Adelaide University

Enhancing Nutrient Removal and Increasing Plant Capacity through Aerobic Granular Sludge

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Contents

Acknowledgements **3**

Executive Summary **4**

Introduction **6**

Background and project rationale 6

Phosphorous removal 8

Aerobic Granular Sludge 9

Aims and approach 11

Methodology **13**

Plant description 13

Operational adjustments to SBR 14

Process adjustments to SBR 14

SBR monitoring (chemical analysis) 15

Bench testing of mixed liquor samples 16

Molecular analysis (16s amplicon sequencing) 16

Results and Discussion **17**

System start-up 17

Long-term phosphate removal performance 18

Phosphate cycle profiling 18

Impact of process changes on SVI and MLSS 20

Nitrification capacity 24

Conclusions and future recommendations **30**

References 34

Enhancing Nutrient Removal and Increasing Plant Capacity through Aerobic Granular Sludge

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Executive Summary

Enhancing phosphorus removal is a future objective for the Mt Compass WWTP as it prepares to meet anticipated environmental discharge requirements associated with regional growth. This study investigated whether enhanced biological phosphorus removal (EBPR) could be achieved within the existing sequencing batch reactor (SBR) system through relatively minor operational modifications, providing a potentially cost-effective alternative to chemical phosphorus dosing.

The study assessed the efficacy of introducing an anaerobic feed phase, reduced settling time, and modified sludge wasting strategy to support EBPR and improve biomass settleability. Full-scale SBR performance was subsequently evaluated under the modified operating conditions to determine the capacity of the existing system to achieve biological phosphorus removal and to assess the resulting impacts on mixed liquor characteristics and sludge settleability.

Following the introduction of an anaerobic feed phase at the Mount Compass WWTP, substantial improvements in phosphorus removal and sludge settleability were observed. Improvements in PO_4–P removal were evident within the first 1–2 months of implementation, with reductions of approximately 50–100% compared with baseline conditions, under which little to no phosphorus removal was observed. Long-term monitoring demonstrated that the incorporation of anaerobic feeding, combined with progressive reductions in settling time (from 60 to 20 minutes), reduced mean effluent PO_4–P concentrations from 11.0 ± 0.94 mg P/L to 3.3 ± 3.4 mg P/L, representing an overall reduction of approximately 70%.

Monitoring of PO_4–P concentrations throughout the SBR cycle provided strong evidence that the observed phosphorus removal was driven by enhanced biological phosphorus removal. The characteristic pattern of phosphate release under anaerobic feeding conditions followed by phosphate uptake during the subsequent aeration phase was consistently observed, indicating sustained activity of phosphorus-accumulating organisms (PAOs) at full scale. Laboratory batch tests using mixed liquor suspended solids (MLSS) sampled from the reactor provided further confirmation, demonstrating the presence of a well-established and metabolically active PAO population when substrate loading and oxygen availability were not limiting.

The modified operating strategy also resulted in substantial improvements in sludge settleability. The introduction of anaerobic feeding, combined with stepwise reductions in SBR settling time, reduced the sludge volume index (SVI) from approximately 250–300 mL/g to 75–90 mL/g. Importantly, adequate MLSS concentrations of 3500-4000 mg/L were maintained despite the shorter settling times, demonstrating that the improved settling characteristics enabled reductions in settling time without significant biomass washout.

16S amplicon sequencing of DNA extracted from mixed liquor samples identified microbial communities that supported observations made at full-scale and in batch studies. Several functional groups relevant to reactor performance were identified, particularly organisms associated with

fermentation and biological phosphorus removal. Fermentative microorganisms represented a substantial component of the community, with selected taxa accounting for approximately 3.8–18.5% of the total relative abundance. A particularly strong signal was observed for PAOs. *Tetrasphaera* was the dominant identifiable PAO-associated taxa and progressively increased from <1.0% prior to the study to 2.8% three months after anaerobic feeding was initiated, reaching approximately 7% after 11 months. *Candidatus Accumulibacter* was also detected, but at considerably lower abundance. Identifiable ammonia-oxidising bacteria, represented less than 0.02% of the community, while nitrite-oxidising bacteria, were not identified at the genus level. Their lack of detection is consistent with the limited nitrification capacity measured in both the full-scale SBR and batch studies.

Outcomes from this study are expected to provide a cost-effective and readily implementable pathway for improving wastewater treatment performance within existing SBR infrastructure, particularly at plants that lack anoxic selectors, with the approach readily transferable to other SBR-based wastewater treatment plants. Although fully developed aerobic granular biomass was not observed, the minor operational modifications achieved two of the key treatment benefits targeted by aerobic granular sludge (AGS) processes. Specifically, the study demonstrated enhanced biological phosphorus removal, reducing reliance on chemical phosphorus dosing and associated chemical and operational costs, while improving biomass settleability and controlling sludge bulking through the selective retention of denser, faster-settling biomass.

Introduction

Background and project rationale

Mount Compass WWTP was upgraded at its current site in 2017 and consists of preliminary treatment via a mechanical screen, followed by a balance tank and a sequencing batch reactor (SBR) for biological treatment. Treated effluent subsequently passes through a lagoon and is disinfected using chlorination prior to reuse. The Mount Compass sewerage network is a full sewer system, and treated effluent is currently disposed of via land application on an area adjacent to the WWTP as a temporary arrangement while a long-term recycled water reuse option is being developed. The intended future use of the recycled water includes municipal irrigation, potential supply to local businesses, and internal operational uses.

The SBR was originally designed to provide treatment for biochemical oxygen demand (BOD_5) and suspended solids (SS) only, with the aeration capacity sized to meet these treatment requirements at maximum flows of approximately 225 kL/day. As nitrification was not a key design objective, the available aeration capacity is insufficient to consistently support nitrification, and dissolved oxygen (DO) concentrations within the reactor are heterogeneous throughout the reactor depth. The SBR operating cycle also does not include dedicated anaerobic or anoxic phases, and the original design does not incorporate an anoxic selector because there was no requirement for denitrification, given that nitrification was not an intended treatment objective.

The absence of an anoxic selector is likely to have contributed to the development and persistence of filamentous bacteria and the associated poor sludge settling characteristics. Anoxic phases and anoxic selectors can help control filamentous growth through several mechanisms by creating conditions that favour floc-forming bacteria over filamentous organisms (Chudoba et al., 1973; Wanner et al., 1987). At Mount Compass, the lack of this selective environment is likely to have favoured filamentous growth and contributed to foaming (**Figure 1**) and the historically poor settling performance of the SBR, with settlers ranging from 910-990 mL at 30 minutes.

During periods of excessive foaming, the current decant arrangement can result in foam and surface scum becoming trapped within the SBR. The absence of a floating decant arm or surface weir means that the surface layer is not effectively removed during the decant phase. Instead, the fixed decant off-take is positioned below the water surface, allowing surface foam and scum to remain within the reactor and accumulate over successive cycles. This can further compound foaming issues.



Figure 1. Filamentous bacteria foaming event captured within Mount Compass WWTP Sequencing Batch Reactor.

The plant was also not designed to provide phosphorus removal. Typical phosphate-P (PO_4-P) concentrations in the treated effluent are approximately 11 mg/L, with monitoring indicating little to no phosphorus removal through the SBR. While the plant is currently operating at approximately 65% of its design capacity, projected increases in wastewater flows and associated phosphorus loads are expected to result in phosphorus inputs exceeding the capacity of the receiving land and crops to assimilate phosphorus through plant uptake. Without implementation of specific phosphorus removal processes, continued increases in phosphorus loading may therefore result in phosphorus accumulation in the land receiving recycled water and potentially presents regulatory risks. This highlights the need to consider phosphorus management as part of any future upgrade or optimisation of the treatment process, particularly if land application of effluent is expanded.

Future options for phosphorus management may also include treatment strategies that improve sludge settleability and reduce filamentous bulking. Whether these objectives can be achieved concurrently through optimisation of the SBR process was a key focus of this study.

Phosphorous removal

Phosphate removal at wastewater treatment plants is generally achieved through one of two approaches: chemical precipitation or biological phosphorus removal.

