigated the potential of non-ligninolytic and ligninolytic fungi for carbamazepine degradation. Fungi Cunninghamellaelegans ATCC 9254 and Umbelopsisramanniana R-56 resulted in only 43% and 26% of carbamazepine degradation respectively after 25 days at similar condition [16]. The result showed non-ligninolytic fungi only capable to degrade less than 50% degradation. From the study, it can be hypothesized that ligninolyticfungi was more efficient in degrading carbamazepine where the efficiency reached 99% when ligninolytic fungi Pleurotusostreatus was used [19].

2.1.2. Bioreactor

Different types of bioreactor used in carbamazepine biodegradation include:

2.1.2.1. Standard Bioreactor

Trametesversicolor ATCC resulted in 54% carbamazepine degradation after 15 days with glucose as additional carbon source [11].

2.1.2.2. BiocatalyticMembrane Bioreactor

Laccase of Trametesversicolor immobilized on titanium oxide (TiO_2) only resulted in 40% carbamazepine degradation after 96 hours [10].

2.1.2.3. Membrane Hybrid Bioreactor

Laccase of Trametesversicolor immobilized on titanium oxide (TiO_2) resulted in 68% carbamazepine degradation at similar condition as biocalytic membrane bioreactor [10].

2.1.2.4. Aerated Batch Bioreactor

TrametesversicolorNRRL 66313 degraded less than 60% carbamazepine after 8 days. Additional carbon source of 5 gL^{-1} glucose was supplied [14].

2.1.2.5. Sequence Plate Bioreactor

Fungi Phanerochatechrysosporium BKM F-1767 resulted in 60-80% carbamazepine degradation after 100 days [17].

2.1.2.6. Countercurrent Seepage Bioreactor

Immobilized Phanerochatechrysosporium BKM F-1767 degraded 80% of carbamazepine within 165 days [21].

2.1.2.7. Rotating Suspension Cartridge Bioreactor

Immobilized Phanerochatechrysosporium BKM F-1767 managed to degrade more than 90% of carbamazepine after 160 days [18].

2.1.2.8. Biocatalyst

Magnetic biocatalyst was conducted using immobilized laccase of Trametesversicolor on chitosan nanoparticles failed to degrade carbamazepine after 12 hours [20].

2.1.2.9. Solid State Fermentation

Fungi Pleurotusostreatus PC9 was used for solid state fermentation using cotton stalk and able to degrade more than 80% carbamazepine after 60 days [9].

2.1.2.10. Summary

Among all methods used in degradation of carbamazepine using fungi, Pleurotusostreatus in batch culture was the most effective where 99% degradation was achieved. Membrane hybrid bioreactor was also effective since laccase of fungi Trametesversicolor capable to degrade carbamazepine with 60% efficiency within short time which was 96 hours. Sequence plate

bioreactor, countercurrent seepage bioreactor, rotating suspension cartridge bioreactor and solid state fermentation using cotton stalk also resulted in high degradation efficiency. However, these experimental methods were time consuming.

2.2. Carbamazepine Degrading Enzymes from Fungi

Carbamazepine-degrading enzymes produced by fungi include:

- Cytochrome P450 enzyme [8, 19]
- Manganese peroxidase [14, 17-19]
- Versatile peroxidase [19]
- Laccase[10-14, 20]
- Lignin peroxidase [14, 18, 22]

All of the conducted studies on carbamazepine degrading enzymes are qualitative study. These enzymes play important role in carbamazepine degradation pathways. A study by [16] proposed that carbamazepine degraded through mixed mono-oxidation reactions (hydroxylation and epoxidation). Both fungi Cunninghamellaelegans ATCC 9254 and Umbelopsisramanniana R-56 produced 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-EP) as major metabolite and 3-hydroxycarbamazepine as one of the minor metabolites. C. elegans also produced 2-hydroxycarbamazepine, while U. ramanniana exhibited new metabolites of hydroxycarbamazepine and 4-hydroxycarbamazepine.

