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Tetracycline Biodegradation by *Pseudomonas sp.* Isolated from Wastewater Treatment Plant

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Abstract
The discovery and the usage of antibiotics in the early 20th century have expanded human life expectancy from around 40 to 60 years old. Where antibiotics solved bacterial infections including Pneumonia, Tuberculosis, Syphilis, and Meningitis to name a few. However, this triumph victory of the long-raging war against pathogenic bacteria builds a future problem which is the accumulation of Tetracycline waste in environments as the result of incomplete degradation after human consumption. This issue may introduce microbial communities to build resistance using an Antibiotics Resistance Gene (ARG) which imposes the threat that may dull the effectiveness of Antibiotics as an essential drug against bacterial infection. This study tries to explore the biodegradation of Antibiotics using *Pseudomonas sp*. as a solution for the accumulation of Tetracycline in the environment. Biodegradation by bacteria was chosen as an alternative solution compared to physical and chemical methods such as Photolysis, Fenton process, and Hydrolysis using an advanced treatment system due to its more affordable cost and produce less toxic by-product. The experiment started by growing *Pseudomonas sp.* as selected bacteria in LB batches with the addition of Tetracycline to ensure its capability to endure stress. Then in the period of 24h the sample of the batches is taken for OD quantification using a spectrophotometer, quantifying Tetracycline concentration using HPLC and obtaining the by-product of the biodegradation process using Q-TOF LC/MS. The findings from this experiment show a successful quantification of the growth profile of *Pseudomonas sp.* under tetracycline stress where the Log phase starts from hour 10th up to hour 20th with an estimation of bacteria cells of 1.073.741.824 at the end of Log phase. The next finding was the Tetracycline biodegradation rate was quantified and the result shows that after 24 hours of incubation, there is 52.26% of Tetracycline gets degraded by *Pseudomonas sp*. Lastly, Q-TOF LC/MS results show a possible by-product of Tetracycline biodegradation, where after analysing the possible results using KEGG software, it shows that 16C Sphinganine has the possibility to be the by-product of this degradation process, then Tetracycline enzymatic reaction was proposed. The outcomes of this experiment shall inform the importance of the biodegradation process towards environmental pollutants by choosing bacteria, another importance of this experiment outcome is to further study the mechanism pathway of Tetracycline biodegradation as the findings are only a proposed possible pathway.
Keywords: Tetracycline; *Pseudomonas sp.*; Biodegradation; Tetracycline By-product; Metabolic Pathway

Introduction

The early usage term of ‘antibiotic’ could be traced back as early as 1890 by the prominent scientist at the time Paul Vuillemin in his publication referring to the antagonistic interaction/ symbiosis between different microorganisms. Later on, the term antibiotic is applied towards the naturally occurring secondary metabolism product by certain bacteria and fungi possessing properties either growth inhibitory (bacteriostatic) or killing (bactericidal) (Manyi-Loh et al., 2018). On September 1928, Alexander Fleming went back to his Laboratory in Paddington, London at St. Mary’s Hospital after his vacation and observed the Staphylococcus Aureus colony he has left is contaminated with a type of fungus called *Penicillium notatum*, currently more known as *Penicillium chrysogenum*.

Even though this finding has occurred by other scientists he did not dispose of the cultures without further analysis. Fleming tries to grow the fungus in pure culture on other pathogenic bacteria and found a similar effect, where there is a clean area in the media without the growth of the bacteria, especially the Gram-positive one and conclude that the fungus could produce a certain substance that could prevent the growth of bacteria and named it Penicillin in March 1929. Later in 1945, Alexander Fleming won the Nobel Prize in Physiology or Medicine for the discovery of Penicillin and its curative effect on various infectious diseases.

In today's age, the wide commercial application of Antibiotics is not just to cure human infections, antibiotics also help agricultural activities towards growth promotion, disease prevention and increasing product quality (Manyi-Loh et al., 2018). Many developing countries keep the high-rate usage of this compound to maintain a healthy state, increasing product quality and quantity. This persistent culture gets worse due to no clear regulation that controls the intensity of the usage of 2 Antibiotics in many developing countries (Manyi-Loh et al., 2018). Antibiotic waste in wastewater plants can't be naturally degraded by humans or cattle and it could seep through groundwater and soil that causes ecotoxicity. Eventually, the intense exposure of antibiotics towards microorganisms would create Antibiotic Resistance Bacteria (ARB) as a form of survival effort and these mutated microbes could also perform gene transfer to other microorganisms in a different environment (Manyi-Loh et al., 2018). Thus, this study aims to figure out the biodegradation of Tetracycline by using bacteria as a safer alternative (Pazda et al., 2019).

The focus of this study is to help degrade tetracycline that can be found in wastewater plants by using *Pseudomonas sp.* as a safer alternative. It is important to prevent unnecessary problems that could happen in the future. Preventing Antibiotic Resistance Bacteria (ARB) could be achieved by degrading the Tetracycline compound with the help of *Pseudomonas sp.* as a chosen microorganism. The usage of a biodegradation agent is preferred due to its low cost, efficiency, lesser side effects and more affordable choice compared to other chemical or physical methods proposed by other studies. The mechanism *Pseudomonas sp.* used to degrade Tetracycline as Carbon-source will also be thoroughly studied as a part of the experiment.