Chemical phosphorus removal is typically achieved by dosing a metal salt, most commonly ferric chloride, ferric sulfate or aluminium-based coagulants such as alum, into the wastewater or activated sludge process (Yeoman et al., 1988). The metal ions react with soluble phosphate to form insoluble metal–phosphate precipitates, which are subsequently captured within the sludge and removed through sludge wasting (Yeoman et al., 1988). Chemical dosing infrastructure generally requires chemical storage tanks, dosing pumps, bunding and safety systems, flow-paced dosing controls, and appropriate mixing points to ensure effective contact between the coagulant and wastewater. Additional sludge handling capacity may also be required because chemical precipitation increases sludge production. The main advantage of chemical phosphorus removal is that it is relatively simple, reliable and readily controllable, and can achieve substantial phosphorus reductions without major changes to the existing biological process. However, chemical dosing increases operating costs associated with chemical purchase, storage and dosing, produces additional sludge requiring disposal, and may require pH or alkalinity management. Overdosing can also adversely affect treatment performance and sludge characteristics. For Mount Compass, chemical phosphorus removal could therefore provide a relatively straightforward pathway for reducing phosphorus concentrations, although the additional infrastructure, ongoing chemical requirements and increased sludge production would need to be considered against biological phosphorus removal and broader process optimisation options.

Biological phosphorus removal in wastewater treatment plants relies on the activity of phosphorus-accumulating organisms (PAOs), which are specialised bacteria capable of storing phosphorus within their cells at levels much higher than required for normal growth. Effective phosphorus removal requires alternating anaerobic and aerobic conditions as outlined in **Figure 2**. During an anaerobic feed phase, readily biodegradable organic matter is converted by fermentation processes into volatile fatty acids (VFAs), which are the preferred carbon source for PAOs (Bassin et al., 2012a; Beun et al., 1999; De Kreuk et al., 2005). In the absence of oxygen, PAOs take up VFAs such as acetate and store them as intracellular carbon reserves as polyhydroxyalkanoates (PHAs) (Jeon and Park, 2000; Randall and Liu, 2002). The energy needed for this uptake is obtained by breaking down stored polyphosphate, resulting in the release of phosphate into the wastewater.

When the biomass is subsequently exposed to aerobic conditions, PAOs oxidise the stored PHAs to obtain energy for growth and for replenishing their intracellular polyphosphate stores. During this aerobic phase, they take up phosphate from the wastewater in amounts greater than that previously released, thereby achieving a net removal of phosphorus. The phosphorus-rich biomass is then removed from the system through sludge wasting, which removes phosphorus from the treatment process. This cyclic metabolism of phosphorus release under anaerobic conditions and enhanced

phosphorus uptake under aerobic conditions forms the basis of enhanced biological phosphorus removal (EBPR). In practice, biological phosphorus removal in an SBR can be achieved through the use of Aerobic Granular Sludge.

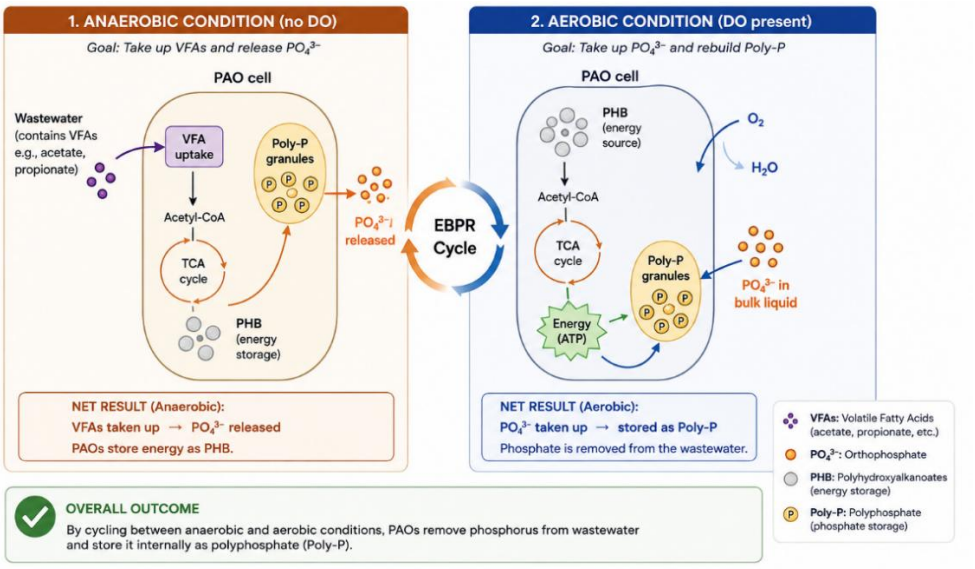


Figure 2. Biological phosphorus removal mechanism: alternating anaerobic and aerobic conditions drive phosphorus uptake and storage by polyphosphate-accumulating organisms (PAOs). AI generated using ChatGPT.

Aerobic Granular Sludge

The use of Aerobic Granular Sludge (AGS) in wastewater treatment is one means of developing a system capable of achieving biological phosphorus removal. AGS is a rapidly emerging secondary wastewater treatment technology that utilises rapidly settling microbial granules in place of the floc-based biomass used in conventional activated sludge systems. Selection for AGS formation is commonly achieved using SBRs. Two key conditions are typically required for AGS establishment, as outlined in **Figure 3**. The first is an anaerobic feed phase, which drives fermentation and selects for slow-growing granule-forming organisms such as PAOs and glycogen-accumulating organisms (GAOs) (Bassin et al., 2012a; Liu and Liu, 2006; Thwaites et al., 2018). Fermentation is critical because it converts readily biodegradable organic matter within the sewage into VFAs, which are the preferred carbon source for PAOs and GAOs. These organisms store VFA's under anaerobic conditions and therefore gain a competitive advantage over floc-forming microorganisms and filamentous bacteria. The second requirement is a short settling time (<30 minutes), which promotes the washout of fast-growing floc-forming organisms (van den Akker et al., 2014; Van Den Akker et al., 2015).

These operating conditions select for fast-settling biomass that gradually evolves into dense microbial granules with excellent settling properties. In addition to improved sludge settleability, AGS offers several operational advantages. Shorter settling times enable shorter overall cycle times, which can substantially increase hydraulic treatment capacity or reduce the physical footprint of the reactor. Furthermore, unlike conventional activated sludge flocs, aerobic granules develop pronounced oxygen concentration gradients through the depth of the granule (Bassin et al., 2012b; De Kreuk et al., 2007; De Kreuk et al., 2005). These gradients create aerobic, anoxic, and anaerobic microenvironments that facilitate nitrification, denitrification, and biological phosphorus removal within the same biomass structure (Bassin et al., 2012b; De Kreuk et al., 2005). As a result, simultaneous nutrient removal can be achieved without the need for separate reactor compartments or biomass recirculation, such as the use of anoxic selectors.

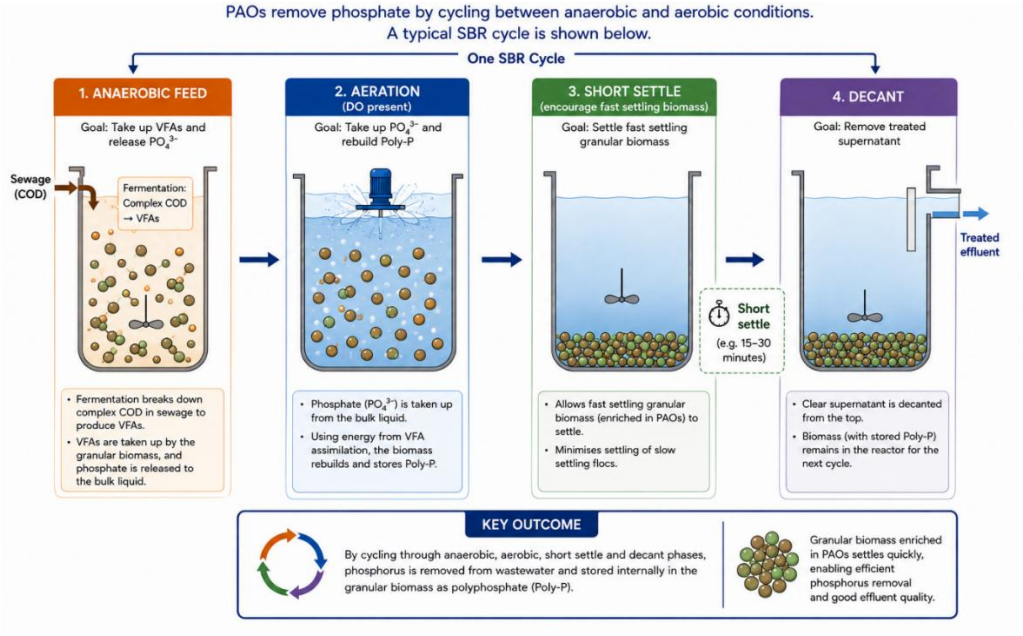


Figure 3. Biological phosphorus removal achieved in practice through modified Sequencing Batch Reactor (SBR) operation incorporating an anaerobic feed phase and a short settling phase. AI generated using ChatGPT.

