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PII: S1385-8947(18)32294-0
DOI: https://doi.org/10.1016/j.cej.2018.11.060
Reference: CEJ 20362

To appear in: *Chemical Engineering Journal*

Received Date: 29 August 2018
Revised Date: 5 November 2018
Accepted Date: 8 November 2018

Please cite this article as: H. Vashi, O.T. Iorhemen, J.H. Tay, Extensive studies on the treatment of pulp mill wastewater using aerobic granular sludge (AGS) technology, *Chemical Engineering Journal* (2018), doi: https://doi.org/10.1016/j.cej.2018.11.060

Extensive studies on the treatment of pulp mill wastewater using aerobic granular sludge (AGS) technology

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ABSTRACT: Aerobic granular sludge (AGS) offers the potential to degrade tannin/lignin found in pulp and paper wastewater (PPW). In this study, aerobic granules were cultivated in a sequencing batch reactor (SBR) with tannin/lignin present in the range of 50-200 mg/L. The mature granules were transferred to a real PPW wastewater system containing tannin/lignin concentration up to 500 mg/L. Biodegradation and biosorption were observed to be the two pathways for the removal tannin/lignin. Biosorption was a primary form of removal at lower concentrations, achieving 74% removal at 50 mg/L. The biosorption ability reduced to 58% removal at 200 mg/L. This reveals that biodegradation prevails at these higher concentrations. The Haldane kinetic parameters were: V_{max} = 0.93 (g tannin/lignin/g VSS· day), K_s = 1910 mg/L, and K_i = 27 mg/L. Various adsorption kinetic models and isotherms were fitted to the system. The Langmuir isotherm coefficients were: $(x/m)_{max}$ = 21.5 (mg tannin/lignin/g SS), b = 0.00386 L/mg. The Freundlich isotherm had coefficients of n = 1.172, K = 0.1174. The study also delves into applying the technology towards real wastewater, achieving a COD removal of 79% and a tannin/lignin removal of 56%. Furthermore, experimental runs in warmer and more humid temperature conditions revealed higher removal efficiencies, achieving about 80% tannin/lignin degradation at a concentration of 130 mg/L. Results from this study will help ascertain an appropriate design protocol for full-scale industrial applications.

Keywords: Aerobic granular sludge; industrial wastewater; pulp and paper wastewater (PPW); tannin/lignin; wastewater treatment.

1. Introduction

The pulp and paper industry is often misunderstood to be a dying industry with the introduction of digital media. However, this is not the case; this industry is still growing, albeit at a slower pace than before. Packaging is growing all around the world, along with the need for hygiene and tissue products. Although this is still a relatively small market, pulp can also be useful for the textile industry. A whole range of new applications is emerging for this industry as newspapers,

and books are becoming a thing of the past. Therefore, it is still necessary to understand the environmental implications of the pulp and paper industry.

Pulp and paper wastewater (PPW) contains a variety of toxic pollutants, from resin acid, to chlorinated phenols and organic halogens. The compounds present in the wastewater are dependent on the pulping method used. For example, in the case of tissue products, an additional bleaching step may be introduced to ascertain a white colour that is necessary to market these products. Therefore, it can be quite difficult to deal with this wastewater due to the complex nature of this industry.

1.1 PPW Characteristics

Pokhrel and Viraraghavan [1] reviewed the treatment of PPW using various technologies. In their review, the authors mention the issue of high organic loading. The chemical oxygen demand (COD) of PPW can range anywhere from 7000 mg/L to 20,000 mg/L depending on the application. Sulfite processes tend to have higher COD concentrations as compared to Kraft pulping. Additionally, the biodegradability of this wastewater is quite low, with biochemical oxygen demand (BOD)/COD ratios typically less than 0.4 [1]. Complex organics such as resin acid, tannins, lignin, and other fatty acids can decrease the biodegradability of this type of wastewater.

Additionally, there are also issues with nutrients in PPW. Nutrient limitation in PPW has been known to cause operational issues such as sludge bulking and poor solid-liquid separation [2]. Therefore, in many cases, nutrients must be added as a conditioning step prior to biological treatment to satisfy the bacterial growth requirements.

1.1.1 Tannin/Lignin

For the purposes of this study, two compounds were particularly emphasized in PPW, tannin/lignin. Lignin is a complex heteropolymer that was first mentioned as a fibrous, tasteless material that is insoluble in water and alcohol, but is soluble in weak alkaline solutions. It was later determined that the compound could be precipitated by acid. Lignin constitutes 30% non-fossil organic carbon, but the composition varies from species to species. Lignin is extremely difficult to degrade due its lack of defined primary structure. It is most commonly seen as a strengthening compound capable of aiding in the formation of the cell wall in vascular plants.

Lignin is a cross-linked polymer with a large molecular mass and is rich in aromatic units. The three main lignols that crosslink are: 4-hydroxy-3-methoxyphenylpropane, 3,5-dimethoxy-4-hydroxyphenylpropane and 4-hydroxyphenylpropane [3].

Tannin is another polyphenolic molecule that serves as a modulator, protecting plants against infections and herbivores with its acidic taste [4]. The chemical structure of tannin is slightly simpler than that of lignin and can be split into three major monomers: gallic acid, phloroglucinol, flavan-3-ol. Typically, tannin molecules require at least twelve hydroxyl groups and at least five phenyl groups to function as protein binders [5]. Examples of possible tannin/lignin structures are given in Fig. 1.

