Effect of feeding strategies on anaerobic digestion of fish waste

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Fish and other seafood consumption in the world showed a massive increase as it contains various essential nutrients contributing to a healthy diet. UN Food and Agricultural Organization (FAO) stated that worldwide fish and seafood consumption increased from 9.0.1 kg/capita in 1961 to 20.2 kg/capita in 2015 (Cappola *et al.*, 2019). However, many fish parts, including the head, fins, tail, backbone, scale, and viscera, generated a large amount of fish waste during fish processing. Those parts are less valuable economically and account for approximately 40 - 60% of the fish weight. Considering bioeconomy of fish waste, some portion of it is further processed for fish food or other by-products such as extracting collagen, pectin, and chitin. Meanwhile, another considerable portion of it remains as waste that must be treated to avoid serious environmental problems.

Compared to other approaches that can degrade organic compounds, anaerobic digestion (AD) is commonly implemented because of its benefits. Fish waste contains a high concentration of biodegradable organics, mainly protein, making it a suitable substrate for AD. However, an accumulated intermediate generated from protein degradation, which is ammonia, causes system instability and even process failure (Solli *et al.*, 2018). Ammonia inhibition is one of common causes of system instability that leads to the inefficiency of AD for energy production (Liu *et al.*, 2019). As ammonia accumulation in AD system is affected by feeding strategy, therefore this study was conducted to investigate the effect of feeding strategy on the performance of anaerobic digesters fed with fish waste and how bacterial community responds it.

The AD systems were operated in sequencing batch reactor (SBR) mode with three different reaction time (7, 9 and, 13 days) and three different feeding strategies (exponential, sigmoid, and stepwise increase of substrate concentration). The reactors fed with the same initial and final substrate concentration, 3 and 11 g-COD/L respectively. Substrate concentration for exponential shape increased was 3.0, 3.2, 4.0, 5.5. 8.0, and 11.0 g-COD/L. Substrate concentration for sigmoid shape increased was 3.0, 3.5, 5.5. 8.5, 10.5, and 11.0 g-COD/L. Substrate concentration for stepwise increased was 3.0, 5.0, 7.0, 9.0, and 11.0 g-COD/L. One course of reaction time is called one cycle. The experiment was conducted in 9 cycles. The first 6 cycles correspond to increasing substrate concentration period and the remaining 3 cycles correspond to confirmation period where the reactors fed with 11 g-COD/L of fish waste. Every treatment was conducted in duplicate reactors, and physicochemical analyses were performed every 2 or 3 days.

Based on cumulative biogas production (CBP), sigmoid strategy performed the best compared to other feeding strategies both on 9 d and 13 d digestion times. Cumulative methane production (CMP) results were in line with CBP. On 9 d and 13 d digestion time, sigmoid strategy produced highest cumulative methane compared to other feeding strategies. On the other hand, SCOD of stepwise feeding with 9 d digestion time and SCOD of exponential feeding with 13 d digestion time generally showed increasing trend until end of confirmation period. Supporting SCOD result, VFAs accumulation on those strategies was also observed. Therefore, it is expected to cause a decrease in methanogenic activities which explains the low CMP. Physico-chemical analyses suggested that sigmoid feeding and exponential feeding were the best and the worst performed feeding strategies for AD of fish waste, respectively.

Next generation sequencing was performed on initial and end point of sigmoid and exponentials to assess the effect of feeding strategies on microbial network (Fig. 1). The dashed line that connects the samples with the bacterial genus (edge) indicates relative abundance and the edge thickness represents relative abundance. The thicker the edge, the higher the relative abundance is and vice versa. Microbial network analysis was performed using force-directed algorithm to position the bacterial genus that have high relative abundance to be closer to its corresponding samples. The result revealed that *Syntrophomonas* sp. and *Alkaliphilus* sp. were responsible for stable performance of sigmoid strategy as the two genera only exist on endpoint of sigmoid feeding. The detailed results will be presented on the conference. The result of this study is beneficial for process control AD system treating fish waste as substrate.



Fig. 1. Microbial network analysis of initial and end points of sigmoid and exponential feeding strategies

References

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Acknowledgement

This work was financially supported by Human Resource Program for Sustainable Environment in the 4th Industrial Revolution Society.