Influence of Pharmaceutical Residue in Domestic Wastewater on the Removal Efficiency of COD and Ammonium in an Anaerobic Batch Reactor

S Sudarno¹, N Hardyanti¹ and F A A Pradhita¹

¹ Department of Environmental Engineering, Faculty of Engineering, Diponegoro University, Semarang
sudarno_utomo@yahoo.com

Abstract. At present, the use of medicines, especially antibiotics, has increased significantly, and this affects domestic waste. The human population contributes to the spread of antibiotic residue into the environment via excretion of pharmaceuticals during the ordinary course of treatment. This study aims to study the effect of pharmaceutical residues, particularly antibiotics, on the removal efficiency of ammonium and COD in domestic wastewater, using an anaerobic batch reactor. The reactor inoculated with a septic tank and an artificial waste. The stages of this research were seeding, acclimatization, and running. This research was conducted for 30 days with the reactor volume at 8.5 litres of seeding and acclimatization time and 1.5 litres of running time. Efficiency removal of COD were 89.66%; 88.24%; 85.21%; 78.27% and 77.72%. Meanwhile, the efficiency removal of ammonium are 55.08%; 53.30%; 48.13%; 40.36% and 32.16%. The reactor with the highest pharmaceutical residue did not contribute to the efficiency of COD removal but affected the effectiveness of ammonium removal.

1. Introduction
In recent years, the production and consumption of PPCP (Pharmaceutical Personal Care Product) increased substantially. Medicines and personal products or commonly known as PPCP, are substances used by individuals for personal health and cosmetic reasons. PPCP itself is divided into two groups, namely the Pharmaceutical Care Product and Personal Care Product. According to international statistical data, the global pharmaceutical market has increased and is estimated to have produced $956 billion medicines in 2011 and grew to $1.2 trillion in 2015. The growth of the pharmaceutical market increased by about 5-8% worldwide [1].

PPCP enters the environment through human activities, including the residue from the use of hospitals and communities. Drugs are often not processed correctly by the human body. After it is excreted, the drug can enter the environment through disposal. There are about 65% of the antibiotic content in the whole world in the deformed environment [2]. Amoxicillin is one of the most widely used antibiotic types [3], and the residue is found in large quantities of surface water of 171 mg/L [4]. Pharmaceutical residues merge into domestic waste through the disposal system.
High levels of COD and ammonium dominate the domestic waste pollution parameter. The provision of organic material is efficiently set aside by anaerobic methanogenic processes that also produce biogas. Also, the removal for nitrogen in wastewater can be made through physical and biological processes [6]. But in its application, the biological process is commonly used because it is more effective and profitable in financial terms. Batch reactor is one of the wastewater treatment technology using suspended growth principles. Steve [6] mentions that the embedded biomass system has better microorganism activity than the suspended biomass system. This activity can occur because the microorganisms that make up the biofilms layer have a low substrate concentration and provide a higher likelihood of reaction with nutrients outside of the substrate [7].

In previous research conducted by Chelliapan et al. [8], biologically processing using an up-flow anaerobic stage reactor by adding tylosin as residue antibiotics showed a relatively small influence on Organic material removal. At high concentrations of Tylosin (600-800 mg/l) causes minor inhibition in the performance of reactors and microorganisms in the reactor. This research will examine the effect of pollutants in the form of pharmaceutical residues, especially amoxicillin-type antibiotics, to the COD and ammonium removal in the domestic wastewater, where the results and parameters are analyzed and Impact.

2. Research Methodology
The study uses an anaerobic batch reactor with a reactor capacity of 8.5 L. Wastewater used is artificial wastewater that has a concentration of COD 300 mg/L and ammonium amounted to 40 mg/L. For the Running stage, the reactor is varied with amoxicillin concentration. There are 5 reactors in the running phase, namely control reactors without the addition of amoxicillin, then AMX 5 for variations of amoxicillin 5 mg/L, AMX 10 for variations of amoxicillin 10 mg/L, AMX 40 for variations of amoxicillin 40 mg/L and AMX 80 for variations amoxicillin 80 mg/L.