The conversion of slow-settling flocs into rapidly settling granules has previously been shown to be highly dependent on the inclusion of a prolonged anaerobic feed phase, rather than the aerobic feed phase commonly employed in most existing secondary treatment SBRs (Van Den Akker et al., 2015). For newly constructed granular sludge SBRs, the anaerobic feed phase can be integrated with the decant phase (Pronk et al., 2015; Thwaites et al., 2017). In this configuration, wastewater is introduced in plug flow from the bottom of the reactor through the settled biomass bed, allowing extended

anaerobic contact while treated effluent is simultaneously withdrawn from the reactor surface (Pronk et al., 2015). Coupling the anaerobic feed and decant phases therefore enables shorter overall cycle times.

When retrofitting existing SBRs for AGS operation, simultaneous anaerobic feed and decant can present significant engineering challenges and may be costly to implement, as it generally requires modification of the inlet and decant systems to maintain plug flow conditions. For existing SBRs that cannot be reconfigured in this way, the most practical approach is typically the inclusion of a separate dedicated anaerobic feed phase at the beginning of the treatment cycle (**Figure 3**). Although this approach does not reduce the total cycle time, because the time saved through a shorter settling phase is largely offset by the additional anaerobic feed step, it can still provide substantial operational benefits. In particular, the anaerobic feed phase promotes the selection of well-settling biomass and can substantially reduce or eliminate sludge bulking, which is a common operational problem in many conventional SBRs that were designed without an anoxic selector for filamentous bacteria control. The anaerobic feed phase effectively performs a selector function by favouring storage-capable organisms such as PAOs and GAOs over filamentous microorganisms. A second major benefit is that it creates the conditions necessary for biological phosphorus removal, thereby improving nutrient removal performance without the need for additional reactor compartments or chemical dosing with metal salts for phosphorus precipitation, both of which can add significant capital and operational costs.

Aims and approach

Enhancing phosphorus removal is a future objective for the Mt Compass WWTP as it prepares to meet anticipated environmental discharge requirements associated with regional growth. This study aimed to assess whether enhanced biological phosphorus removal could be achieved within the existing SBR system through minor operational modifications, providing a cost-effective alternative to chemical phosphorus dosing, while supporting broader sustainability and process optimisation objectives. In particular, the study aimed to:

- Assess the feasibility of retrofitting the existing SBR process for EBPR through minor operational modifications. This included the introduction of an anaerobic feed phase (30-50 minutes) in the absence of plug flow. Two feeding configurations were assessed, with sewage introduced at either the top or bottom of the reactor.
- Evaluate selective sludge wasting as a means of improving biomass characteristics. The waste activated sludge (WAS) wasting point was relocated from the bottom of the reactor to the upper portion of the settled sludge bed (approximately 1500mm depth). This approach was intended to preferentially remove lighter, slower-settling biomass while retaining denser, faster-settling biomass within the reactor.

- Assess full-scale SBR performance under the modified operating conditions, including the capacity of the existing system to achieve phosphorus removal biologically and the resulting changes in mixed liquor characteristics and biomass settleability.

Collectively, the study evaluated whether relatively simple operational changes could enhance phosphorus removal and biomass performance within the existing infrastructure, avoiding the need for additional reactor capacity or chemical phosphorus dosing.

Methodology

Plant description

Mount Compass WWTP, shown in **Figure 4** comprises preliminary treatment via a mechanical screen, followed by a balance tank and a SBR providing biological treatment. Treated effluent is subsequently discharged to a lagoon before chlorination and reuse. The SBR was designed primarily for the removal of BOD_5 and SS, with target effluent concentrations of less than 20 mg/L BOD_5 and 30 mg/L SS, respectively. The treated effluent is decanted by gravity. The original SBR design did not incorporate specific nutrient removal processes. The aeration demand was also not calculated to provide capacity for nitrification. These design characteristics impose constraints on the potential for biological nitrogen removal. A waste activated sludge (WAS) storage tank is also provided to store sludge generated from the activated sludge process. Supernatant from the WAS tank is returned to the balance tank, allowing residual soluble loads in the supernatant to re-enter the treatment process. Overall, the existing infrastructure reflects the original design intent of achieving conventional BOD_5 and SS removal, with limited provision for nutrient removal or process conditions specifically intended to optimise sludge settleability and biological phosphorus removal.

A)



B)

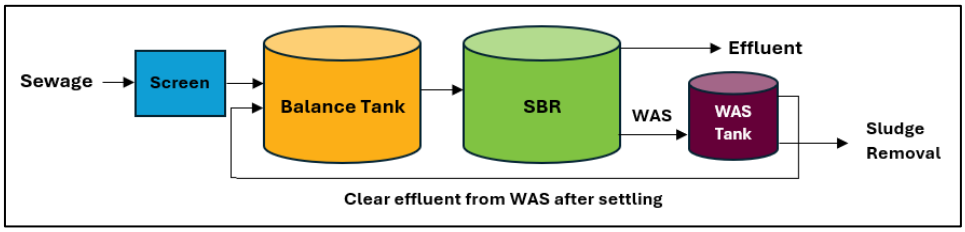


Figure 4. Aerial photograph of Mount Compass WWTP **(A);** and a line diagram showing key treatment processes **(B)**.

Operational adjustments to SBR

The SBR cycle at the Mount Compass Wastewater Treatment Plant was modified to incorporate an anaerobic feeding phase, which was not included in the standard operational cycle (**Figure 5**). This modified cycle (termed the "new cycle") was designed to promote the enrichment of PAOs, which utilise VFAs, such as acetate, to form intracellular storage polymers under anaerobic conditions, providing them with a competitive advantage over filamentous bacteria. The extended anaerobic feed phase was intended to promote the fermentation of organic matter to VFAs and to enable subsequent uptake by PAOs, which is a key prerequisite for phosphorus removal during the subsequent aerobic phase.

Although the overall cycle duration had increased to accommodate the additional anaerobic phase, it was hypothesised that improved sludge settleability would occur over time due to suppression of filamentous bacteria growth, which enabled a substantial reduction in settling time (from 60 to 20 minutes). The combination of the anaerobic feed phase and reduced settling time is referred to as the "advanced cycle" (**Figure 5**).

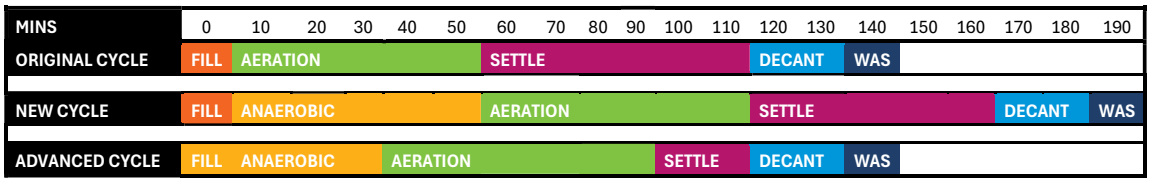


Figure 5. Modifications made to the SBR cycle phases during the study.

Process adjustments to SBR

To support the enrichment of PAOs and address the sludge bulking that commonly affects this type of treatment system, the SBRs sludge wasting strategy was modified. Specifically, the sludge wasting point was relocated from the bottom of the reactor to the upper portion of the settled sludge bed (1500mm depth). This approach preferentially removes lighter, slower-settling biomass while retaining the denser, faster-settling sludge that is more likely to be enriched in PAOs. We also hypothesised that, over time, this selective wasting strategy would impose a settling-based selection pressure, reducing the abundance of filamentous (bulking) biomass by favouring the retention of rapidly settling biomass within the system. In addition, the feed pipe was repositioned to the bottom of the SBR late in the trial to maximise 'feast' conditions for the denser, well-settling sludge, supporting the development and selection of PAO's. The timeline of key process changes made to the SBR are outline in **Figure 6**.