Materials and methods

The bacteria, Pseudomonas sp. was a courtesy from Athena Dana (PhD student) which has been isolated from wastewater treatment at Taman Harmoni 1 (Effluent sample) and was sent to Apical for 16S rRNA identification and kept freeze drying at around -80°C.

The chosen media of Nutrient agar and broth is used due to its simplicity of preparation and effectiveness in growing *Pseudomonas* bacteria (Devika et al., 2021; Eyler, 2013; Al-Saffar & Jarallah, 2019). The first step is to clean the bench using 70% of Alcohol solution to avoid any contamination from unwanted microbes and prepare the tools including digital weighing, spatula, beaker, Scott bottle, agar plate and magnetic stirrer (Eyler, 2013; Nepali et al., 2019). Meanwhile, the ingredients are to take Yeast extract, NA powder, NaCl powder, Tryptone, and distilled water (Eyler, 2013; Al-Saffar & Jarallah, 2019). The step to prepare a 250mL solution of NA agar is first to weigh 14g of NA powder, 1.2g of Tryptone, 1.2g of NaCl, and 0.25g of Beef extract then put it on a beaker then add 250ml of distilled water and put it on a magnetic stirrer, adjust the intensity to around 100 rpm and the temperature on 50°C (Eyler, 2013; Al-Saffar & Jarallah, 2019; Nepali et al., 2019). When the solution is lukewarm the next step is to change the pH to around 7 by checking the solution on the pH meter, if it exceeds the range, add NaCl powder into the solution 1 tbsp then stir it on the magnetic stirrer (Nepali et al., 2019; Eyler, 2013). The next step is to pour the solution into a Schott bottle then autoclave it to kill any possible contaminants at 120°C for 15 minutes then let it cool down then the solution is poured into an agar plate inside a laminar flow machine, then the plate is kept in a room temperature until the agar is fully solidified, after that the last step is to seal the NA plate using parafilm paper to prevent any contamination of the agar, then the last step is to write the date and NA name on the side of the plate using a marker (Nepali et al., 2019; Eyler, 2013).

For the Nutrient Agar broth, the step is quite similar to the NA agar (Eyler, 2013). Still, the main difference is the solution is needed in the form of liquid, which means the NA powder is not needed during the preparation, and the solution can be kept in a Scott bottle instead of getting poured into an

agar plate (Nepali et al., 2019; Eyler, 2013; Al-Saffar & Jarallah, 2019). The NA broth is then kept in a 4°C fridge until its usage (Nepali et al., 2019; Eyler, 2013).

For preparing 100 ml of Tetracycline solution, the required tools to be prepared are a beaker, Scott bottle, spatula, measuring cylinder, weighing scale, magnetic stirrer, 0.22 µm syringe filter and syringe (Pizan-Aquino, 2020). Meanwhile, the ingredients for the Tetracycline Hydrochloride solution are a TC hydrochloride powder and 100 ml of sterile water (Pizan-Aquino, 2020). The first step is to weigh 1g of Tetracycline hydrochloride powder on a weighing scale, then add 100 ml of sterile water, the next step is to still the solution using a magnetic stirrer until the Tetracycline is fully dissolved in the water, then using a syringe and syringe filter to filter the solution into Scott bottle, then the solution can be kept into 4°C fridge (PizanAquino, 2020).

Using NA agar and broth to grow the pure culture of *Pseudomonas*, the frozen *Pseudomonas* pure culture was taken from the freeze-drying fridge and put inside a laminar until ¼ of its ice melted (Eyler, 2013; Al-Saffar & Jarallah, 2019). Then preparing the agar plate containing NA agar, bunsen burner and inoculation rod for streaking purposes. Lit the burner then spray the inoculation rod using alcohol to kill bacteria, then burn the rod until it turns orange in colour then let it cool down for 2 minutes (Eyler, 2013; Al-Saffar & Jarallah, 2019). After that open the sealed pure culture of *Pseudomonas* and take a dip using the rod into the tubes, closed the tubes and perform streaking on the surface of NA agar, the proper method to perform streaking is to make sure the hands are in the middle of the laminar, streak the rod gently, making 3 - 5 quadrants of the streaking, and open the agar plate near the bunsen burner (Eyler, 2013; Al-Saffar & Jarallah, 2019).

Repeat the process into 5 different agar plates and 3 empty agar plates with no streaking performed as a control group. The next step is to seal the NA agar plate inside the incubator at 37°C for around 24 hours to perform the incubation process (Eyler, 2013). Take out the plate then observe and take photos as documentation and proof. The next step is to take a single colony of each NA agar plate and then put it into NA broth for further steps (Eyler, 2013; Al-Saffar & Jarallah, 2019).

Using the prepared NA broth then pour 9.9 ml of it into 10 different falcon tubes, 5 of them as a control group, then the other 5 get an additional ingredient of TC hydrochloride for 10 mL/L as the exposure of the Tetracycline (Eyler, 2013; Al-Saffar & Jarallah, 2019). Then take a single colony of the *Pseudomonas* bacteria using a rod and dip it into the broth and repeat it for each agar plate that has been made and incubate the Falcon tubes for 24 hours under 37°C (Eyler, 2013; Al-Saffar & Jarallah, 2019).