Another study proposed a detailed pathway, where 24 metabolites were identified during carbamazepine degradation [9]. The three pathways involved were oxidation, hydrolysis and methoxylation. The main pathway was oxidation of carbamazepine into 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-EP). This reaction was aided by enzymes cytochrome oxidase and manganese peroxidase. Then, hydrolysis reaction converted the metabolite to dihydroxide carbamazepine (diOH-CBZ). It was suggested that epoxide hydrolase involved in this reaction. The process was followed by methoxylation resulting in formation of 10-methoxy-carbamazepine. The major metabolite identified in this study is similar to [16].

3. BIODEGRADATION USING BACTERIA

Bacteria are also one of the microorganisms group which capable to degrade organic

pollutants [7]. Since bacteria grow rapidly, application of this microorganism group can result in fast degradation of carbamazepine. Studies on biodegradation of carbamazepine using bacteria are limited. There were various carbamazepine biodegradation studies that utilized bacteria such as Streptomyces MIUG 4.89 [23-24], Streptomyces SNA [24], Serratia sp. [25], Rhodococcusrhodochrous[15], Pseudomonas CBZ-4 [3], mixed culture of Aquicella sp., Microvirga sp. and family Rhodobacteraceae[26], mixed culture of Spinghomonas sp., unclassified family of Spinghomonadaceae and Xanthomonandaceae[26], mixed culture of Acetinobacter US1, Bacillus halodurans, Micrococcus SBS-8 and Pseudomonas putida[27], mixed culture of Spinghobacterium sp., Chryseobacterium sp. and Alcaligenes sp. [28] and Paraburkholderiaxenovorans LB400 [29].

Table 2 showed summary of carbamazepine degradation by various bacteria species. This table is classified according to efficiency, different types of system applied, experimental condition and enzyme involved.

Table 2. Bacterial degradation of carbamazepine

Bacteria	Efficiency	System Applied	Condition	Enzyme Involved	References
Streptomyces MIUG 4.89	30%	Culture: Batch reactor Extraction: Centrifugation (supernatant retain) Analysis: High performance liquid chromatography	Initial concentration: 0.2 mgL^{-1} Period: 7 days Temperature: 25 °C pH: 6 Medium: Basal media (liquid) Additional carbon: 6.5 gL^{-1} glucose, 2 gL^{-1} yeast	Not stated	[23]
Streptomyces MIUG 4.89	35%	Culture: Batch flask culture Extraction: Not stated	Initial concentration: 0.2 mgL^{-1} Period: 7 days	Laccase Phenoloxidase	[24]

		Analysis: High performance liquid chromatography	Temperature: 25 °C pH: Not stated Medium: Minimal media (liquid) Additional carbon: 5 gL^{-1} glucose		
Streptomyces SNA	30%	Culture: Batch flask culture Extraction: Not stated Analysis: High performance liquid chromatography	Initial concentration: 0.2 mgL^{-1} Period: 7 days Temperature: 25 °C pH: Not stated Medium: Minimal media (liquid) Additional carbon: 5 gL^{-1} glucose	Laccase	[24]
Rhodococcus rhodochrous	15%	Culture: Batch flask culture Extraction: Centrifugation (retain supernatant) Analysis: High performance liquid chromatography-diode array detector	Initial concentration: 9.5 mgL^{-1} Period: 7 days Temperature: 26 °C pH: Not stated Medium: Minimum mineral salt media (liquid) Additional carbon: 3 gL^{-1} glucose	Not stated	[15]
Pseudomonas CBZ-4	46.6 %	Culture: Aerobic batch flask culture Extraction: Ethyl acetate	Initial concentration: 9.5 mgL^{-1} Period: 144 hours	Not stated	[3]