![Reactor schemes](image)

Both of the seeding and acclimatization stages as well as the running stage, the operation of the reactor is carried out for 15 days, by measuring the parameters of pH, temperature, the concentration of COD, ammonium, nitrite, and nitrate. The research carried out at the Diponegoro University Environmental Engineering campus, Semarang, Central Java. Measuring temperature and pH every day using a thermometer and pH meter. COD, ammonium, nitrite and nitrate tests are carried out every three days using spectrophotometric methods.
3. Result and Discussion

3.1. Stages of seeding and acclimatization

Seeding and acclimatization are necessary to familiarize the bacteria with the environment or in other words, to make the bacteria adaptable to the new environment before the bacteria is used for the next phase of research. At this stage, artificial wastewater that has been made with COD concentrations of 300 mg/l and ammonium amounted to 40 mg/L is fed into a reactor containing 1.2 litres of septic tank sediment for 15 days.

Figure 2. The graph of ammonium, nitrite, nitrate and pH concentrations in seeding and acclimatization

Based on the graph above, the ammonium concentrations in the reactor fluctuate. The decrease in ammonium concentration occurs on day 2 to day 6. However, on the 7th day, there is an increase in ammonium concentrations. However, on the 7th day of the ammonium concentration has decreased, and continues to occur until the 15th day. This condition proves that it takes time for adaptation to remove ammonium from wastewater. Lowering ammonium concentrations can also be caused by ammonium used as a supporting substrate by microorganisms [9].

After analysis, it is known that the average stage rate of ammonium is 1.45 mg NH$_4^+$-N/L. Day, with a maximum ammonium removal rate of 7.88 mg NH$_4^+$-N/L. The day occurred on Day 8. Furthermore, the efficiency of the ammonium removal achieved from the first day to the 15th of 52.31%. The maximum accumulation of nitrite occurs on the 7th day, namely 16.88 mg NO$_2^-$-N/L, with the concentration of nitrate formed for 15 days ranging from 1.79 – 14.34 mg NO$_3^-$-N/L.
Figure 3. The graph of Ammonium, nitrite, nitrate and pH concentrations in seeding and acclimatization

From the data contained can be found that the initial concentration of COD is 349.11 mg/l with an ammonium concentration of 41.49 mg/L. During 15 days of seeding process and acclimatization, COD concentration continues to decline to be able to set aside 80.52%, i.e. to 68 mg/L. COD removal that occurs due to the activity of anaerobic organisms in wastewater derived from septic tank sediment. This removal is due to the occurrence of microbial metabolism, where microbes need high carbon as a source of energy to grow and adapt so that there is a decrease in COD value. These microorganisms can degrade the organic material contained in the wastewater optimally until it reaches a removal of 80.52%, with an average COD removal of 18.74 mg/l per day. The results of the same study were in research conducted by [10] which suggests that batch reactors in anaerobic can set aside COD by 76.6% on the 14th day.

The results of the same study were in research conducted by [10] which suggests that batch reactors in anaerobic can set aside COD by 76.6% on the 14th day. The pH data resides in the range 6-7 as listed in the table. On day 0, the pH of wastewater is 7.48. The pH value then drops and tends to sour on day two and rises back on the 4th day. This activity is possible due to the biological activity of microorganisms contained in the reactor that makes the surrounding environment acidic. Because in this process, there is an acidogenesis process where the organic material that exists is transformed into volatile acidic acids by microorganisms. The existence of this acid causes a decrease in the pH reactor.

The pH data resides in the range 6-7 as listed in the table. On day 0, the pH of wastewater is 7.48. The pH value then drops and tends to sour on day 2nd and rises back on the 4th day. This activity is possible due to the biological activity of microorganisms contained in the reactor that makes the surrounding environment acidic. Because in this process, there is a process of resuffle the organic material into volatile acids by existing microorganisms. The existence of this acid that causes a decrease in the pH in the bacterial concentration reactor increased on the 6th day. It is interesting to review because the increase in biomass concentration related to the contact time between bacteria and wastewater will result in microbial growth. The VSS value increases from the number 694.6 mg. Biomass/L to 720 mg. Biomass/L, this means the condition in the reactor supports the rate of microbial growth. After experiencing the increase, the concentration of VSS decreased on day 15. This decline is due to the lack of substrate and growth-limiting nutrient less supportive for microbial growth.

