Original Article

Effect of Polymer on the Granular Biomass Activity, Size, and Morphology in the EGSB Reactor of the SWTP

Ghulam Mustafa^{1*}, Karen Jones¹, Cathy Parra¹, Paul Chappell¹, Allan Bate¹, Andrew Smith¹, Nicolas Daniel Spiccia², Felix Sharpe³

> *¹Opal Paper & Recycling Mill, Quality Department, Sydney, Australia ²Opal Research & Development Centre, Melbourne, Australia ³ University of Sydney, School of Chemical Engineering, Sydney, Australia*

> > *Corresponding Author : ghulam.mustafa@opalanz.com*

Received: 23 June 2024 Revised: 26 July 2024 Accepted: 12 August 2024 Published: 30 August 2024

Abstract - Different doses of polymer (Solutrix100) on the biomass activity showed that increasing concentration up to 20 ppm did not affect the microbial activity (0.93 g/g Vs.d) as compared to control (0.83 g/g Vs.d). Instead, an increase of 21% was found in biomass activity with 20 ppm dose. However, an 18% decrease in biomass activity was found at 30 ppm as compared to control. Two polymer doses, 20 and 30 ppm, were applied, and the biomass activity was compared with the control. Different levels of COD were tested, ranging between 4869 to 6673 ppm, through the mill wastewater to see the effect of the polymer on the different parameters. Solutrix100 dose of 20 ppm increased by 2% pH. However, this dose slightly increased VFA production (51%) compared to the respective control (46%). sCOD removal efficiency decreased due to the addition of polymer from 80 to 64 %. The reduction in Ca concentration was 11% at 20 ppm during the treatment. Visual observations of granules revealed that granules were black and shiny and did not affect the shiny surfaces due to the increasing Solutrix100 concentrations. The shape of the granules was round to oblong in the case of 20 ppm, and they were not shiny but oblong at 30 ppm treatments compared to the control. However, the granular diameter was reduced (40%) because of the Solutrix100 application as compared to the control, but it showed a positive effect on biomass activity at a lower dose only. Gelatinous material around the granules was observed due to the 30 ppm dose. This was fibrous filamentous and different from the inside of the granule porous material. This aspect requires further study to explore.

Keywords - Biomass, Solutrix100, BAT, COD, Volatile fatty acids, Gelatinous material.

1. Introduction

Anaerobic digestion using Extended Granular Sludge Bed (EGSB) reactors is a very well-established and efficient process for the treatment of contaminated wastewater. This process decomposes organic matter, including acetic acid, to generate biogas. When used to produce electricity, it reduces greenhouse gas emissions due to the consumption of methane. Furthermore, the anaerobic treatment of wastewater is widely accepted and extensively used nowadays as a proven technology. The introduction of high-rate reactors proved to be successful in which biomass retention and liquid retention are uncoupled [9, 14]. The wastewater treatment process of combining anaerobic digestion and aerobic treatment also produces excess biomass sludge. The dewatered biomass sludge was found to be rich in mineral nutrients such as nitrogen and phosphorus, particularly to improve the soil properties with excellent biofertilizer characteristics [11]. It is important to provide optimum growth conditions to granular microbes in the EGSB reactor of the Secondary Water Treatment Plant (SWTP) [15]. The two-stage EGSB reactor

enhances the acidogenic and methanogenic activities because of hydrolysis, fermentation, and acidification [17]. A variety of authors encouraged the use of this type of EGSB reactor all over the world around 20 years ago [4,12]. Furthermore, granular growth in the EGSB reactor and biomass activity may be improved by the addition of flocculating chemicals or polymers [16]. However, no literature is available to support the enhanced granulation or deterioration of granules due to the addition of polymers in the EGSB reactor or through the addition of aeration tank wastewater into the EGSB reactor to reduce Ca concentration. pH is one of the important factors for running an EGSB reactor successfully. In the field conditions, we maintained a pH of 7.0-7.2 to get maximum biomass yield, healthy microbial growth, and high acidogenic and methanogenic bacterial activities in addition to the other parameters, such as temperature. However, efficient biogas production in an anaerobic digestion reactor was found in the range of 6.8-7.4 [12]. Volatile Fatty Acid (VFA), low carbon chain compounds are produced due to the hydrolysis and acidogenesis of high carbon compounds, which further