		Analysis: High performance liquid chromatography	Temperature: 10 °C pH: 7 Medium: Dominic and Graham media (liquid)		
Serratiasp.	0%	Culture: Batch flask culture Extraction: Chloroform application Analysis: High performance liquid chromatography	Initial concentration: 0.75 mgL^{-1} Period: 20 days Temperature: Not stated pH: Not stated Medium: Bushnell Haas media (liquid)	Not stated	[25]
Aquicellasp., Microvirgasp., Rhodobacteraceae mixed bacteria culture	Aerobic: 12.8-14.5 % Anaerobic: 6.2-14.9%	Culture: Aerobic and anaerobic batch amber bottle culture Extraction: QuECHERS for soil extraction followed by solid phase extraction for liquid Analysis: Liquid chromatography mass spectrometry	Initial concentration: 50 ngg^{-1}, 500 ngg^{-1}, 5000 ngg^{-1} Period: 14 days Temperature: Not stated pH: Not stated Medium: Soil (solid)	Not stated	[26]
Spinghomonassp.,unclassified family of Spinghomonadaceae and	More than 25%	Culture: Aerobic and anaerobic batch amber bottle culture Extraction: QuECHERS for soil extraction followed by solid phase	Initial concentration: 5000 ngg^{-1} Period: 14 days Temperature: Not stated pH: Not stated	Not stated	[26]

Xanthomonadaceae mixed bacteria culture		extraction for liquid Analysis: Liquid chromatography mass spectrometry	Medium: Soil (solid)		
Acetinobacter US1., Bacillus halodurans, Micrococcus SBS-8, Pseudomonas putida mixed bacteria culture	60%	Culture: Batch flask culture Extraction: Solid phase extraction Analysis: High performance liquid chromatography	Initial concentration: 100 μgL^{-1} Period: 12 days Temperature: 30 °C pH: 7 Medium: LB media (liquid)	Not stated	[27]
Sphingobacterium sp., Chryseobacterium sp., Alcaligeness p. mixed bacteria culture	With acetate: 20% No acetate: 10%	Culture: Batch flask culture Extraction: Not stated Analysis: High performance liquid chromatography-diode array detector	Initial concentration: 25 μgmL^{-1} Period: 14 days Temperature: 25 °C pH: Not stated Medium: Minimal media (liquid) Additional carbon: 100 μgmL^{-1} acetate	Not stated	[28]
Paraburkholderiaxenovorans LB400	100%	Culture: Batch serum bottle culture (direct biological method) Extraction: Ethyl acetate	Initial concentration: 10 mgL^{-1} Period: 24 hours Temperature: 25 °C	Biphenyl dioxygenase, dihydrodi	[29]

	Analysis: High performance liquid chromatography-diode array detector, High performance liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry	pH: 7 Medium: Phosphate buffer (liquid)	ol dehydrog enase

3.1. Efficiency of Bacteria Degradation According to Methods

Different types of methods used in carbamazepine biodegradation using bacteria include batch flask culture, bioreactor, batch amber culture bottle and batch serum culture bottle.

3.1.1.Batch Flask Culture

Bacteria Streptomyces MIUG 4.89 degraded only 35% carbamazepine during 7 days of experimental period when 5 gL^{-1} of glucose was added as carbon source [24]. Meanwhile, bacteria Streptomyces SNA resulted in lower carbamazepine degradation which is 30% during similar experimental period and condition. Bacteria Rhodococcusrhodochrous managed to degrade only 15% of carbamazepine after 7 days eventhough additional carbon source of 3 gL^{-1} glucose was added [15].Another study demonstrated that Pseudomonas CBZ-4 capable to degrade only 46.6% of carbamazepine after 144 hours period [3]. Bacteria Serratia sp. failed to degrade carbamazepine eventhoughit was cultured during a long period of 20 days [25].

Individual flask culture study was less efficient in degrading carbamazepine. None of the individual flaks culture study achieved more than 50% degradation. Moreover, addition of carbon source did not enhance degradation of carbamazepine.

