Rapid start-up of expanded granular sludge bed (EGSB) reactor using granulated anaerobic bacteria in pharmaceutical wastewater treatment: pilot scale

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Abstract. The start-up process is one of the most critical processes in wastewater treatment. The performance of a biological agent (bacteria) and/or the system can be predicted when the start-up process is carried out. The purpose of the start-up process is an adaptation of biological agent, and also as a granulation process for bacteria to form granule. The aim of this research was to study the effect of granular sludge in enhancing start-up time. The research was done using a 7 m tall stainless-steel reactor with 1.2 m x 1.2 m cubic shape-based and seeded with granulated anaerobic bacteria. The Reactor was operated continuously with configuration as follows: 4 h of HRT with up-flow velocity ($V_{up}$) of 1.75 m/h and recirculation ratio of 1:2. The main parameters observed were pH, alkalinity, and COD. The result showed that the effluent pH increased from 5.1 (inlet) to 6.9 on the first day of start-up and achieved pH 8.2 on the 4th day, the alkalinity also showed an increase from 219.05 CaCO$_3$ mg/l to 470.3 CaCO$_3$ mg/l. and the reduction of COD was 41.1% on the 4th day. It can be concluded that seeding the reactor with granulated anaerobic bacteria had successfully accelerated the start-up process.

1. Introduction
Pharmaceutical industries are some of the most important industries to support human life, yet the activity of the industry would give a negative impact on the environment and even more on human health if the wastewater is not treated well. Most pharmaceutical industries producing antibiotics will have antibiotics in their wastewater. The presence of antibiotics in wastewater will disrupt the biological wastewater treatment and lead to inefficient wastewater treatment processes. While the chemical treatment is not suitable for treating pharmaceuticals wastewater [1], there are numbers of technologies that could be used to treating pharmaceuticals wastewater such as photocatalytic [2], mixed aerobic-physical technology (membrane filtration/reverse osmosis) [3], ozone and peroxide oxidation [4]. Recently, anaerobic technologies have started to be a promising option for treating pharmaceuticals wastewater due to their high degradation efficiency and lower operational cost.

Anaerobic digestion for treating industrial wastewater has been developed for more than 30 years, Pol and Lettinga [5] have proposed anaerobic digestion as a promising technology for treating industrial wastewater. By now, most industries have been using or at least trying to use this technology for treating their wastewater. The reason that industries prefer to use this technology is due to high effectiveness, minimum maintenance, and operational cost, no chemical needed, and also less to none dangerous sludge production compared to other conventional treatments such as aerobic and physical-chemistry treatments [6]. A lot of research about anaerobic technology has been done to achieve
higher treatment efficiency and a more versatile system. Expanded granular sludge bed (EGSB) is the latest anaerobic technology that has been introduced to industries, which is an improvement from the famous up-flow anaerobic sludge blanket (UASB) technology. EGSB was first introduced in the mid-1990s, the major difference between EGSB and UASB is higher up-flow velocity and it can handle a wider variety of the wastewater characteristic such as low to high strength organic loading rate (OLR) or even wastewater that contains recalcitrant substances [7].

In the application of advanced anaerobic treatment such as UASB and EGSB, granules formation is extremely important to ensure the effectiveness of the anaerobic degradation process. Anaerobic granules are important because they do not only support active biofilm but also provide settleability to allow maximum contact between anaerobic microorganism and wastewater. Even though the granulation process is essential to UASB and EGSB system, yet it is one of the major concerns in the start-up process. Anaerobic granulation is known as having a long startup process, it depends on the source of bacterial seed, desired operational condition, and wastewater characteristic. The granulation time varies from 30 [8] to 120 days [9] but is mostly done for more than 30 days.