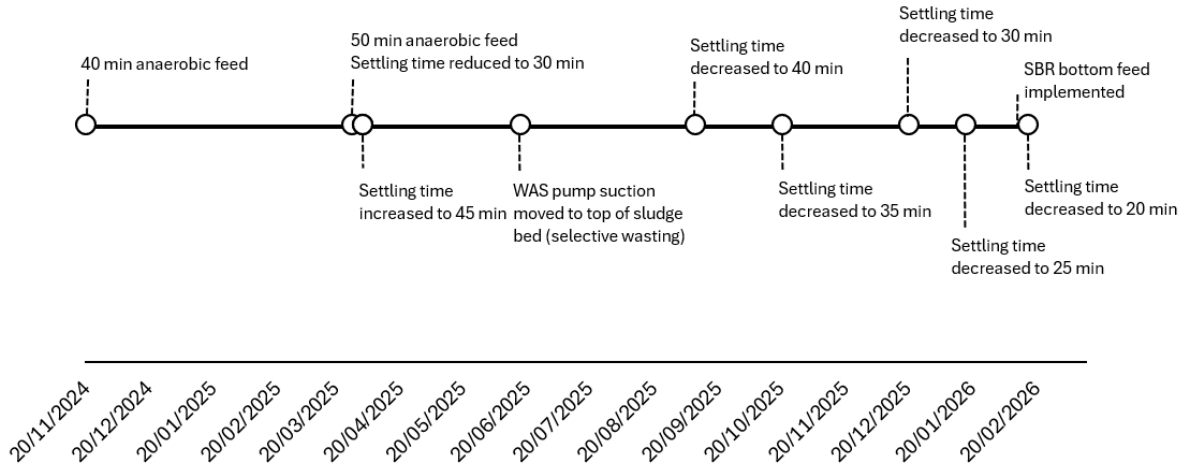


Figure 6. Timeline of process changes made to the SBR.

SBR monitoring (chemical analysis)

Concentrations of phosphate-P, ammonium-N, nitrate-N, and nitrite-N were measured in filtered samples collected throughout the SBR cycle (influent, before and after the anaerobic feed phase, during aeration, and during decant) to characterise EBPR and nitrification performance within the full-scale plant. Analysis was performed using HACH methods, 10020 (nitrate-N), 8153 (nitrite-N), 8114 (reactive phosphorus), and 10031 (ammonia-N). Chemical oxygen demand (COD) (method 8000) was also measured to assess the availability of readily biodegradable carbon. To ensure representative sampling, samples were collected only during periods of active mixing when the reactor contents were well mixed and homogeneous.

Bench testing of mixed liquor samples

To further evaluate the biological phosphorus removal potential of the biomass, laboratory batch tests were conducted under controlled conditions. One litre of mixed liquor was collected from the SBR at the end of the aeration phase. Batch tests were initiated by adding fresh sewage at a volumetric exchange ratio (VER) of 30% (v/v), compared with the 8% VER used in the full-scale SBR, to settled mixed liquor to simulate the anaerobic feed phase. Following a 60-minute anaerobic incubation period, the mixed liquor was aerated for 120 minutes under elevated DO concentrations to represent an extended aerobic phase under near-optimal conditions for phosphorus uptake. PO_4-P concentrations were monitored throughout the batch tests to assess EBPR activity by measuring the characteristic anaerobic phosphorus release and subsequent aerobic phosphorus uptake by PAOs.

Similar batch tests were conducted to assess the nitrification potential of the mixed liquor. In these experiments, no anaerobic feed phase was simulated. Instead, one litre of mixed liquor collected at the end of the SBR aeration phase was continuously aerated for 150 minutes under elevated DO concentrations (6 mg/L) to provide conditions favourable for nitrification. Concentrations of ammonium-N, nitrite-N, and nitrate-N were measured at 30-minute intervals to characterise nitrification activity and determine the capacity of the biomass to oxidise ammonium under optimal laboratory conditions.

Molecular analysis (16s amplicon sequencing)

Biomass (MLSS samples) were sent to the Australian Water Quality Centre (AWQC) and The University of Queensland's, Australian Centre for Ecogenomics (ACE) for next generation DNA sequencing. These laboratories use different next generation sequencing (NGS) platforms, which were compared. AWQC NGS was performed using an IonTorrent (Life Technologies) platform. In brief, DNA samples were amplified using the 16S Metagenomics Kit containing bacterial 16S primers specific to hyper variable regions. The amplicons are barcoded, pooled and processed using the IonTorrent methodology. The sequences are run through a bioinformatics pipeline curated to the MicroSEQ 16S rRNA reference database and Green genes database. Australian Centre for Ecogenomics (ACE) 16S rRNA amplicon sequencing was performed on a MiSeq i100 Series. The FASTA files were further processed using ACE's QIIM2 pipeline. For the 16s rRNA sequencing bacteria primers amplifying V3 and V4 regions were used (Bac_SSU_341F-806wR). All data is presented as Operational Taxonomic Units (OTUs).

Results and Discussion

System start-up

Following the introduction of the anaerobic phase at Mount Compass WWTP, a noticeable improvement in SBR PO_4–P removal was observed within the first 1-2 months of implementation with reductions ranging from 50% to 100%, compared to baseline conditions where no removal was observed (**Figure 7**). While PO_4–P removal fluctuated as the system stabilised, it showed that a phosphorus removal was possible by incorporating the anaerobic feed. The decline in PO_4–P removal performance observed during February 2025 was likely caused by the substantial reduction in settling time from 60 to 30 minutes, which resulted in biomass washout. This operational change was subsequently reversed, and a more gradual reduction in settling time was adopted, which is presented later.

This start-up period is consistent with the typical timeframe reported for observing noticeable improvements in phosphorus removal following the implementation of anaerobic feeding in batch and continuous flow systems (Li et al., 2016; Ong et al., 2013).

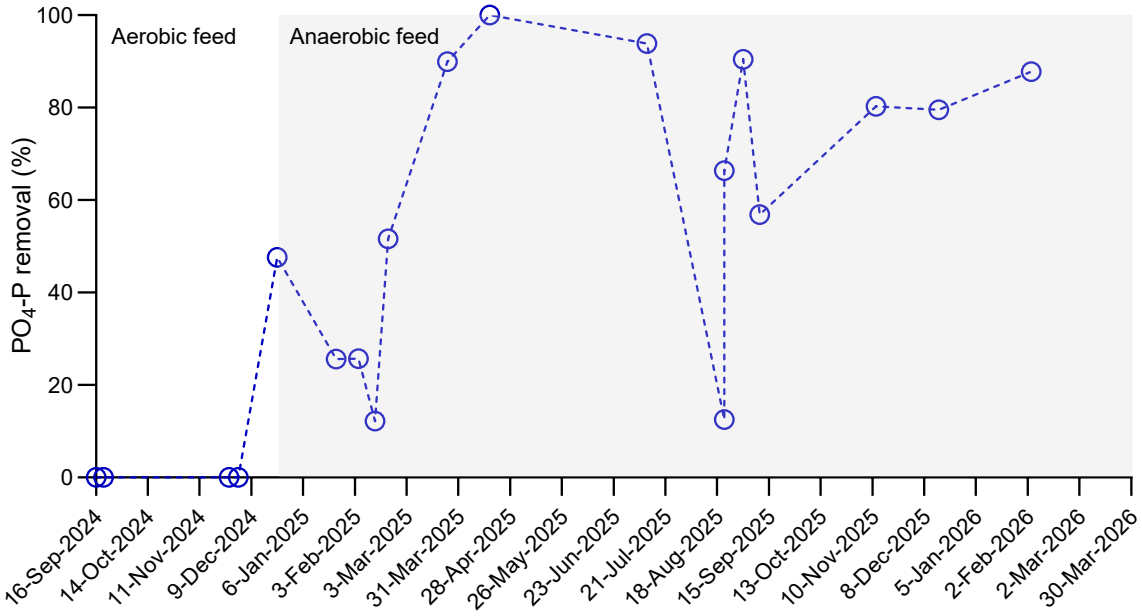


Figure 7. Temporal changes in SBR PO_4–P removal efficiency (%) following the introduction of an anaerobic feeding phase.

Long-term phosphate removal performance

Figure 8 shows the distribution of effluent PO_4–P concentrations achieved throughout the entire study, presented as a box-and-whisker plot. A more detailed assessment of performance under each operational configuration following changes in settling time and sludge wasting position is provided in **Table 1**. Overall, these results demonstrate that the incorporation of anaerobic feed, combined with reductions in settling time, enhanced PO_4–P removal performance, with mean effluent concentrations reducing from 11.0 ± 0.94 mg/L to 3.3 ± 3.4 mg P/L representing an approximately 70% reduction in sewage PO_4–P (**Figure 8**). Effluent PO_4–P values as low as 2.2 ±1.5 and 1.8 ±0.5 mg/L were achieved at steady state once SBR settling times had decreased to 40 and 25 minutes respectively (**Table 1**).