The next step is to quantify the growth rate of the *Pseudomonas* under the NA broth containing the *Pseudomonas* colony, every 2 hours up to 24 hours to make the graph containing the Lag Phase, Log Phase, Stationary Phase and death Phase using the Spectrophotometer in the wavelength of 600 nm OD (Hadwan, 2018). The first step is to clean the cuvette tubes using deionized water and let it dry (Hadwan, 2018). The next step is to prepare the samples inside cuvette tubes for around 1 ml each using micropipettes, which contain a blank sample and the sample containing *Pseudomonas* (Hadwan, 2018). Then turn on the spectrophotometer machine and set it to 600 nm wavelength (Hadwan, 2018). Calibrate the quantification using the blank sample as the control group (Hadwan, 2018). Remove the blank sample and replace it with the sample then run the test after waiting 10 seconds and do the process with the rest of the sample and interval time (Hadwan, 2018).

The first step to running High-Performance Liquid Chromatography is to prepare the mobile phase solution by adding 400 ml of acetonitrile to approximately 1.5 L of purified deionized water and then add 2.4 ml of glacial acetic acid to the solution. Then dilute the solution using deionized water until the final solution becomes 2.0 litre with a final pH concentration of around 2.8 to 3.2. Then adjust the pH to 4.2 by adding 40% of sodium hydroxide (Moudgil et al., 2019; Saridal et al., 2019). Then filter the mobile phase solution using a 0.47-µm Nylon 66 membrane filter under vacuum to degas the solution and to remove solids that could plug the chromatographic column (Moudgil et al., 2019; Saridal et al., 2019). The next step is to prepare standard solutions, in this case, Tetracycline Hydrochloride solution by adding 2ppm of Tetracycline Hydrochloride into a volumetric flask, then dilute using deionized water up to 500 ml mark. Then the next step is to set the HPLC machine by setting the flow rate of the mobile phase to 0.5 ml/min, then set the minimum pressure to 250 psi and maximum pressure to 4.000 psi. Then press ‘zero’ on the detector’s front panel in order to set the blank (the blank is the pure mobile phase) (Arabsorkhi 27 et al., 2018; Tang et al., 2020). Then continued by rinsing a 100-µL syringe with

deionized water and then adding the standard solutions to identify the peak for the Tetracycline sample. The next step is to add the sample solution (Moudgil et al., 2019; Saridal et al., 2019). Using the injector handle, inject 100 µL of the sample solution into the septum port. When ready to start, rotate the injector handle to the inject position and click the button 'start' on the computer data collection program immediately (Arabsorkhi et al., 2018; Tang et al., 2020). When the results come out, save the data using a suitable name. Then remove the syringe from the septum and repeat the process for each of the remaining working standards (Arabsorkhi et al., 2018; Tang et al., 2020).

The first step is to prepare the mobile phase where Acetic acid and Acetonitrile were used. Preparing the pure water/ deionized water by filling 2 1L scotch bottles with distilled water then autoclaving it at 121°C for 15 minutes, add 10mL of acetic acid and acetonitrile to respective bottles, then filter the solution using filter paper for a bulk quantity inside laminar flow.

The next step is to take the Tetracycline biodegradation sample after 24h of incubation where by using a syringe filter pump it with 0.22 microlitre and transfer it into a 1.5 microlitre vial, label the vial with the name then keep it to a 4°C freezer. The last step is to submit all the components to T03 staff to run the Q-TOF LC/MS experiment.

Starting off the Log phase of the graph, it is safe to assume that a single colony was taken and from the literature that *Pseudomonas sp.* can divide every 20 minutes in ideal conditions. Thus, the calculation may go by 2 squares of (the duration of Log phase x 3).

Thus, the formula: $2^{(Log\ phase\ in\ hour\ x\ 3)}$

Interpreting the HPLC results can be done by plotting the graph with the data from area% of HPLC result. Use the average of duplicate results then plot the graph, since the initial concentration of Tetracycline is 2ppm then to calculate the biodegradation results from initial and final, the formula that can be used in determining the concentration of degraded Tetracycline.

Thus, the formula: $\frac{(Initial-Final)conc.}{Initial\ conc.}\ x\ 100\%$

The interpretation of the Q-TOF LC/MS results can be started by finding a similar retention time from the HPLC results because the Tetracycline biodegradation should still have some similarities with its end product, thus the identification of possible products could be achieved. The next step is to remove an unlikely compound that exceeds 22 Carbons in its compound, the choice of 22 Carbons is based on the Tetracycline Carbon number that has only 22 C, where it is most likely that the by-product should have at most 22 C. The next and last step is to analyse those possible by-products in KEGG software to determine the metabolic pathways related to the Tetracycline pathways.

Results and discussion

The first experiment is to grow a *Pseudomonas sp.* sample in Nutrient Agar in order to get pure culture as the below picture shows five replications were done in order to complete this step. After 48 hours of incubation, the growth can be observed by eyes where a zig-zag white line was exhibited. The single colony was then taken using an inoculum rod and it was transferred to Nutrient Broth for further analysis.

The triplicate of Nutrient broth was then incubated and 2 ml of the solutions were taken into cuvettes for every 2 hours interval. The cuvette samples were then quantified by using Spectroscopy. The results that were obtained are shown in Table 1 then a plotted graph from that table is shown in Figure 2.