The speed of the granulation process depends on many aspects such as bacterial morphology, substrate similarity, surface charge compatibility, and ability to produce specific polymers to form flock or granules [7], to achieve functional granules it would require a variety of naturally symbiotic microorganism consortia and it would be a time-consuming process. Previous works done by Liu [10] show a significant difference in the number of the microbial community in seed sludge and granulated sludge after 58 days granulation process. In general, the time required for the startup process is not only affected by the sludge source but also by feed wastewater properties and sludge retention time (SRT) [11]. In addition to microorganism dynamic during the startup process, the conventional startup method uses gradual dilution wastewater as feed, starting from 10% to 90%, this method provides a high chance of startup process but takes more time. During the startup and operational processes, pH, alkalinity, and COD reduction are used as main parameters to control and monitor the stability or health of the anaerobic system.

However, the method to speed up the start-up process remains unknown. Thus this work is aimed at figuring out the use of granulated anaerobic sludge in enhancing the start-up process time in pilot-scale EGSB reactor for treating pharmaceuticals wastewater.

2. Materials and methods

2.1. Materials

2.1.1. Anaerobic bacteria inocula. Inocula used in this research was granulated anaerobic sludge obtained from The Research and technology laboratory, Center of Industrial Pollution Prevention Technology. The granular sludge has 3-7 mm in diameter.

![Figure 1. Anaerobic granules used as inocula.](image-url)
2.1.2. Reactor configuration. The EGSB reactor comprised a cubic shape column of 7 m in total height (6.5 m working height) and 1.2 m x 1.2 m cubic shape base made from 4 mm thick stainless steel 306L with a total working volume of 9.36 m$^3$. Inlet and circulation flow were supplied by a digital pump (DAB water technology), nutrition and phosphate buffer was supplemented using a dosing pump.

![Figure 2. 3D modelling of EGSB reactor units, (a) Wastewater storage tank; (b) Equalization tank; (c) EGSB reactor; (d) Circulation and settling tank; (e) Outlet, and schematic diagrams of reactors flow, (1) Inlet pipe; (2) Settled Granular sludge; (3) Baffle separator/gas capture; (4) gas pipe; (5) Splattered granular sludge; (6) Processed wastewater; (7) Separator/settled baffle; (8) Reactor outlet; (9) Circulation pipe.](image)

2.1.3. Nutrient. To maintain sludge performance and to prevent shock loading during startup process, supplementary nutrition was required. 5 litres of nutrient used in this research was modified methanogenium media (DSMZ 141) consisting of (g/l) : KCl (Merck) 0.34; MgCl$_2$.6H$_2$O (Merck) 4; MgSO$_4$.7H$_2$O (Merck) 3.45; NH$_4$Cl (Merck) 0.25; CaCl$_2$.2H$_2$O (Merck) 0.14; K$_2$HPO$_4$ (Merck) 0.14; NaCl (Merck) 18; Yeast extract (Oxoid) 2; NaHCO$_3$ (Merck) 5, and 2 litres of stock solution of trace elements consisting of (g/l) : MgSO$_4$.7H$_2$O (Merck) 1.5; MnSO$_4$.H$_2$O (merck) 3; NaCl (Merck) 0.5; FeSO$_4$.7H$_2$O (Merck) 1; CoSO$_4$.7H$_2$O (Merck) 0.18; CaCl$_2$.2H$_2$O (Merck) 0.1; ZnSO$_4$.7H$_2$O (Merck) 0.18; CuSO$_4$.5H$_2$O (Merck) 0.01; H$_3$BO$_3$ (Merck) 0.01; NaMoO$_4$.2H$_2$O (Merck) 0.01; NiCl .6H$_2$O (Merck) 0.03. Additional 3 litres stock buffer phosphate containing (g/l) NaHCO$_3$ 3.6 and KH$_2$PO$_4$ 6.8 were also used to help maintain the system pH. Before used in the experiment, all of the nutrients above were stored at 4°C refrigerator to prevent decaying or contamination which could lead to the decrease in its quality.