undergo methanogenic decomposition by the microbial bacteria to produce methane. VFA yield in the form of acetic acid increased due to the addition of biofuel in a Sequence Batch Reactor (SBR) [17]. A Sequence Batch Reactor (SBR) experiment was conducted to see the VFA production and found that VFA yield increased by 43.8% compared to the original VFA content [10]. COD removal efficiency depends on the biomass activity. Therefore, few studies have been conducted elsewhere. For example, zeolite was used in an EGSB reactor to see the effect on chemical oxygen demand (COD) removal, granule formation, and size. The results showed COD removal of 89.1 %. Moreover, the positive effect of Zeolite addition was seen in the formation and size of the granules as compared to the control [13]. A positive correlation was found between divalent calcium and biomass granulation [1]. However, during an in-situ experiment at 37 ⁰C for 30 days [2] found that varying Ca concentrations, such as 150 to 1000 ppm, caused precipitation and had a negative effect on cell-to-cell communication in the EGSB reactor. We were encountering a problem with Ca scaling in our SWTP aeration tank.

The calcium scaling phenomenon was caused by the deposition of Ca from the EGSB effluent water-blocked pipes, which were deposed on the inner surfaces of the aeration tank. We spend extra money to perform Ca descaling after stopping the SWTP. This practice cost us money, too. Solutrix100 was used to mitigate this problem and minimize the impact of calcification on the aeration tank and associated pipes. The research literature on the direct effect of Solutrix100 on biomass activity is not available. Therefore, less existing literature on the current research findings was cited. Solutrix100, a pale-yellow straw-coloured liquid with approximately pH 8.0, acrylic, organic water-soluble polymer, was applied through the EGSB effluent pipeline coming in the aeration tank of Opal's SWTP at various concentrations to accelerate the descaling process. It enhances the removal of calcium as a result of the dissolution process. Polymer is absorbed on the Ca scale surfaces by poorly adhering to the metal surfaces, causing precipitation. These precipitated salts then form a fine suspension. Finally, Ca salts are removed from the aeration tank through the disposal of treated water [6]. Granular biomass is used to remove the pollutants from the paper mill wastewater through the biomass in the EGSB reactor of the SWTP.

As mentioned earlier, Solutrix100 is used to descale Ca deposition in the aeration tank of the SWTP. Sometimes, aeration tank wastewater, low in Ca, is added as a recirculation water in the EGSB reactor to dilute the Ca concentration. Calcium is introduced into the process due to its presence in the wastepaper feedstock. Calcium is used as a mineral filler and in printing inks on many types of paper and cardboard packaging. When the wastepaper is pulped as part of the recycling process, this calcium ends up dissolved in the process water. As a guideline, no literature and research

findings have been available so far. Therefore, the experiment was conducted (i) To find out the effect on the biomass activity of this commercial organic product added into the EGSB reactor through the aeration tank wastewater, (ii) To observe any change in the morphology and size of the biomass granules in the EGSB reactor. (iii) To find out the possibility of using recirculation aeration tank water to mitigate high Ca concentration in the EGSB reactor.

2. Materials and Methods

The granular biomass is kept in the EGSB reactor of the SWTP to treat the mill wastewater before further treatment, followed by disposal of the effluent into the sewer. The study was conducted for 13 weeks. Biomass samples were collected and analysed to examine the visual, physical, and chemical properties in the Opal Paper and Recycling Wet Chemistry Laboratory. Two Solutrix100 doses @ 20 and 30 ppm were used and compared with the control (no Solutrix100). A Eutech® pH meter equipped with combined electrodes was used to determine pH. The temperature was measured using an LCD digital thermometer.

Analytical reagents grade urea and phosphoric acid were used to compensate for the macro nutritional requirements during the biomass activity determinations. Temperature and pH were maintained between 36-37 °C and 6.80-7.00, respectively, during the experiment. The pH was maintained by using analytical reagent grade 0.1 N hydrochloric acid and sodium hydroxide. A homogeneous biomass sample of 200 mL from 1.0 M depth of the EGSB reactor and a paper mill effluent sample of 1800 mL was continuously stirred at 300 - 340 rpm using a Cole-Parmer magnetic stirrer [7] equipped with a hot plate. Biomass activity was measured using the following formula, and the apparatus was assembled in the Opal Wet Chemistry Laboratory, as shown in Figure 1, to simulate the EGSB reactor in-situ.