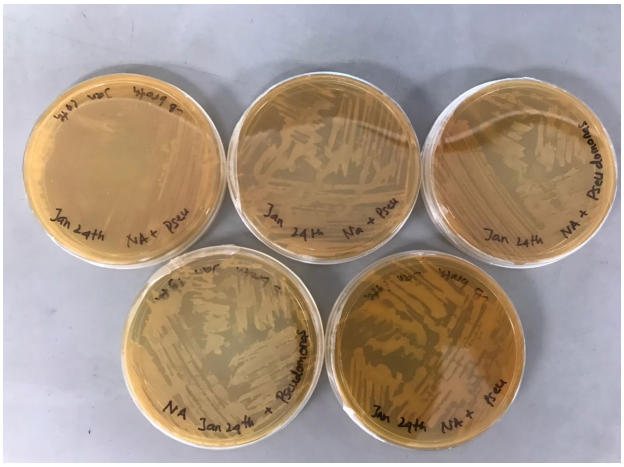

Figure 1 *Pseudomonas sp.* Grown in Nutrient Agar

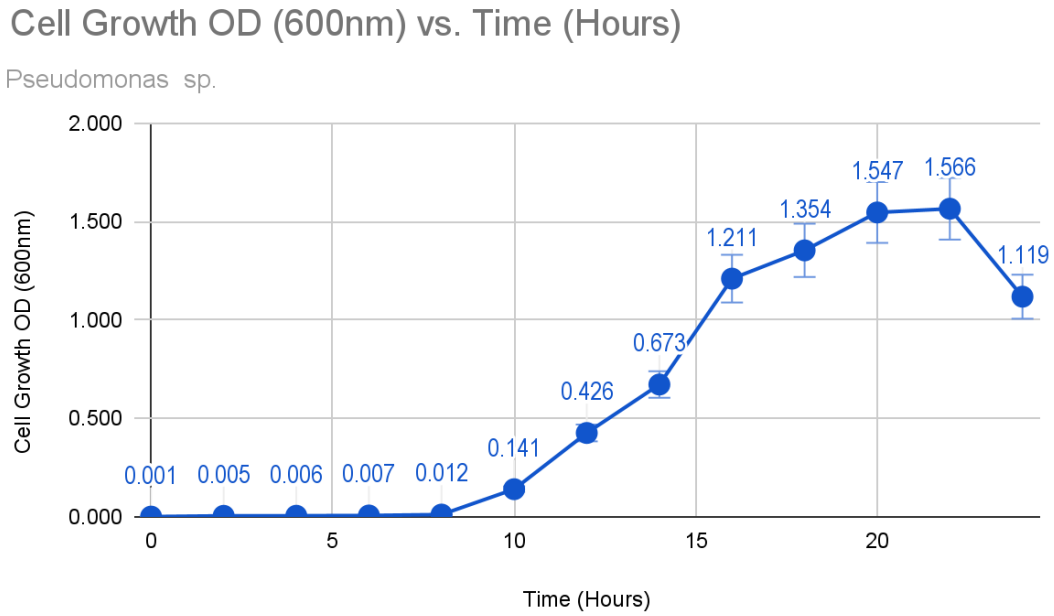

Figure 2 A plotted growth profile of *Pseudomonas sp.*

Table 1: Growth profile of *Pseudomonas sp.*

Time (Hours)	Cell Growth (600 nm)
0	0.001
2	0.005
4	0.006
6	0.007
8	0.012
10	0.141
12	0.426
14	0.673
16	1.211
18	1.354
20	1.547
22	1.566
24	1.119

Table 1 and Figure 2 shows a growth profile of *Pseudomonas sp.* in Nutrient broth media, where it shows that *Pseudomonas sp.* bacteria require around 10 hours of Lag phase as the Optical density remains low for that amount of duration. Based on the literature review, usually *Pseudomonas* bacteria require 1 - 4 hours of lag phase in ideal conditions, but since the Nutrient broth was added with Tetracycline solution, the bacteria require more time to adjust to the new environment.

The next phase is the log phase that is started from hours 10 - 20 where this phase has a characteristic of a fast dividing process which was shown by the sharp increase of Optical density value starting from 0.141 up to 1.547 nm. The accumulation of *Pseudomonas* cells takes up most of the resources and it also accumulates the waste that eventually becomes toxic for their own culture, which it shows in the next step which is the Stationary phase when the rate of cell division is similar to the rate of cellular mortality. Table 1 shows the stationary phase that happened around hours 20 - 22 with an optical density value of 1.547 to 1.566. The last phase is the death phase where the food resource is depleted and the accumulation of waste has become toxic to *Pseudomonas* culture. It starts at hour 22 onwards with an optical density value 34 of 1.566, and in hour 24, it has an optical density value of 1.119. This means that *Pseudomonas sp.* has a growth profile within a 24-hour span in a given nutrient broth resource.

Based on figure 3 where the calculation of *Pseudomonas* cells at the end of Log phase is 1.073.741.824 (One trillion seventy-three million seven hundred fourty one thousand and eight hundred twenty-four), this number is an estimation of the value after Pseudomonas sp. divide itself in Nutrient broth.

$$2^{(10\ x\ 3)} = 1.073.741.824$$

Figure 3 *Pseudomonas* estimation cell number at the end of Log phase.

The concentration of Tetracycline biodegradation rate is steadily declining as shown in figure 3 that the initial concentration of 2 ppm of Tetracycline with the value of concentration 5.505 and after incubation it decreased to 2.628 where after the calculation the degradation value is 52.26% this means *Pseudomonas sp.* bacteria can degrade 1.04 ppm of Tetracycline concentration within 1 day, where final concentration of tetracycline from 2 ppm get reduced to 0.96 ppm after the incubation. The degradation rate of tetracycline which only 52.26% happened due to the short duration of incubation, based on journals that could manage to degrade tetracycline from 70% - 90% happened because the incubation time that was applied starts from 6 to 8 days (Chen et al., 2022). Thus, that is the main reason why in this experiment only 52.26% of Tetracycline gets degraded.