3.2. Running Stage Ammonium

Results of the ammonium concentration test for 15 days in each reactor are outlined in the table and depicted in the picture 2.
Table 1. Ammonium concentrations

| Ammonium concentrations (mg NH₄⁺-N/l) | AMX 01 | AMX 5  | AMX 10 | AMX 40 | AMX 80 |
|--------------------------------------|--------|--------|--------|--------|--------|
| AMX 01                              | 44,15  | 46,40  | 49,47  | 52,16  | 52,73  |
| AMX 5                               | 37,28  | 41,45  | 43,53  | 43,61  | 43,77  |
| AMX 10                              | 36,87  | 38,93  | 39,44  | 34,55  | 46,12  |
| AMX 40                              | 30,66  | 34,52  | 35,59  | 43,00  | 38,15  |
| AMX 80                              | 20,00  | 29,09  | 33,09  | 33,94  | 40,05  |
| Control                             | 19,83  | 21,20  | 25,66  | 31,11  | 35,77  |

Decrease in the ammonium concentrations due to pH and temperature conditions. As the previous researcher's statement [11], that the optimum pH for the denitrification process is 6.5-7.5 and the optimum temperature for the Nitrosomonas bacteria is 25°C-35°C. Another cause of ammonium concentration is the use of ammonium by the COD degrading bacteria as support. This hypothesis is answered by the study of Rajagopal et al. [9], where it is said that the ammonia, which has a concentration of 50-200 mg/L, can support microorganisms. The concentration 200-1000 mg/L does not affect the microorganisms, and the concentration 1500-3000 mg/L is a performance inhibitor or inhibitors of microorganisms at a certain pH. Therefore, the concentration of ammonia which exceeds 3000 mg/L is toxic to microorganisms.

Table 2. Efficiency and decreased rate of Ammonium

| Reactor | Efficiency removal (%) | Decreased rate (mg. NH₄⁺-N/L. Day) |
|---------|------------------------|-----------------------------------|
| Control | 55,08                  | 1,62                              |
| AMX 5   | 53,30                  | 1,61                              |
| AMX 10  | 48,13                  | 1,59                              |
| AMX 40  | 40,36                  | 1,40                              |
| AMX 80  | 32,16                  | 1,13                              |

Based on the data contained in table 2, it can be seen that the ammonium concentrations in each reactor have decreased. However, a decrease in the ammonium concentrations of each reactor has varying levels of removal and reduced rates. In the control reactor where there is no addition of antibiotic concentration, a 15-day absence of the ammonium removal is 55.08% with an average decrease rate value of 1.62 mg NH₄⁺-N/L. Day, and the maximum decrease rate of 3.55 mg NH₄⁺-N/L that occurred on the 12th day.

In the AMX 5 reactor, in which the addition of the concentration of amoxicillin is 5 mg, obtained a decrease in the rate of average ammonium concentrations of 15 days 1.61 mg NH₄⁺-N/L. The day and the rate of decline of the maximum ammonium concentration of 2.63 mg NH₄⁺-N/L that occurred on day 15. Also, the efficiency of the ammonium level removal achieved is 53.30%.

In the AMX 10 reactor, which is given the addition of the concentration of amoxicillin 10 mg, obtained the rate of decrease in the average concentration of ammonium for 15 days amounted to 1.59 mg NH₄⁺-N/L. The day and the rate of decline of the maximum ammonium concentration of 2.48 mg NH₄⁺-N/L that occurred on day 15. Also, the efficiency of the ammonium level removal achieved is 48.13%.

In the AMX 40 reactor, where the addition of the concentration of amoxicillin at 40 mg, obtained the rate of decline of the average ammonium concentration for 15 days by 1.40 mg NH₄⁺-N/L. The day and the rate of decline of the maximum ammonium concentration of 3.02 mg NH₄⁺-N/L that occurred on the 12th day. However, in this reactor, the ammonium concentrations fluctuate, where on day 9, the
concentration of ammonium is increased. Up to the 15th day, the amount of the ammonium level removal is 40.36%.