Fig. 1 Apparatus used for the determination of biomass activity of granular biomass

Biomass Activity (g/g Vs.d) = Volume of reactor (mL) / 1000 x (sCOD₁- sCOD₂) x 24 / ((t₂-t₁) x (Vs biomass (g) x (1000))

Parameters such as ammonium–N, orthophosphate, and sCOD were measured using a [5] DR 3900 spectrophotometer in Opal's Wet Chemistry Laboratory. All the samples were filtered through Whatman folded filter paper 597 [8]. NH3- Nitrogen and sCOD analysis was conducted using TNT 832 and TNT 822 vials, respectively [5]. Whereas Volatile Fatty Acids (VFA) and total alkalinity (TA) were analysed using vials TNT 872 and TNT 870, respectively. Orthophosphate (P) was analysed per HACH method 8178 using a spectrophotometer. The granular size, shape, and colour were also measured in the same laboratory. Gelatinous material around the granules was examined under the Scanning Electron Microscope (SEM) at Opal's Research & Development Centre, Melbourne [3].

3. Results and Discussion

Biomass activity is an indication of biomass health and performance in treating wastewater. It significantly increased due to the application of polymer @ 20 ppm (1.05 g/g Vs.d)) compared to control (0.83 g/g Vs.d). At a lower dose of 20 ppm, an increase of 21 % was found. However, at 30 ppm, an 18 % decrease in biomass activity (0.68 g/g Vs.d) was observed compared to the control. This showed that the polymer had a positive effect on the granular activity but showed a negative effect with the higher dose of 30 ppm (Figure 2). The application of 20 ppm of Solutrix100 reduced biomass granule size. The reduction in biomass granule size was 40% because of the application of polymer dose. The shape of biomass granules in the case of 20 ppm Solutrix100 treatment was comparatively oblong compared to control. However, biomass granules had bright and shiny surfaces before and after the treatment, as shown in Figure 3. A study conducted on the effect of the addition of another type of polymer revealed that the granular size was bigger and more compact than the control. However, these compacted granules, because of polymers, were not quantified [15].

Fig. 2 Effect of polymer on the granular biomass activity

Fig. 3 Granules size, shape, and morphology (a) before (control) (b) after the 20 ppm solutrix100 application

Fig. 4 Granules size, shape, and morphology (a) before (control) (b) after the 30 ppm solutrix100 application

Granule size and shape reduced after 30 ppm Solutrix100 application. Granules were found to be smaller in size, black, and oblong but not shiny. Meanwhile, in control, shiny, black, and round granules were observed, as shown in Figure 4. An efficient biogas production in an anaerobic digestion reactor was found in the pH range of 6.8-7.4 [12]. pH plays an important role in facilitating biomass activity in the EGSB reactor and helps maintain the health of the granule biomass. Therefore, in our experiment, an efficient operation of the simulated EGSB reactor was achieved through pH maintenance in the range of 6.90 to 7.20. Finally, an experiment was conducted to see the effect of polymer on biomass activity and other parameters such as pH, granular size, VFA consumption, and COD removal, showed negligible pH variation $(0.3 - 3 \%)$ due to the application of different polymer doses as shown in Figure 5. VFA consumption increased with the increase of polymer dose from 20 to 30 ppm as compared to control by the microbes in the EGSB reactor. Volatile Fatty Acids were efficiently consumed or in other words, decomposed and were found 51 % at 20 ppm and 72 % at 30 ppm polymer doses, when compared to the respective controls (Figure 6). This showed a positive effect of polymer on the VFA decomposition.

Fig. 6 Effect of different polymer doses on the VFA in the EGSB reactor

During our experiment, COD removal decreased in the case of 20 ppm application of Solutrix100, and this decrease was 64 %, whereas a 78 % COD removal was found at a higher dose of polymer compared to control, as shown in Figure 7. Calcium is a part of the cell membrane and can show an impact on the change in polymer concentration, as depicted in Figure 8. Calcium plays a pivotal role in the development of granular biomass in the EGSB reactor. Therefore, a positive correlation was found between divalent calcium and biomass granulation [2]. In our experiment, 11 % Ca concentration was reduced from the start to end of each dose, including control, and it may be an inhibiting factor for the granulation formation process in the EGSB reactor. Consequently, granule size reduction was seen with the use of polymer compared to the control (Figure 3). As Solutrix100 is a descaling/decalcifying substance, therefore, we have observed a reduction of calcium concentration with the polymer application in the EGSB reactor under the laboratory environment. Similarly, the effect of Ca on the granule activity in the EGSB reactor was also discussed elsewhere [2]. However, semi-quantitative elemental analysis of the biomass after the 30 ppm Solutrix100 application using Energy Dispersive Spectroscopy revealed 33 % more Ca in the biomass than in the biomass filtrate. SEM analysis gave us a clear picture of calcification around the granules and caused a 40 % reduction in granular size (Figures $3 \& 4$) in our study (Table 1). Calcification also reduced biomass activity by 18 %, as shown in Figure 2.