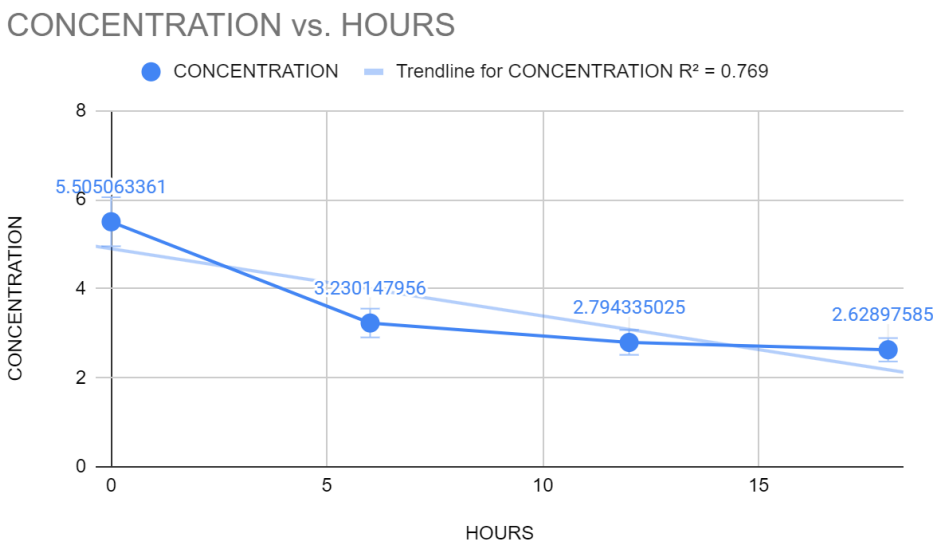

Figure 4 Graph plot of Tetracycline biodegradation rate.

$$\frac{(5.505 - 2.628)}{5.505} \; x \; 100\% = 52.26\%$$

Figure 5 Calculation of final concentration of Tetracycline.

Table 2: Area % of Tetracycline degradation value in HPLC result.

Hour	Duplicate		Concentration of Tec
	D1	D2	
0	56.139	53.725	5.505
6	43.727	54.538	3.230
12	54.561	42.907	2.794
18	43.468	53.965	2.628

The incubated of *Pseudomonas sp.* in a conical flask containing Nutrient broth were still being used in this step. After 24-hour incubation, the sample was transferred into a 1.5 ml vial using a filter syringe. The prepared sample and mobile phase were then submitted to the T03 staff and the result of Q-TOF LC/MS are shown in the figures below.

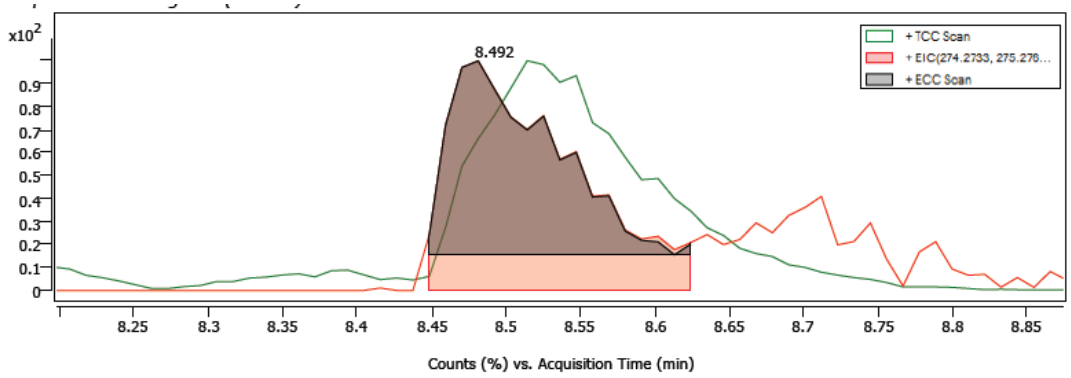

Figure 6 Retention time of C16 Sphinganine (possible by-product).

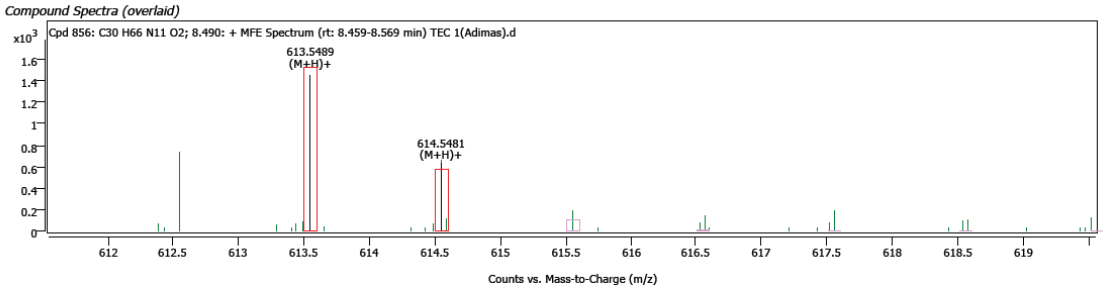

Figure 7 Compound spectra of C16 Sphinganine.