2.2. Methods

2.2.1 Granular sludge seeding. The volume of granular sludge seeded into the reactor was about 28.5% of the total reactor volume, this was the recommended amount of anaerobic sludge inocula [7]. Inoculated sludge was then diluted with tap water until the reactor was filled. Inoculated sludge was then left in the reactor for about 24 hours to settle. On the day after, tap water was added again until the water flowing through the reactor outlet, this was done to wash out debris and other unwanted materials brought from the sludge. Before the startup experiment, nutrition was supplied using a dosing pump to maintain the sludge growth during the startup process.
2.2.2 Wastewater source and experimental configuration. The raw pharmaceutical wastewater was collected from the industry wastewater tank and then pumped into the experiment wastewater storage tank. The startup process was carried out by using diluted pharmaceuticals industry wastewater (50% wastewater: 50% tap water), on the start-up process wastewater maximum loading rate must not exceed 50% [7]. Dilution was carried out in an equalization tank and stirred using an overhead mixer. Experimental configuration was done by diluting wastewater and tap water in an equalization tank, then the influent was pumped into the reactor through an inlet pipe in the base of the reactor, thus make the granular sludge on the base pushed vertically along with wastewater inlet and circulated wastewater. Influent and circulation pump were configured to achieve a vertical up-flow speed (Vup) 1.75 m/hr with inlet: circulation ratio was 1:2 and HRT was kept at 4 hours throughout the startup duration. The reactor was operated continuously for 4 days. Before the experiment began, 5 liters of nutrient was diluted until the final volume was 20 liters, 2 liters of trace elements were diluted until the final volume was 10 liters and 3 liters of buffer phosphate were diluted until 6 liters, all of the materials were then stored in a separate container before injected to the reactor using dosing pump through the inlet channel. Before the experimental startup process, half of the supplementary nutrition was gradually supplemented into the reactor and then recirculated for 24 hours, this was done to maximize the contact between nutrition and anaerobic granules. At the initial startup process, diluted wastewater and supplementary nutrition were pumped into the reactor and the startup process began. Inlet and outlet samples were collected using a clean plastic bottle sample every 4 hours and then the samples were brought to the laboratory to analyze their pH, alkalinity, and COD content.

2.2.3 Analysis. Chemical parameters bicarbonate alkalinity and COD were measured according to APHA 2320 and APHA 5220 D respectively while pH was measured using Krisbow KW06-744 pH and temperature tester according to SNI 6869.11:2009. The data from the tested parameters were then collected and evaluated as per day result, this was due to the characteristic of inlet wastewater that would change every 24 hours depends on the industrial production process.

3. Results and discussion
3.1 pH changes during startup
The acidic condition value that varied from 5.1 to 5.9 (figure 3) in the inlet occurred due to accidental fermentation of sugar and starch content in the wastewater during storage in the industrial wastewater tank and also the mixture of wastewater from antibiotic production facilities which produced very acidic wastewater could lead to the inlet pH becomes acidic[12]. A high level of sugar and starch content in pharmaceuticals wastewater might occur due to the syrup and capsule or tablet production process. The lowest outlet pH value occurred on the first day of the experiment which was 6.9, this happened because the anaerobic granules were still in the adaptation process to the wastewater. On the other hand, on the first day, the circulation of the effluent did not occur yet meanwhile, the main aspect of the EGSB reactor was circulation/recirculation. Circulation was critical to prevent pH drop in the anaerobic system [13]. The effluent pH increased gradually day by day, from 7.3 on the second day to 8.2 on the third and fourth days. Thus gradual increment happened due to the circulation that started and the granules that started to adapt to the system condition. Maintaining a pH above 6.6 in the anaerobic system is very important since the success of the anaerobic system relies on complete degradation processes from fermentation-acidogenesis-acetogenesis and methanogenesis. However, the drop in pH in the system would inhibit methanogens bacteria to grow and utilize acetate to produce methane [14]. Alkalinity in the system would also affect the system pH. Buffer phosphate supplementation also helped the system to maintain pH on the reactor. The works done by Kispfergher [15] show that the fermentation process without bicarbonate supplementation could lead to a pH drop from 6.5 to 5.5.
3.2 Alkalinity