Table 1. Semi-quantitative elemental composition of biomass @ 30 ppm

polymer dose			
Description	$\%Ca$	%C	%F
Biomass	12.6		
Biomass filtrate		38.8	

Fig. 7 Change in COD concentrations because of Solutrix100 application

Fig. 8 Effect of varying doses of Solutrix100 on the Ca concentration

Furthermore, SEM revealed some chunks of Ca-rich material in the biomass (Figure 9). Application of Solutrix100 @ 30 ppm produced some gelatinous material around the granules, which triggered the reduction of biomass activity by up to 18 %, as shown in Figure 10. The production of gelatinous material was not seen in the case of 20 ppm Solutrix100 application.

29211-1-0001 2024/05/30 HM D8.4 x300 300 µm **Fig. 9 SEM image of biomass material showing Ca chunks**

Fig. 10 Gelatinous material formation on the granular biomass due to 30 ppm Solutrix100 application

The biomass granules surrounded by the gelatinous growth material were observed at Opal's Melbourne Research Centre using transmission light microscopy. Analysis of gelatinous material showed that biomass granules themselves have an amorphous texture with a dark appearance, and the gelatinous growth material was a collection of fibrous filamentous material in nature, as shown in Figure 11. Fibrous filaments may be oozed out of the granules because of polymer application. This requires further investigation in the future and is not the scope of this paper.

'Gelatinous growth'

Fig. 11 Transmission optical microscope images showing fibrous filaments of gelatinous growth material

4. Conclusion

- Biomass activity increased by 21 % and decreased by 18 % with 20 ppm and 30 ppm Solutrix100 applications, respectively.
- The COD removal efficiency was found to be 18% less with 20 ppm Solutrix100 as compared to the control.
- A negligible decrease in VFA concentration was observed with the use of polymer.
- Calcification (33%) was observed in the biomass, as proved by using SEM @ 30 ppm polymer treatment rather than filtrate.
- Granular biomass size decreased by 40% in the presence of polymer.
- Granules were porous, while gelatinous growth material around the granules was found in fibrous filaments in nature. Requires further study.
- It is recommended to use aeration tank wastewater, low in Ca and total dissolved solids, as a recirculation water to reduce the Ca concentration in the EGSB reactor up to 20 ppm Solutrix100 dose.

Acknowledgments

This work was conducted in the Wet Chemistry Laboratory, Opal Paper and Recycling Mill, Sydney, Australia. The author thanked and appreciated Opal Paper and Recycling Mill management for supplying the necessary resources, moral support, financial assistance, and equipment to conduct this research.