The chosen possible by-product of Tetracycline biodegradation was C16 Sphinganine. The reason to choose this compound is due to several reasons, the number of Carbon atoms that is less than 22, the similar retention time it has with Tetracycline retention time and the clear metabolic pathway it has in KEGG software.

From the Q-TOF LC/MS result, it shows that the detected compound has 16 Carbons which means it has less than 22 C from Tetracycline. The retention time of 8.490 is also similar to the retention time of Tetracycline which is around 8.7. The KEGG metabolic network shows that this compound has an extensive metabolism pathway in the software. Based on Table 3 The C16 Sphinganine has a molecular formula of C16H35NO2 with a mass of 273.2661, mass-to-charge ratio of 274.2 m/z and retention time of 8.490.

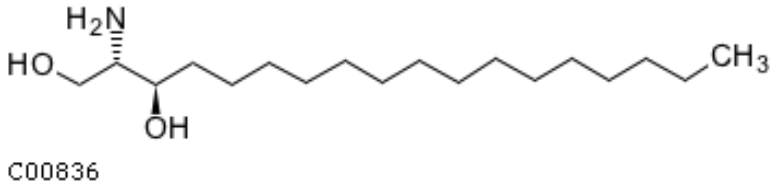

Figure 8 Sphinganine chemical structure.

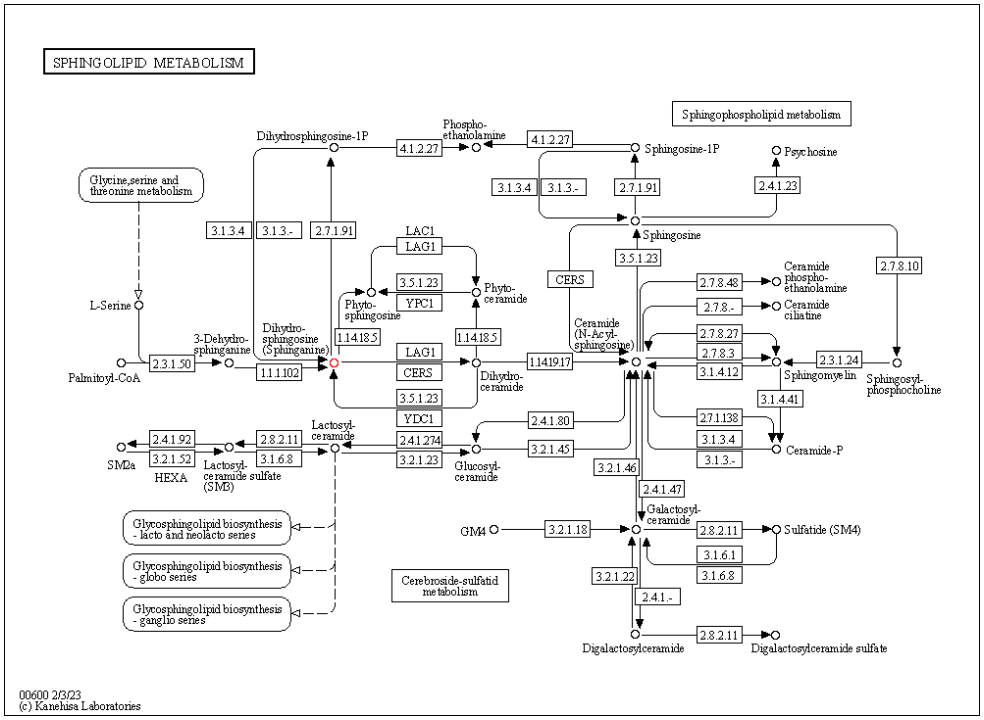

Figure 9 Sphinganine metabolism. Retrieved from KEGG software.

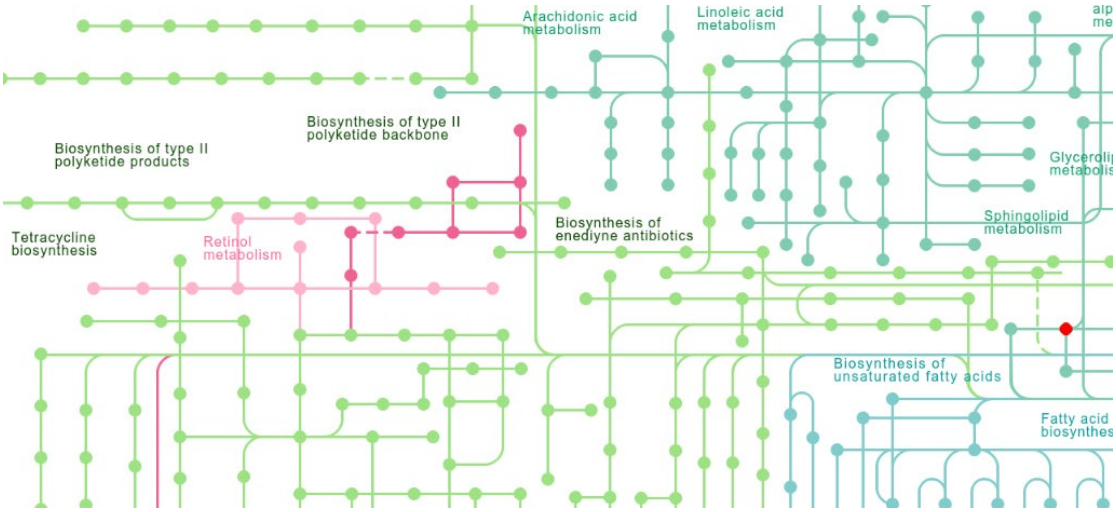

Figure 10 Location of Sphingolipid in Metabolism Network. Retrieved from KEGG software.