In the AMX 80 reactor, where the addition of the concentration of amoxicillin at 80 mg, obtained the rate of decline of the average ammonium concentration for 15 days by 1.13 mg NH$_4^+$-N/L. The day and the rate of decline of the maximum ammonium concentration of 2.99 mg NH$_4^+$-N/L that occurred on day 3rd. However, in this reactor, the ammonium concentrations fluctuate, were on the 6th day, the ammonium concentrations increased. The same thing happens on the 12th day where the ammonium concentrations are re-increased. Until the day K1 15, the efficiency of the ammonium level removal is 32.16%.

From the above data, the smallest removal and rate of decline is found in the AMX 80 reactor in which a reactor has a variation of amoxicillin with the most significant pollutant load of AMX 80 mg/L. This result corresponds to the research of Zhang et al. [5], where it was said that the ammonium removal process could tolerate the presence of amoxicillin to a rate of 60 mg/L. When the level is at 80 mg/l, then amoxicillin will be an inhibitor in the ammonium stage. It can be seen from picture 3, in reactor 5 occurs fluctuations in the ammonium removal, where this process is interrupted by the presence of amoxicillin. After declining, ammonium concentrations increased again. This is attributed to the rupture of the cell, releasing the intracellular substance into the extracellular chamber, thereby increasing the ammonium [5].

In addition to inhibiting the ammonium stage process, antibiotics can also impede the reduction of nitrite and nitrate because of the undoing of biochemical processes in microorganisms so that microbial activity is under stressful conditions due to antibiotics Disrupt the metabolism of biochemistry and affects their population [12]. Also, the presence of amoxicillin concentrations that indirectly increase the concentration of COD affects the ammonium stage process. COD does not give direct influence, but the bacterial activity that utilizes COD is affecting the ammonium stage process. The addition of organic substances, such as COD will support the activity and growth of heterotrophic bacteria that utilize organic carbon so that the growth rate of heterotrophic bacteria will be much faster than bacteria with slow growth rates such as nitrifying bacteria [13].

The growth rate of heterotrophic bacteria is 2 to 3 times as much as the growth rate of nitrification bacteria, even reaching 5 times more [14]. The number of heterotrophic bacteria then raises competition and inhibits the growth of nitrifying bacteria due to the ammonium absorption that is used for biosynthesis until the ammonium used by nitrifying bacteria is reduced.

![Figure 4. Graph of pH parameters](image-url)
The initial pH is on the five reactors in an acidic setting but still within the recommended pH range for anaerobic processes. However, more prolonged pH is increasingly acidic due to the influence of antibiotic residue given. This condition, of course, can disrupt the process and is a condition that is not optimal for anaerobic bacteria in setting off COD. Then, to condition pH to be in the optimal pH range for the anaerobic process back, then the pH solution is given as a pH buffer. The pH buffer is performed on the third day after measurement. Viewable, for the fourth day pH, starts to hike and start to the desired pH range.

![Graph of temperature parameters](image)

**Figure 5.** Graph of temperature parameters

Based on the picture above can be seen that the temperature in the five reactors relatively stable. It indicates the potential for the bacteria to live.

![Graph of COD parameters](image)

**Figure 6.** Graph of COD parameters

From the data contained in the table it can be seen that the initial COD concentrations for the control reactors, AMX 5, AMX 10, AMX 40, and AMX 80 are 303 mg/l, 351.33 mg / l, 369.67 mg / l, 466.33 mg / l, and 559.67 mg / l. During the 15 days of the running process, COD concentrations continued to decrease until they were able to set aside by 271.67 mg / l, 310 mg / l, 315 mg / l, 365 mg / l and 435 mg /
l for each reactor. COD removal that occurs due to anaerobic organism activity in wastewater from septic tank sediments. This activity happens because of the process of microbial metabolism, in which microbes require high carbon as an energy source to grow and adapt. These microorganisms can degrade organic material contained in wastewater optimally with an average COD removal rate of 18.11 mg/l per day, 20.67 mg/l per day, 21 mg/l per day, 24.33 mg/l per day, and 29 mg/l per day for each reactor.