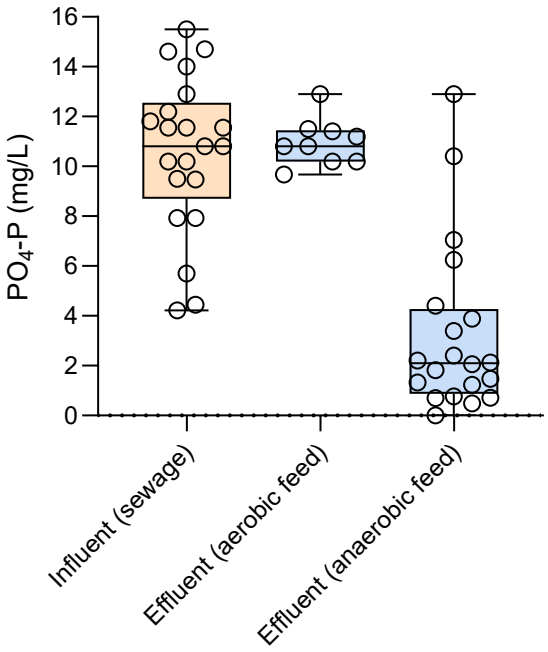


Figure 8. Box-and-whisker plot showing phosphate-P concentrations measured in sewage and full-scale decanted effluent, comparing overall system performance under aerobic and anaerobic feed conditions.

Phosphate cycle profiling

The profiling of phosphate concentrations during the SBR cycle (**Figure 9**) taken across multiple days (n = 12 events) provided evidence of PAO activity being constantly responsible for the observed phosphorous removal at full-scale. A clear increase in PO_4–P concentration occurred (from 9.9 ± 3.2 to 16.2 ± 8.0 mg/L) during the anaerobic feeding phase is consistent with the expected release of

phosphorus by PAOs as these microorganisms hydrolyse intracellular polyphosphate to generate energy for uptake and storage of VFAs. Following the subsequent aeration phase, phosphate then decreased to 3.3 ± 2.8 mg/L, achieving a net mean removal of 67%. This demonstrates that the biomass is capable of taking up phosphate beyond normal growth requirements (luxury uptake), which is the defining mechanism of EBPR.

The laboratory batch tests (n = 6) provide stronger evidence of EBPR because the biomass was sampled directly from the full-scale SBR and assed for PAO activity under optimal operating conditions, comprising of a higher volumetric exchange ratio (VER) of 30% and an extended aeration phase of 2 hours, to evaluate the maximum EBPR potential (**Figure 9**). During these tests, the anaerobic feeding phase resulted in phosphate release, increasing PO_4–P concentrations to 30 ± 7.0 mg/L, which was almost double the peak concentrations observed in the full-scale SBR due to the substantially higher substrate loading (VER of 30% vs. 8%). This was followed by rapid phosphate uptake during aeration, with PO_4–P concentrations decreasing to 8.9 ± 6.7 mg/L after 1 hour, and 2.8 ± 4.6 mg/L after 2 hours of aeration. This characteristic pattern of anaerobic phosphate release followed by aerobic phosphate uptake is a hallmark of EBPR activity and highlights the high phosphorus removal potential of the biomass under optimal operating conditions, when substrate loading and oxygen availability were not limiting. These findings also provide strong evidence that a well-established and metabolically active population of PAOs was present rather than the observed phosphorus removal being solely due to chemical precipitation or biomass assimilation.

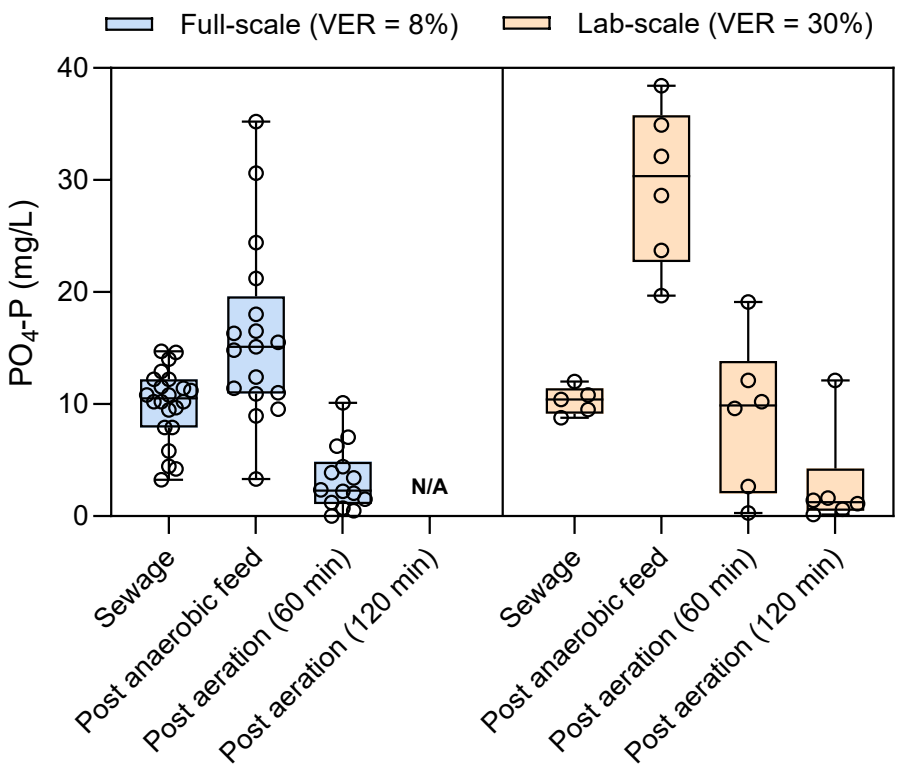


Figure 9. Box-and-whisker plot showing the distribution of phosphate-P concentrations measured throughout the full-scale SBR cycle and the lab-scale batch test cycle of the same biomass. The full-scale SBR was operated at a volumetric exchange ratio (VER) of 8%, whereas the lab-scale batch test was operated at a higher VER of 30%.

Impact of process changes on SVI and MLSS

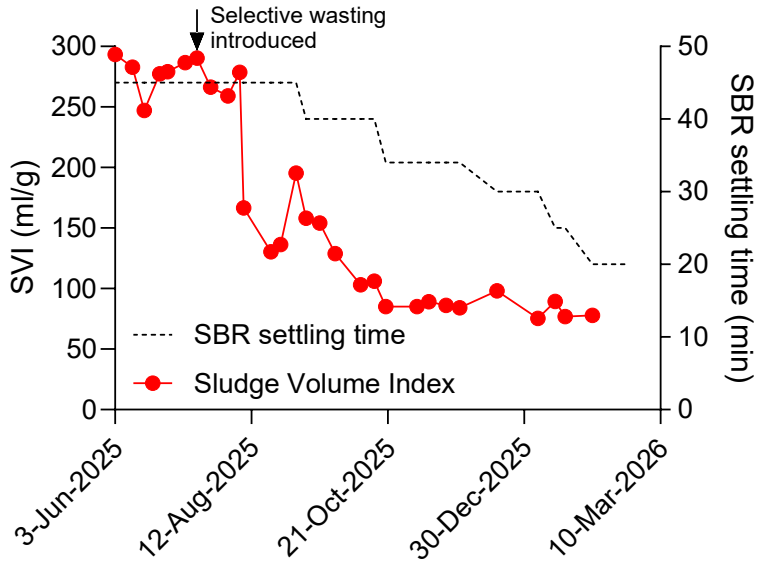
Figure 10 illustrate the impact of the anaerobic feed and subsequent reductions in SBR settling time on temporal changes in biomass concentration, represented as mixed liquor suspended solids (MLSS), and sludge settleability, expressed as the sludge volume index (SVI). Selective wasting of MLSS was also implemented in mid July 2025, by positioning the WAS pump extraction point from the bottom to the top of the settled sludge bed in attempts to remove light, filamentous bacteria and select for denser fast settling biomass. Mean values are also reported in **Table 1**, which provides a more detailed breakdown of SBR performance across the different phases in which the SBR configuration and operation were modified.