References

- [1] You-Wei Cui, Ji-Lin Huang, and Fakhri Alam, "Fast Granulation of Halophilic Activated Sludge Treating Low-Strength Organic Saline Wastewater Via the Addition of Divalent Cations," *Chemosphere*, vol. 264, 2021. [\[CrossRef\]](https://doi.org/10.1016/j.chemosphere.2020.128396) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=5041870894616596299&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/S0045653520325911?via%3Dihub)
- [2] Maria Cristina Gagliano et al., "Calcium Effect on Microbial Activity and Biomass Aggregation During Anaerobic Digestion at High Salinity," *New Biotechnology*, vol. 56, pp. 114-122, 2020. [\[CrossRef\]](https://doi.org/10.1016/j.nbt.2020.01.001) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=14111975619554507347&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/S1871678419301189?via%3Dihub)
- [3] Masako Nishimura, Kaori Ichikawa, and Masahiko Ajima, "Features and Applications of Hitachi Tabletop Microscope TM3030Plus," *Hitachi High-Tech Corporation*, vol. 7, pp. 71-77, 2016. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Features+and+applications+of+Hitachi+tabletop+microscope+TM3030Plus&btnG=) [\[Publisher Link\]](https://www.hitachi-hightech.com/global/en/sinews/technical_explanation/07071/)
- [4] Freda R. Hawkes, T. Donnelly, and G. K. Anderson, "Comparative Performance of Anaerobic Digesters Operating on Ice-Cream Wastewater," *Water Research*, vol. 29, no. 2, pp. 525-533, 1995. [\[CrossRef\]](https://doi.org/10.1016/0043-1354(94)00163-2) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=2188893284183769052&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/0043135494001632?via%3Dihub)
- [5] Manufactures Water Quality Testing and Analytical Instruments and Reagents, Hach, Australia, 2015. [Online]. Available: shttps://au.hach.com.
- [6] BASF-Australia, Basf.com, 2024. [Online]. Available: https://basf.com.au
- [7] Cole-Parmer, Coleparmer.com, 2024. [Online]. Available: https://www.coleparmer.com
- [8] [Online]. Available: https://www.whatman.com
- [9] G. Lettinga et al., "Use of The Upflow Sludge Blanket (USB) Reactor Concept for Biological Wastewater Treatment, Especially for Anaerobic Treatment," *Biotechnology and Bioengineering*, vol. 22, no. 4, pp. 699-734, 1980. [\[CrossRef\]](https://doi.org/10.1002/bit.260220402) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=5875650175400493795&hl=en&as_sdt=0,5) [\[Publisher](https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.260220402) [Link\]](https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.260220402)
- [10] Jing Xiang Lim, and Vel Murugan Vadivelu, "Enhanced Volatile Fatty Acid Production in Sequencing Batch Reactor: Microbial Population and Growth Kinetics Evaluation," *AIP Conference Proceedings*, vol. 2124, no. 1, pp. 1-15, 2019. [\[CrossRef\]](https://doi.org/10.1063/1.5117100) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=7160111298590682909&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://pubs.aip.org/aip/acp/article/2124/1/020040/667251/Enhanced-volatile-fatty-acid-production-in)
- [11] Daniela Lovarelli et al., "Agricultural Small Anaerobic Digestion Plants: Combining Economic and Environmental Assessment," *Biomass and Bioenergy*, vol. 128, 2019. [\[CrossRef\]](https://doi.org/10.1016/j.biombioe.2019.105302) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=4283974554391102466&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/S096195341930251X?via%3Dihub)
- [12] Duc Nguyen et al., "Chapter 31 Biogas Production by Anaerobic Digestion: Status and Perspectives," *Biomass, Biofuels, Biochem*i*cals*, pp. 763-778, 2019. [\[CrossRef\]](https://doi.org/10.1016/B978-0-12-816856-1.00031-2) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=3552184914991845994&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/B9780128168561000312?via%3Dihub)
- [13] Tania Pérez-Pérez et al., "Performance of EGSB Reactor Using Natural Zeolite as Support for Treatment of Synthetic Swine Wastewater," *Journal of Environmental Chemical Engineering*, vol. 9, no. 1, 2021. [\[CrossRef\]](https://doi.org/10.1016/j.jece.2020.104922) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=262629045402519032&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/S2213343720312719?via%3Dihub)
- [14] Arjen Rinzema, Herbert van Veen, and Gatze Lettinga, "Anaerobic Digestion of Triglyceride Emulsion in Expanded Granular Sludge Bed Reactors with Modified Sludge Separators," *Environmental Technology*, vol. 14, no. 5, pp. 423-432, 1993. [\[CrossRef\]](https://doi.org/10.1080/09593339309385310) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=16344815004236121930&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.tandfonline.com/doi/abs/10.1080/09593339309385310)
- [15] S Uyanik, P. J. Sallis, and G. K. Anderson, "The Effect of Polymer Addition on Granulation in An Anaerobic Baffled Reactor (ABR). Part I: Process Performance," *Water Research*, vol. 36, no. 4, pp. 933-943, 2002. [\[CrossRef\]](https://doi.org/10.1016/S0043-1354(01)00315-3) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=16155506481584864905&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/S0043135401003153?via%3Dihub)
- [16] Randall A. Wirtz, and Richard R. Dague, "Enhancement of Granulation and Start-Up in The Anaerobic Sequencing Batch Reactor," *Water Environment Research*, vol. 68, no. 5, pp. 883-892, 1996. [\[CrossRef\]](https://doi.org/10.2175/106143096X127893) [\[Google Scholar\]](https://scholar.google.com/scholar?cluster=16155506481584864905&hl=en&as_sdt=0,5) [\[Publisher Link\]](https://onlinelibrary.wiley.com/doi/10.2175/106143096X127893)
- [17] Tengyu Zhang, Endashaw Workie, and Jingxin Zhang, "Chapter 8 Bioreactors for Enhanced Anaerobic Digestion for Bioenergy and Biochemicals," *Biomass, Biofuels, Biochemicals*, pp. 159-178, 2022. [\[CrossRef\]](https://doi.org/10.1016/B978-0-323-90633-3.00009-2) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Bioreactors+for+enhanced+anaerobic+digestion+for+bioenergy+and+biochemicals&btnG=) [\[Publisher Link\]](https://www.sciencedirect.com/science/article/abs/pii/B9780323906333000092?via%3Dihub)