In Figure 6, it can be seen that the COD concentration in the five reactors has decreased. This result indicates the active bacterial activity. However, the reduction in COD concentration in each reactor has different efficiency and reduction rates. In the control reactor, where there is no increase in the concentration of amoxicillin, the average rate of COD concentration decrease for 15 days is 18.11 mg/l/day, and the maximum rate of reduction of COD concentration is 40.56 mg/l/day which occurs at day 6. Besides, the removal efficiency of COD levels achieved was 89.66% with COD levels set aside for 15 days of reactor operation at 271.67 mg/l.

In the AMX 5 reactor, where an increase in amoxicillin concentration of 5 mg was obtained, the average COD concentration decrease rate for 15 days was 20.67 mg/l/day, and the rate of reduction of the maximum COD concentration was 41.67 mg/l/day which occurred on day 6. Besides, the removal efficiency of COD levels achieved was 88.24% with COD levels set aside for 15 days of reactor operation amounting to 310 mg/l. In the AMX 10 reactor, where an increase in amoxicillin concentration of 10 mg is obtained, the average rate of COD concentration decrease for 15 days is 21 mg/l/day, and the maximum rate of decline in COD concentration is 34.45 mg/l/day that occurs on day 3. Besides, the removal efficiency of COD levels achieved was 85.21% with COD levels set aside for 15 days of reactor operation at 315 mg/l. In the AMX 40 reactor, where an increase in amoxicillin concentration of 40 mg is obtained, the average COD concentration decrease rate for 15 days is 24.33 mg/l/day, and the maximum rate of COD concentration decrease is 51.11 mg/l/day which occurred on day 3. Besides, the removal efficiency of COD levels achieved was 78.27% with COD levels set aside for 15 days of reactor operation of 365 mg/l.

In the AMX 80 reactor, where an increase in amoxicillin concentration of 80 mg is obtained, the average COD concentration decrease rate for 15 days is 29 mg/l/day, and the maximum rate of decrease in COD concentration is 77.79 mg/l/day that occurs on day 3. Besides, the removal efficiency of COD levels achieved was 77.72% with COD levels set aside for 15 days of reactor operation at 435 mg/l.

Based on Table 4.10, the best COD reduction rate is found in the AMX 80 reactor, followed by the AMX 40, AMX 10, AMX 5 reactors and finally the Control reactor. The maximum removal rate between the control reactor and AMX 5 is different from the AMX 10, AMX 40 and AMX 80 reactors. This condition can be caused by differences in the speed of microbial growth in the control reactor and AMX 5 with the AMX 10, AMX 40 and AMX 80 reactors.

The maximum removal rate at the control reactor and AMX 5 was reached on day 6, while at the AMX 10, AMX 40 and AMX 80 reactors the maximum removal rate was reached faster on day 3. This phenomenon indicates that the AMX 10, AMX 40 and AMX reactors 80 enters the stationary phase more quickly than the control reactor and AMX 5. More substrates will accelerate the bacterial growth phase so that the stationary phase occurs faster, and the COD removal will be higher. Conversely, on fewer substrates, the bacterial growth phase will be slower, and the stationary phase reached longer so that the COD removal is lower than the higher organic load.

The difference in removal efficiency and the rate of decrease in COD concentration occur because the number of substrates in each reactor is different, with the highest AMX 80 reactor compared to other reactors. Based on the Monod equation that is \( \mu = \mu_{\text{max}} \cdot S / (K_s + S) \), the substrate (S) is a function of the growth rate (\( \mu \)). A small amount of substrate can cause limiting of substrate or limiting substrate because the substrate which slightly limits the rate of waste degradation. The low substrate causes the growth speed (\( \mu \)) is directly proportional to the substrate (S) so that the growth speed becomes slower and steady
conditions are achieved longer. More substrates will accelerate the bacterial growth phase so that the stationary phase occurs more quickly, and the COD removal is higher. Conversely fewer substrate, the bacterial growth phase will be slower and stationary phase is achieved so that the COD removal is lower than the higher organic load.