Alkalinity is an important parameter to support methanogenesis in the anaerobic system. Inlet alkalinity varied from 219.05 mg/l to 277.63 mg/l (figure 4), the fluctuation of inlet characteristic happened due to the different quantity or different products produced by the industry. Overall, alkalinity in our EGSB reactor effluent increased from 470.3 on the first day to 519.1 and 499.83 mg/l on the third and fourth days respectively. Theoretically, the proposed amount of alkalinity to enhance bio methanation in the anaerobic process is about 2500 mg/l, it is needed to supply the system for the necessary co-substrate and to prevent pH drop below 6.5 [16]. The alkalinity of CaCO3 is also important to support the anaerobic system if there is excess production of volatile acids and other acids from the fermentation process and acidogenesis or acetogenesis. CaCO₃ alkalinity was not only provided from the addition of buffer phosphate but also the regeneration of VFA (volatile fatty acid) alkalinity during acetate to methane conversion [17].

Figure 3. pH fluctuation on inlet and outlet during the experiment.

Figure 4. Alkalinity during experimental setup compared to outlet pH.
Figure 4 shows that during the startup process, pH and alkalinity started to elevate on the second day. This phenomenon proves that the supplementary nutrition and phosphate buffer had a significant role in preventing bacterial community to collapse when introduced to wastewater. On the third day of the experiment, the trend of alkalinity outlet was relatively stagnant even though inlet alkalinity was fluctuating, and buffer phosphate supplementation stopped. This showed that the anaerobic system configuration was capable to maintain system stability during the startup process.

3.3 **COD reduction during startup**

COD is an essential chemical parameter in the wastewater treatment plant as the success of wastewater treatment is determined by COD reduction. Figure 5 shows the diluted wastewater used in this research was relatively stable varied from 1198.68 mg/l at the lowest and 1284.85 at the highest. The lowest COD reduction percentage was on the first day of the startup process which was 33.75% due to system shock loading. Shock loading on the first day of the startup is already understood by many researchers, thus made the percentage of COD reduction was low. Nutrition and phosphate buffer supplementation on the first day of startup helped the system to deal with shock loading. The data (figure 5) shows that after surpassing the first day, anaerobic granules recovered thus lead to the increasing of COD reduction percentage to 38.34%, 38.5%, and 40.14% in the second, third, and fourth days respectively. Thus, it is proven that with the current COD loading rate, the EGSB system supplemented with anaerobic granular sludge could easily adapt to the wastewater characteristic and successfully surpass the startup process in a short time.

![Figure 5. COD reduction during experimental startup.](image)

COD reduction in the anaerobic system or start-up period was determined by the reactor setup such as HRT, COD inlet, OLR, and wastewater: diluter mixtures. Another start-up configuration done by Liu [10] shows a 70% of soluble COD reduction in 21 days startup using the EGSB reactor. However, the result was obtained by using longer retention time (12 hours) lower \( V_{up} \) (0.1 m/h) and lower OLR, and longer startup time. This setup could be useful when the source of the seed was not a granulated sludge and the targeted wastewater was low-strength wastewater. Another study done by Zhang [18] found an accelerated EGSB reactor start-up that was done in 34 days using stored annamox (anaerobic ammonium oxidation bacteria) sludge for treating wastewater with high nitrogen content. The other configuration of the anaerobic startup process using the UASB reactor for treating the pharmaceutical wastewater has successfully gained 80-85% COD reduction during 120 days of startup time [19].
4. Conclusion
The result of this work concluded that the start-up process using the EGSB system using directly 50:50 wastewater to diluter (tap water) ratio and supplemented with granulated sludge has successfully accelerated the start-up time to less than a week. Supplementing proper nutrition, trace elements, and buffer phosphate at the initial startup process has been proved to prevent seed to collapse due to shock loading stress.

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