Results showed that the implementation of anaerobic feed, when combined with stepwise reductions in SBR settling time, resulted in substantial improvements in sludge settleability, with SVI decreasing from highs of approximately 250–300 mL/g to 75–90 mL/g. Importantly, adequate MLSS

concentrations were maintained under the reduced settling times, indicating that biomass washout did not occur despite the shorter settling times.

One of the key drawbacks of these wastewater systems is the lack of an anoxic selector to suppress filamentous bacterial growth and minimise sludge bulking, both of which are common operational challenges that can result in elevated effluent suspended solids concentrations. These results highlight the benefits of introducing an anaerobic feed, not only to enhance biological phosphorus removal, but also to improve biomass settleability. The shorter settling times provides a selective pressure favouring faster-settling biomass, while the anaerobic feed promotes the growth and activity of slow-growing organisms, such as PAOs. Under anaerobic conditions, PAOs can rapidly uptake and store readily biodegradable substrates, such as acetate, providing them with a competitive advantage over filamentous organisms and contribute to improved sludge characteristics. Importantly, this work demonstrates that relatively simple operational modifications can be readily incorporated into existing systems to compensate for the absence of an anoxic selector.

A)



B)

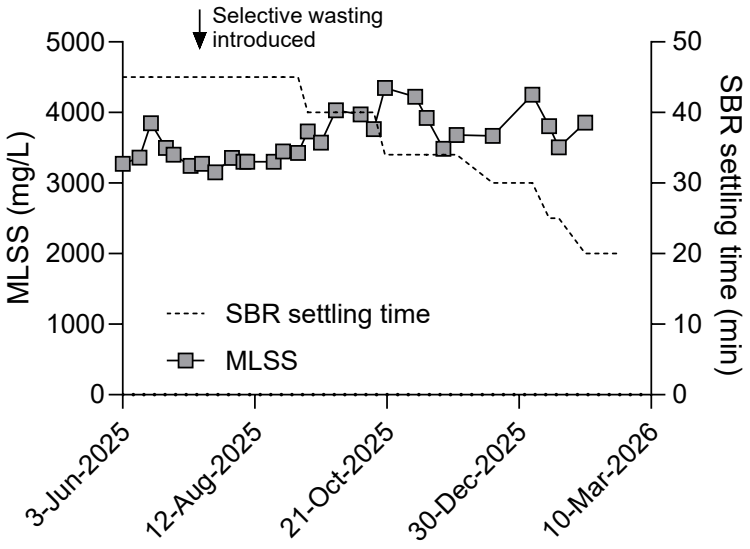


Figure 10. Temporal changes in full-scale SBR sludge volume index (SVI) **(A)**; and MLSS concentration **(B)**, following the onset of anaerobic feeding phase of 40 minutes. Step changes in SBR settling time are also shown.

Table 1. Comparison of SBR performance in terms of effluent PO_4–P concentration, mixed liquor suspended solids (MLSS) concentration, and sludge settleability, expressed as the sludge volume index (SVI), under different operational configurations. Values are presented as the mean (± 1 standard deviation).

	SBR configuration			
Parameter	**• Aerobic feed • Settling time of 50 min**	**• Anaerobic feed • Settling time of 45 min**	**• Anaerobic feed • Settling time of 45→40 min • Selective wasting**	**• Anerobic feed • Settling time of 34→20 min • Selective wasting**
Effluent PO_4-P (mg/L)	11.0 (0.9)	2.7 (3.2)	2.2 (1.5)	1.8 (0.5)
SVI (ml/g)	195 (113)	242 (58)	212 (72)	85 (7.0)
MLSS (mg/L)	5907 (1887)	3370 (164)	3487 (259)	3875 (309)

Nitrification capacity

Cycle profiling of ammonium-N concentrations in the full-scale SBR indicated that minimal ammonium removal occurred during treatment (**Figure 11**). Measurement of oxidised nitrogen species (nitrite and nitrate) showed that only a small proportion of the ammonium removed was recovered as nitrite or nitrate during the aeration phase, indicating limited nitrification capacity (**Figure 12**). Online DO measurements confirmed that surface DO concentrations typically peaked at 2–6 mg/L, depending on the day and cycle, although during periods of higher morning peak loading, DO concentrations often peaked at only approximately 1 mg/L. It was possible that any nitrite or nitrate produced was simultaneously reduced through denitrification. Depth profiles of DO measured using a portable DO probe confirmed a depth-wise reduction in DO concentrations, with lower DO observed at greater depths. This indicated some degree of poor mixing but also suggested the presence of conditions capable of supporting simultaneous nitrification–denitrification, whereby nitrification can occur in oxygenated regions while denitrification occurs concurrently in oxygen-depleted depths.

The poor level of ammonium removal was somewhat unexpected, as the MLSS concentration (3-4 g/L) and corresponding sludge age (10-20 days) were considered sufficient to sustain a nitrifying population. Furthermore, the relatively low volumetric exchange ratio (VER; ~8%) and sewage COD concentration (~190 mg/L) suggested that the organic loading should not have limited nitrification. It was therefore hypothesised that the 60-minute aeration period was insufficient to achieve higher levels of ammonium oxidation.

It can also be seen that influent ammonium concentrations varied substantially, ranging from approximately 55 to 100 mg N/L. These differences are likely due to the return of supernatant from the WAS tank and the extended sludge retention within the tank leading to ammonification (mineralisation). The step change in influent ammonium concentrations observed between August and October 2025 coincided with the WAS tank reaching capacity and an associated increase in sludge retention time.

To investigate the nitrification potential of the biomass, mixed liquor samples were collected and laboratory batch tests were conducted under fully aerobic, non-limiting conditions (dissolved oxygen 4–6 mg/L), with the aeration period extended from 60 to 150 minutes (**Figure 13**). Despite these favourable conditions, only minimal ammonium removal was observed, consistent with the performance observed in the full-scale SBR. Furthermore, negligible amounts of the ammonium removed was recovered as nitrite or nitrate, providing further evidence that an established nitrifying population was absent.

To further validate these findings, 16S rRNA gene amplicon sequencing was undertaken to determine the relative abundance of nitrifying microorganisms within the activated sludge community.

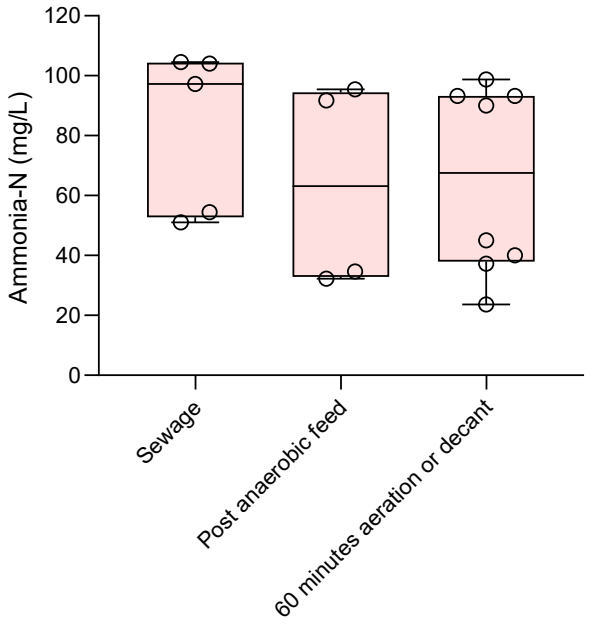


Figure 11. Box-and-whisker plot showing the distribution of ammonium-N measured across the full-scale SBR.

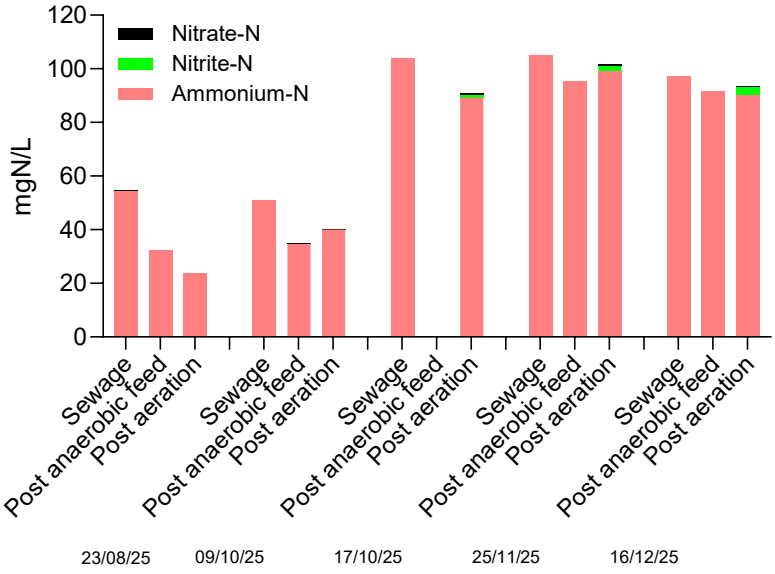


Figure 12. Ammonium-N, nitrite-N, and nitrate-N concentrations measured in the full-scale SBR influent (sewage) and decanted effluent.