Next, mixed bacteria culure studies were also conducted. Mixed bacteria culture of Acetinobacter US1, Bacillus halodurans, Micrococcus SBS-8 and Pseudomonas putida degraded 60% of bacteria within 7 days of experimental period [27]. This revealed that mixed bacteria was more efficient compared to individual flask culture study. However, another mixed bacteria culture study of Spinghobacterium sp., Chryseobacterium sp. and Alcaligenes

sp. only able to degrade 20% of carbamazepine when 100 µgmL^{-1} of acetate was added as additional carbon source. Meanwhile, biodegradation efficiency decreased to 10% when acetate was absence [28]. Mixed bacteria culture may provide synergistic effects or antagonistic effects depending on bacteria species. Additional nutrient source effect study is limited and further investigation should be continued.

3.1.2. Bioreactor

Standard bioreactor was used to degrade carbamazepine using bacteria Streptomyces MIUG 4.89. This strain only degraded 30% of carbamazepine after 7 days with additional carbon source of 6.5 gL^{-1} glucose and 2 gL^{-1} yeast [23].

3.1.3. Batch amber Bottle Culture

Degradation experiment was conducted by comparing aerobic and anaerobic condition of batch amber bottle culture in soil medium. Mixed bacteria culture of Aquicella sp., Microvirga sp. and family Rhodobacteraceae only degraded 12.8-14.5% of carbamazepine in aerobic condition within 14 days and only degraded 6.2-14.9% of carbamazepine in anaerobic condition within the same experimental period and condition. Another mixed bacteria culture study of Spinghobacterium sp., Chryseobacterium sp. and Alcaligenes sp. only managed to degrade more than 25% of carbamazepine in soil medium within 14 days [26].

3.1.4. Batch Serum Bottle Culture

Bacteria Paraburkholderiaxenovorans LB400 achieved complete degradation of carbamazepine within 24 hours [29].

3.1.5. Summary

Based on various studies, different biodegradation methods have no significant effects on carbamazepine degradation by bacteria. Bacteria Paraburkholderiaxenovorans LB400 degraded 100% carbamazepine due to its ability to release various types of enzymes that play important roles in carbamazepine degradation. Mixed bacteria culture of Acetinobacter US1, Bacillus halodurans, Micrococcus SBS-8 and Pseudomonas putida also showed efficient degradation with 60% degradation rate. Other individual culture and mixed bacteria culture only degraded less than 50% carbamazepine. In mixed bacteria culture, there was limited nutrient, thus, bacteria die and resulted in inefficient degradation. Hence, bacteria characteristics and its adaptability in carbamazepine environment need to be determine before

study is conducted.

3.2. Carbamazepine Degrading Enzymes of Bacteria

Carbamazepine-degrading enzymes produced by bacteria include:

- Laccase[24]
- Phenoloxidase[24]
- Biphenyl dioxygenase[29]
- Dihydrodiol dehydrogenase [29]

In [29] proposed a pathway of carbamazepine degradation by bacteria Paraburkholderiaxenovorans LB400. The main metabolites includecis-10,11-dihydroxy-10,11-dihydrocarbamazepine and cis-2,3-dihydroxy-2,3-dihydrocarbamazepine. The enzyme involved was biphenyl dioxygenase. Further reaction utilized dihydrodiol dehydrogenase enzyme and coverted the cis-dihydrodiols into carbamazepine-diol and 2-hydroxycarbamazepine. For laccase and phenoloxidase enzymes, the study only identified the presence of enzymes qualitatively hence, no pathway is proposed.

4. CONCLUSION

Various fungi and bacteria are capable in degrading carbamazepine. The difference in degradation efficiency is mainly affected by fungi and bacteria properties and its ability to release enzymes that aid in carbamazepine degradation. For fungi, different types of methods affect degradation efficiency. Meanwhile, for bacteria applications of advanced methods do not necessarily enhance the degradation efficiency. There are gaps of study in addition of carbon source utilization by both fungi and bacteria. Utilization of carbon source differs according to species. Thus, further study is needed on this topic. Next, for enzymes study, to authors' best knowledge, there is no proposed pathway that involved laccase enzyme in carbamazepine degradation using bacteria and fungi. Hence, detailed study on pathways involving this enzyme is needed. This study leads to further development and application of carbamazepine bioremediation.

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