Table 3: Characteristics of C16 Sphinganine.

Parameter	Value
Name	C16 Sphinganine
Formula	C16 H35 N O2
Mass	273.2661
Mass-to-charge ratio	274.2734 m/z
Retention time	8.490

Even though C16 Sphinganine was chosen as a possible by-product for Tetracycline biodegradation. There are possible other by-product candidates for this biodegradation, but they all

share similarities with C16 Sphinganine which is a lipid-based compound. Which are Phytosphingosine and Xestoaminol C. These two are categorized as Sphingolipid compounds which still belong to the same lipid class as C16 Sphinganine. The reason Phytosphingosine and Xestoaminol C was not chosen as the possible by-product is that they are commonly found in plant and fungi organisms and less likely can be produced by *Pseudomonas* bacteria.

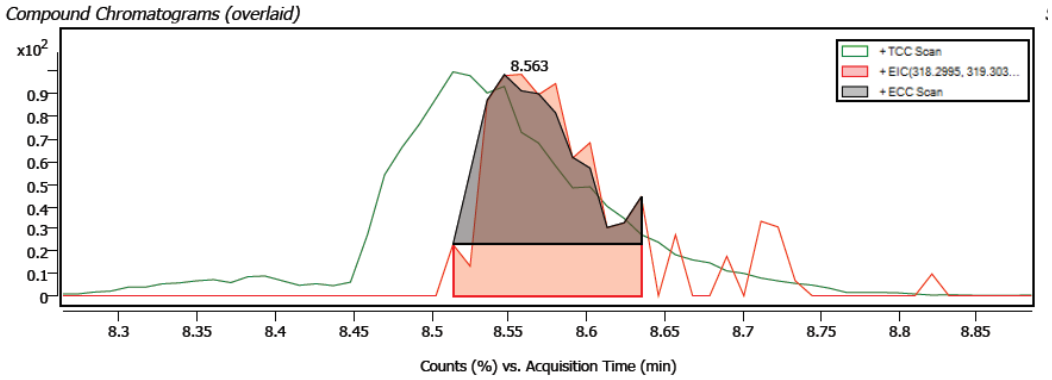

Figure 11 Retention time of Phytosphingosine with value 8.563

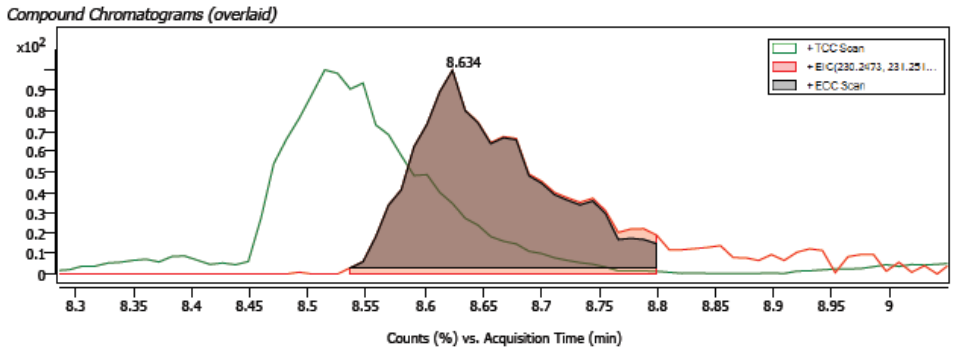

Figure 12 Retention time of Xestoaminol C with value 8.634

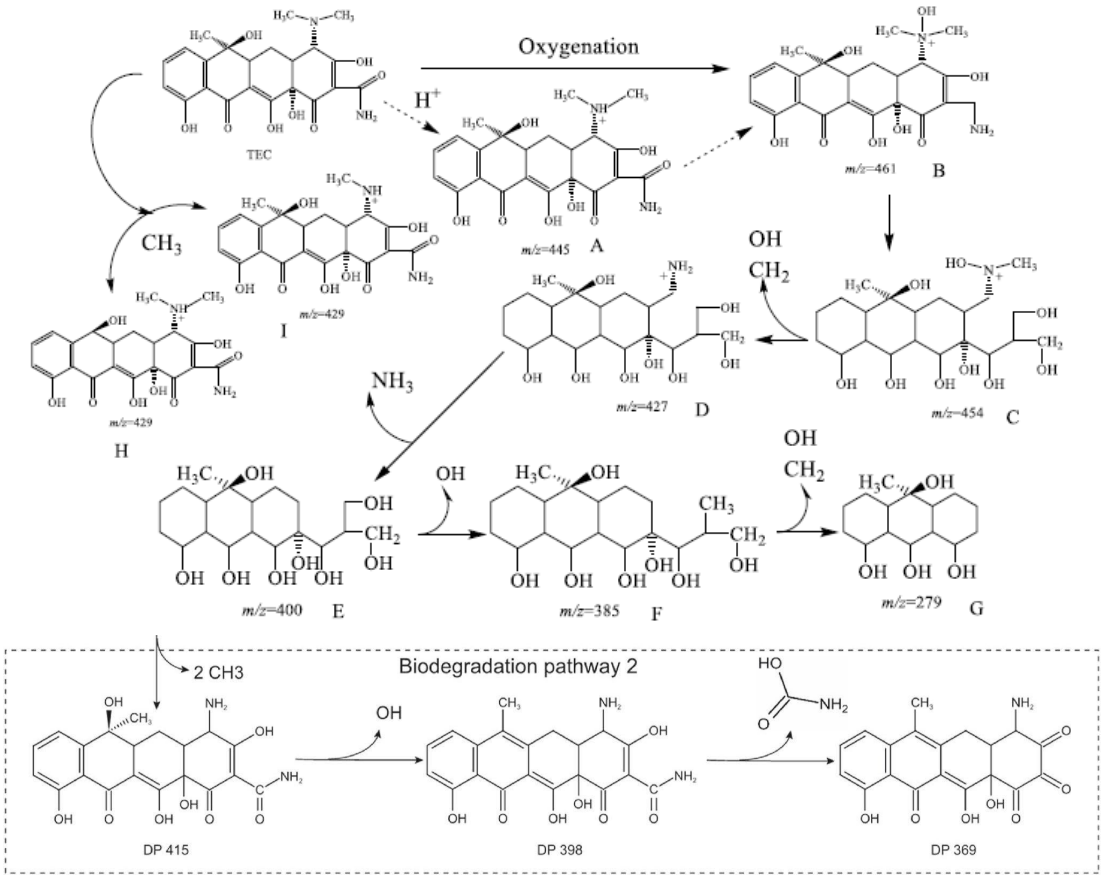

Figure 13 Proposed pathway of Tetracycline biodegradation (Chen et al., 2022)

Tetracycline biodegradation that was influenced by enzymes such as tetracycline 11a-monooxygenase will alter the molecular structure of the Tetracycline ring. There are multiple pathways that involves in Tetracycline biodegradation such as hydrolysis that was precursor by hydrolase and esterases, oxidation that uses enzyme flavin-containing monooxygenases (FMOs), and reduction that is typically performed by anaerobic bacteria as shown in figure 14 below (Chen et al., 2022).

ENZYME: 1.14.13.231

Help

Entry	EC 1.14.13.231 Enzyme
Name	tetracycline 11a-monooxygenase; tetX (gene name)
Class	Oxidoreductases; Acting on paired donors, with incorporation or reduction of molecular oxygen; With NADH or NADPH as one donor, and incorporation of one atom of oxygen into the other donor BRITE hierarchy
Sysname	tetracycline,NADPH:oxygen oxidoreductase (11a-hydroxylating)
Reaction(IUBMB)	tetracycline + NADPH + H+ + O2 = 11a-hydroxytetracycline + NADP+ + H2O [RN:R11449]
Reaction(KEGG)	R11449; (other) R11525 R11526 Reaction
Substrate	tetracycline [CPD:C06570]; NADPH [CPD:C00005]; H+ [CPD:C00080]; O2 [CPD:C00007]
Product	11a-hydroxytetracycline [CPD:C21391]; NADP+ [CPD:C00006]; H2O [CPD:C00001]
Comment	A flavoprotein (FAD). This bacterial enzyme confers resistance to all clinically relevant tetracyclines when expressed under aerobic conditions. The hydroxylated products are very unstable and lead to intramolecular cyclization and non-enzymic breakdown to undefined products.

Figure 14 Tetracycline 11a-monooxygenase enzyme. Retrieved from KEGG software.