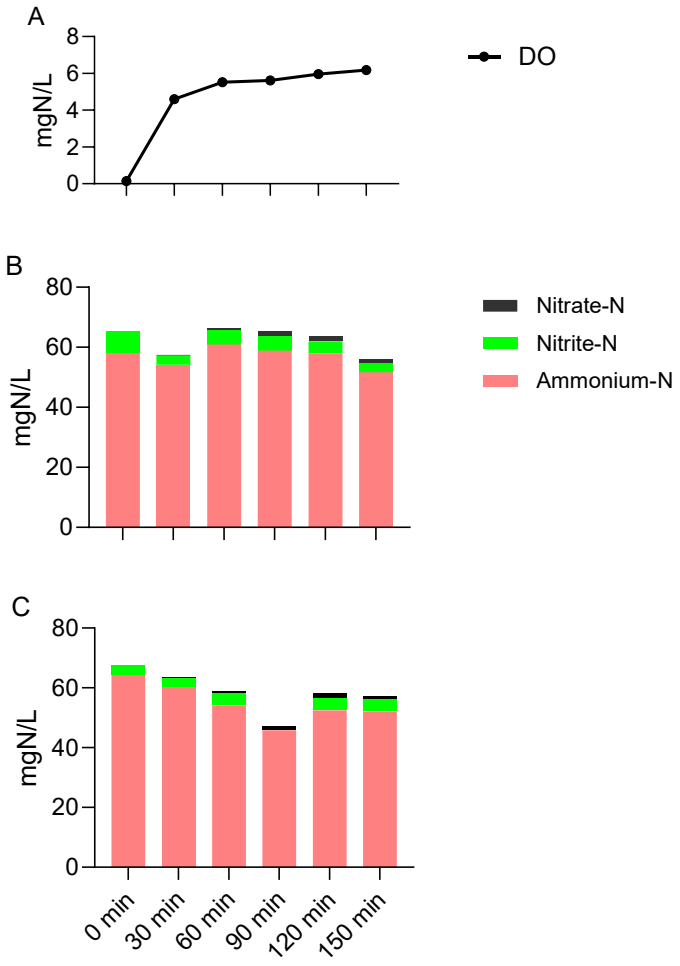


Figure 13. Concentration profiles of dissolved oxygen **(A)** and ammonium-N, nitrite-N, nitrate-N **(B-C)** measured during repeat nitrification batch test under aerobic conditions.

Microbial community composition and functional groups associated with SBR performance

16S rRNA sequencing of samples collected between 2021 and 2025 and analysed using the Ion Torrent platform showed that fermentative organisms became more prominent in the later samples following implementation of the anaerobic feed, particularly *Propionivibrio* and *Trichococcus*. The relative abundance of fermenters increased from 0.32% prior to implementation of the anaerobic feed to 7–13% following its implementation. This increase provides evidence of the establishment and enrichment of fermentative microorganisms following introduction of the anaerobic feed, which is an important step in supporting EBPR.

Interestingly, the PAO-associated community also appears to have changed substantially over the study period. Prior to implementation of the anaerobic feed, the identifiable PAO-associated community was dominated by *Dechloromonas*, which reached approximately 6–11% relative abundance, while *Tetrasphaera* was virtually absent. Following implementation of the anaerobic feed, *Tetrasphaera* became considerably more prominent, reaching 5.85% relative abundance, alongside 5.05% *Dechloromonas*. This suggests a substantial shift in the composition of the PAO-associated community. Both *Dechloromonas* and *Tetrasphaera* are recognised PAO-associated genera, however it is important to note that these genera encompass diverse metabolic capabilities, and relative abundance based on 16S rRNA sequencing does not provide direct evidence of polyphosphate accumulation or PAO activity. *Candidatus Accumulibacter*, a well-established PAO in EBPR systems, was not detected in any of the samples.

The signal for conventional nitrifying taxa remained very weak, with *Nitrosomonas* detected at only approximately 0.01–0.05% relative abundance in some samples, while the NOB genera *Nitrospira* and *Nitrobacter* were not identified in these datasets. Glycogen-accumulating organisms (GAOs), such as *Candidatus Competibacter*, which can compete with PAOs for readily biodegradable carbon, were also not detected. However, the apparent scarcity or absence of these organisms should be interpreted cautiously, as 16S rRNA sequencing may not adequately resolve all populations and relative abundance does not provide direct evidence of microbial functional activity. Furthermore, their absence from the sequencing results may reflect the taxonomic resolution of the analysis, including the 16S rRNA region and primers used, and does not necessarily indicate that these organisms were absent from the bioreactor.

Follow-up 16S rRNA sequencing, performed using the MiSeq i100 Series platform on ten bioreactor samples collected between August 2025 and June 2026, identified a dynamic microbial community containing several functional groups relevant to fermentation and biological phosphorus removal (Table 2). Clear temporal changes were observed, indicating substantial microbial succession over the monitoring period.

Consistent with the Ion Torrent sequencing results, fermentative microorganisms represented a substantial component of the community, with selected taxa accounting for approximately 3.8–18.5% of total relative abundance. Major fermenters included *Trichococcus*, *Paludibacter*, *Propionivibrio*, *Saccharofermentans*, *Macellibacteroides*, *Anaerolinea* and *Leptolinea*. A clear shift occurred over time. *Trichococcus* dominated during August–October 2025, reaching approximately 12% in August, but subsequently declined. In contrast, *Paludibacter* increased from 0.14% in August to 11.26% in November and remained relatively abundant thereafter. This suggests that fermentative capacity was maintained through much of the study despite changes in the organisms contributing to this function. Such organisms are potentially important for EBPR because fermentation can generate readily available carbon substrates that support PAO metabolism.

A particularly strong signal was observed for PAO-associated organisms. *Tetrasphaera* was the dominant identifiable PAO-associated genus and increased progressively from 2.40% relative abundance in August to approximately 7% in October 2025. Following the October peak, *Tetrasphaera* declined to 3.85% in November, 2.52% in December, 0.77% in April 2026 and 0.30% in June, where as *Dechloromonas* became more prominent during the later sampling period (0.3–0.7%), indicating a shift in the PAO-associated community over time. In contrast to the Ion Torrent sequencing results, *Candidatus Accumulibacter* was also detected but at considerably lower abundance (0.1–0.3 %).

In contrast to the Ion Torrent sequencing results, GAOs, which can compete with PAOs for readily biodegradable carbon, were detected in the MiSeq i100 sequencing results. *Candidatus Competibacter* represented approximately 3–4% of the community between August and October, before declining markedly to approximately 0.6% in November–December. It subsequently increased to 2.69% in April 2026, which coincided with a major plant failure that occurred due to a power failure over the weekend where there was no aeration available for 3 days. These changes suggest that the balance between PAOs and GAOs also varied substantially over the operating period.

Similar to the Ion Torrent results, MiSeq i100 sequencing results provided limited evidence of conventional nitrifying microorganisms. Identifiable ammonia-oxidising bacteria, including *Nitrosomonas* and a Nitrosomonadaceae lineage, represented less than 0.02% of the community. Classical nitrite-oxidising bacteria, including *Nitrospira* and *Nitrobacter*, were not identified at genus level, and no clear ammonia-oxidising archaea (AOA) were detected. Their absence from both sequencing datasets is consistent with the poor nitrification capacity measured in the full-scale SBR and batch studies.

Overall, the results indicate a strong fermentative and EBPR-associated microbial community, with progressive enrichment of *Tetrasphaera* between August and October 2025 followed by substantial community restructuring from November onwards. The decline in *Tetrasphaera*, changes in fermenter composition and fluctuations in GAOs suggest that operating or environmental conditions may have altered microbial selection within the SBR, which requires further examination.

Table 2. Temporal changes in the relative abundance of key functional microbial groups in the SBR based on MiSeq i100 sequencing of samples collected during 2025–2026.