4. Conclusion
Based on the results and discussions that have been carried out, it can be concluded that amoxicillin concentration variation affects the removal of ammonium quite noticeably difference in each reactor. Ammonium removal efficiency in control reactors, AMX 5, AMX 10, AMX 40, and AMX 80 were 55.08%; 53.30%; 48.13%; 40.36%; and 32.16%. The effect of variations in the concentration of amoxicillin on COD removal is relatively small. In the Control reactor, AMX 5, AMX 10, AMX 40 and AMX 80 were able to set aside COD in a row of 271.67 mg. COD / l, 310 mg. COD / l, 315 mg. COD / l, 365 mg. COD / l and 435 mg. COD/l.

References
[1] Thomais V and Athanasios V 2013 Pharmaceuticals and Personal Care Products as Contaminants in the Aquatic Environment A Category of Organic Wastewater Pollutants with Special Characteristics Pharmakeftiki 25 p 16-23
[2] L J Githinji, M K Musey and R O Ankumah 2001 Evaluation of The Fate of Ciprofloxacin and Amoxicillin in Domestic Wastewater Water and Soil Pollut. 219 p 191-201
[3] G Marliere, M Ferraz and J Q dos Santos 2000 Antibiotic Consumption Patterns and Drug Leftovers in 6000 Brazilian Households Adv. Ther. 17 p 32-44
[4] Z Chen, H Wang, Z Chen, N Ren, A Wang, Y Shi and X Li 2011 Performance and Model of A Full-Scale Up-Flow Anaerobic Sludge Blanket (UASB) to Treat the Pharmaceutical Wastewater Containing 6-APA and Amoxicillin J. Hazard. Mater. 185 p 905-913
[5] Zhang Z Z, Zhang Q Q, Guo Q, Chen Q Q, Jiang X Y and Jin R C 2015 Anaerobic ammonium-oxidizing bacteria gain antibiotic resistance during long-term acclimatization Bioresource Technology 192 p 756–764
[6] Goh C P 2008 Comparative Study Of Sequencing Batch Biofilm Reactor and Sequencing Batch Reactor In p-Nitrophenol Removal (Thesis: University Sains Malaysia)
[7] Tri S, et al 2013 Anaerobic Fixed Bed Reaktor Untuk Menurunkan COD, Fosfat (PO4) dan Detergen (Las) (Skripsi: Program Studi Teknik Lingkungan Fakultas Teknik Sipil dan Perencanaan UPN)
[8] S Chelliapan, T Wilby and P J Sallis 2006 Performance of An Up-Flow Anaerobic Stage Reactor (UASR) in the Treatment of Pharmaceutical Wastewater Containing Macrolide Antibiotics Water Res. 40 p 507-516
[9] Rajagopal R, Masse D I and Singh G 2013 A critical Review on Inhibition of Anaerobic Digestion Process by Excess Ammonia Bioresource Technology 143 p 632-641
[10] S Florence, Halimatuddahildana and Amir H 2018 Pengolahan Limbah Cair Tahu Menggunakan Bioreaktor Anaerob Satu Tahap dan Dua Tahap Secara Batch (Skripsi: Program Studi Teknik Lingkungan Fakultas Teknik Universitas Sumatera Utara)
[11] Chaerunisah and R N Sopiah 2006 Laju Degradasi Surfactan Linear Alkil Benzena Sulfonat (LAS) pada Limbah Detergen secara Anaerob pada Reaktor Lekat Diam Biromedia Sarang Tawon Jurnal Teknologi Lingkungan 7 (3): 243-250
[12] Ahmad M, Vithanage M, Kim K, Cho J-S, Lee Y H, Joo Y K and Ok Y S 2014 Inhibitory Effect of Veterinary Antibiotics on Denitrification in Groundwater: A Microcosm Approach The Scientific World Journal p 1–7
[13] Fu L, Huang T, Wang X, Su L, Li C and Zhao Y 2017 Toxicity of 13 Different Antibiotics Towards Freshwater Green Algae, Pseudokircheriella subcapitata and their Modes of Action Chemosphere 168 p 217-222

[14] Grady L C P, Daigger G T and Lim H C 1980 Biological Wastewater Treatment 2nd Edition (Marcel Dekker Inc)