Conclusion

In summary, this thesis looked at the biodegradation of tetracycline by a species of *Pseudomonas* found in a wastewater treatment facility. The objective of the study was to comprehend *Pseudomonas sp*.'s potential as a bioremediation tool for environments polluted with tetracycline. The research results showed that *Pseudomonas sp*. had a high capability to digest tetracycline effectively through a variety of laboratory experiments and analyses. Tetracycline was completely degraded as a result of the bacteria's capacity to use it as a carbon and energy source. This was demonstrated by both the gradual drop in tetracycline concentration and the presence of breakdown by-products.

The initial concentration of tetracycline, temperature, and pH all had an impact on the biodegradation process. The ideal conditions for *Pseudomonas sp.* to break down tetracycline were studied, offering important information for prospective uses in wastewater treatment systems. The study also investigated the processes of tetracycline biodegradation, offering information on the implicated enzymatic pathways and the probable function of certain enzymes in the degradation of the antibiotic. The findings of this study have important ramifications for environmental protection and wastewater treatment methods. Tetracycline is a persistent antibiotic that has a negative environmental impact. By using *Pseudomonas sp.*'s tetracycline degrading abilities, it is feasible to improve the removal of this antibiotic from wastewater. The findings present a viable strategy for the reduction of antibiotic contamination and add to the body of knowledge on microbial bioremediation.

To fully understand the potential of *Pseudomonas sp.* in actual wastewater treatment systems, more study is necessary. For this bacterial genus to be successfully used, long-term studies evaluating its stability, effectiveness, and usability are needed. Tetracycline removal from wastewater may be improved by looking at the possible effects of other co-existing pollutants and the viability of combining different remediation technologies. The potential for using *Pseudomonas sp.* to biodegrade tetracycline to clean up tetracycline-contaminated wastewater is highlighted by this thesis, which broadens our understanding of the process. The results have significant ramifications for environmentally friendly wastewater treatment procedures and highlight the value of utilising microbial bioremediation techniques to successfully manage antibiotic contamination.

Acknowledgement

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