Sampling date	Fermenters (%)	Total PAO associated taxa (%)	Ca. Competibacter (%)
6-Aug-25	10.40%	2.58%	2.93%
23 Aug 2025a	13.60%	3.98%	3.98%
23 Aug 2025b	13.20%	4.09%	3.86%
10-Sep-25	11.70%	5.12%	3.81%
17 Oct 2025a	14.30%	7.23%	3.68%
17 Oct 2025b	14.40%	7.51%	3.99%
26-Nov-25	18.50%	4.54%	0.64%
16-Dec-25	13.70%	3.52%	0.65%
16-Apr-26	3.80%	1.63%	2.69%
19-Jun-26	17.30%	1.04%	0.90%

Conclusions and future recommendations

Outcomes from this study are expected to provide a cost-effective and readily implementable pathway for improving wastewater treatment performance within existing SBR infrastructure. Although fully developed aerobic granular biomass was not observed during the study, the operational modifications produced two of the key treatment benefits targeted by AGS processes: enhanced biological phosphorus removal and substantially improved biomass settling characteristics. These improvements were achieved through minor operational modifications rather than major capital upgrades or increased chemical dosing, demonstrating the potential to improve treatment performance and move existing SBR systems towards AGS-type process benefits. Specifically, the study demonstrated the potential to:

- **Enhance biological phosphate removal**, reducing reliance on chemical phosphorus dosing and associated chemical and operational costs.
- **Improve biomass settleability and control sludge bulking**, through selective retention of denser, faster-settling biomass. It is anticipated that this will reduce effluent suspended solids by improving sludge settleability and clarification performance. Reduced carryover of SS and BOD_5 is also likely to improve downstream chlorine disinfection performance.
- **Improve overall process stability** and treatment performance without requiring significant changes to existing infrastructure.
- **Provide a transferable operational strategy** that could be readily adopted at other SBR-based wastewater treatment plants.

Lessons from this study have also identified several design considerations for existing and newly constructed plants that could further enhance process performance, including phosphorus removal, solids separation, effluent SS and disinfection performance, as well as overall plant stability and treatment capacity. These considerations are detailed below.

Option for an anaerobic phase

Including an anaerobic phase in the reactor cycle is critical to both the removal of phosphorus, but also to preference better settling sludge. The Mount Compass WWTP included additional coding to enable this phase during site design, however many of the other SBRs at Alexandrina Council do not include this option, and it is likely that is the case at many other sites across SA.

Ensuring that code is available for an anaerobic feeding phase is a simple addition when designing a site but can be complicated to add later in the process. This should certainly be included when designing any SBR in future with the ability to disable if not desired; however, this project demonstrated the benefits of enabling this phase.

Bottom feed

As an extension of the anaerobic phase, optimal SBR design should incorporate controlled influent feeding at the base of the reactor to maximise fermentation and promote contact between readily biodegradable COD (rCOD) and the settled PAO biomass. In newly constructed SBRs, the anaerobic feed phase can be integrated with the decant phase (Pronk et al., 2015). Under this configuration, influent wastewater is introduced at the bottom of the reactor and passes upward through the settled biomass bed in a plug-flow manner, providing an extended anaerobic contact period while treated effluent is simultaneously decanted from the surface. Coupling the feed and decant phases can therefore reduce overall cycle times while maintaining favourable conditions for EBPR. If bottom feeding is implemented in existing SBRs, it is important to fully understand the pump sizing and reactor hydraulics to avoid excessively turbulent flows.

WAS pump location/control

Current intent of wasting at the Mount Compass WWTP is to remove as much solid material as possible within the allocated timeframe, hence the wasting is completed at the end of the settling phase and drawn from the bottom of the reactor. This however preferentially removed the denser sludge from the process, and leaves the poor settling sludge in suspension. It would be better practice to either select for the poor settling sludge, or waste from reactor when mixed.

First preference would be to move the wasting step to the end of the aeration phase. This would maximise the removal of accumulated phosphorus while ensuring that a representative, homogenised biomass is wasted. This approach avoids unnecessary changes to plant operation and provides a consistent wasting strategy.

Second preference is to change the location (depth) of sludge wasting to preferentially remove lighter, filamentous sludge from the settled sludge bed. However, this approach would increase the operational workload, as the operations team would need to confirm that wasting occurs each day and monitor for the formation of cavities in the sludge bed when using a single extraction point.

Fermenting/digesting in WAS tank

As the WAS tank is a ‘sludge thickening tank’, it works on the principle that solids will concentrate at the bottom of the tank, and the effluent supernatant will overflow back into the head of the plant until the WAS tank is filled with enough solids that it can thicken no more (solid overflow); This can take ~60-90 days depending on the wasting methodology. At this point the WAS tank is emptied and trucked offsite.

As there is no oxygen present in this tank, it inadvertently becomes a cold anaerobic digester and would facilitate the growth of methanogenic bacteria over time. This process produces a high concentration of ammonia that is released into the liquid phase, as well as re-releasing any captured phosphorus into the liquid phase, which is then returned to the head of the plant.

This practice not only results in the continuous recirculation of phosphorus within the process, but also increases ammonium concentrations in the balance tank when the retention time in the WAS tank is at its longest. The extent of WAS digestion should be investigated since a further consideration is the potential production of VFAs, which could increase the availability of readily biodegradable COD and further support biological phosphorus removal.

To successfully achieve phosphorus removal across the entire WWTP process, several options could be considered in future plant design:

- Remove the WAS tank from the process and dewater waste activated sludge immediately, thereby minimising phosphorus release during sludge storage.
- Empty the WAS tank prior to overflow, although this would increase operational costs.
- Target chemical dosing for phosphorus removal in the WAS tank overflow to prevent soluble phosphorus from being returned to the treatment process.
- Investigate additives that maintain phosphorus in the solid phase during WAS storage, allowing phosphorus to be removed with the waste sludge through the normal solids-handling process.

Fixed decant vs floating decanter

This site has a fixed decanting ring that sits ~300mm below the surface water level. The time to wait for the sludge to settle beyond this point (needs to settle ~500mm to sit below the bottom of the pipe) could be improved if a floating decant was used instead of this ring. As the sludge blanket naturally fluctuates over time, this would reduce the risk of solids carryover. Additionally, this may support the selective removal of filamentous bacteria and scum that accumulate at the top of the reactor, helping to prevent the entrapment and accumulation of foam.

Aeration

The Mount Compass reactor was designed primarily for BOD_5 and SS removal, with the aeration system sized accordingly. However, adequate aeration is essential for maintaining a healthy and diverse microbial population, and the ability to achieve nitrification can serve as an indicator of adequate biological activity and process health. Where aeration is consistently limited to the minimum required for BOD_5 removal, conditions may favour the proliferation of filamentous bacteria, increasing the risk of sludge bulking. Breakthrough of ammonium also increases the chlorine demand for disinfection.

The surface aerator was shown to have high concentrations of DO at the surface of the reactor, however when measuring lower than 2m (4m tank), the DO dropped below 0.5mg/L. This oxygen gradient throughout the tank likely has an impact on the overall process, and could be avoided via a

different method of aeration (diffusers/etc.). There also may be consideration to the shape of the reactor, as it's height to width ratio is much higher than many of the other reactors.

Reliability and Adequate Redundancy

In April, a power failure left the plant without aeration for three days, resulting in a marked shift in the microbial community. A separate mechanical failure of the aerator occurred four weeks later. Because the plant has no duty–standby aeration arrangement, these incidents placed the biological treatment process at significant risk: prolonged loss of aeration can rapidly compromise the microorganisms responsible for nutrient removal. This effect was evident in the April and June samples, which showed an increased relative abundance of fermenters and reductions in PAOs and nitrifiers following the failures.

This vulnerability is not unique to Mount Compass. The sensitivity of biological nutrient removal (BNR) processes is compounded by the limited redundancy incorporated into many treatment plant designs across South Australia. Visibility of treatment performance is also limited where nutrient monitoring is not a mandatory regulatory requirement, and microbial community analysis is generally not undertaken during commissioning or routine operation. As a result, deterioration in nutrient-removal capacity may remain undetected until broader process performance is affected.

Future plant designs should therefore incorporate sufficient redundancy to maintain critical treatment functions during power or equipment failures. Depending on plant scale and risk, provisions may include additional reactor capacity, duty–standby pumps, motors and aeration equipment, backup power, and appropriate monitoring and alarm systems.

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