Due to the aromatic units contained within these large compounds, both tannin/lignin are extremely difficult to degrade. They require multiple steps because unlike many other simpler aromatic molecules, each reaction with these compounds can result in side products that may be even more complex than their parent compound. Chemical oxidation with strong oxidizing agents such as hydrogen peroxide can cause the enzymes involved to attack any accessible sites, thus resulting in oxygenation (which reduces the molecular weight of the compound) but may also result in dehydrogenation which would cause the coupling of two adjacent phenolic molecules resulting in an even stronger C-C bond [6].

Therefore, biodegradation is often the most sought-after form of removal, as it can even result in 100% removal efficiency. Several microbial species, including fungi, can be quite potent against tannin/lignin. Asina et al. [7] reviewed the microbial treatment of industrial lignin and some of the successes and challenges in the area. In their article, the authors mention that the species *Sphingomonas* and *Phanerochaete chrysospoprium* were able to degrade model lignin compounds. There has also been some work on the ability of conventional activated sludge (CAS) to treat tannin/lignin from Kraft mill wastewater, achieving only 36% removal after 23 days of operation [1]. Tannin/lignin are high molecular weight compounds, so it is not surprising that the ability of CAS to degrade these compounds is poor. The results also indicated that the removal efficiencies are strongly affected by the retention time. To achieve even 51% removal, a 10-hour HRT had to be employed. Therefore, there is a large gap in this line of work, and there is an imminent need for a new biotechnology that can help degrade these complex pollutants.

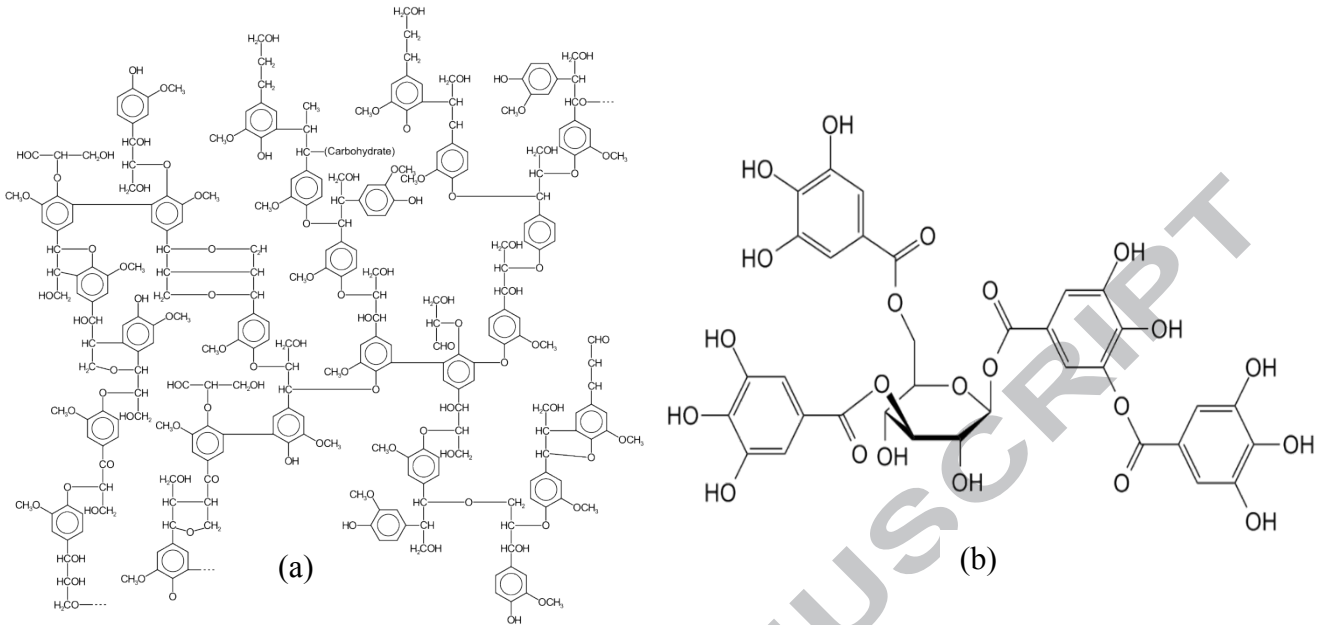


Fig. 1. (a) Potential lignin molecule on the left and (b) tannin molecule on the right

1.2 Aerobic granular sludge for the treatment of PPW

Aerobic granulation is a process of self-immobilized microorganisms developing a dense, microbial community through high shear force in the form of upflow aerobic velocity. Aerobic granular sludge (AGS) technology yields a cost-effective solution to many industrial wastewater applications. Due to the excellent settling ability of the granules, there is no need for an additional clarification tank thus reducing both operating and start-up costs [8].

The granules are cultivated using the ability of bacterial species to generate extracellular polymeric substances (EPS) when a high organic loading rate and superficial velocity are employed. EPS is a binding substance that enables the aggregation of a wide range of bacterial species capable of degrading complex aromatics [9]. Most studies are carried out in a sequential batch reactor (SBR) in order create a timely schedule involving the following processes: reaction/aeration, settling, decant, and fill.

AGS has been used towards the treatment of paper-making wastewater on a few occasions, and a recent review by Vashi et al. [10] described the potential of using this technology towards the treatment of PPW. However, there has not been an in-depth scientific analysis to-date on the ability for AGS to be used on pulp mill effluent containing tannin/lignin.

1.3 Biosorption

Biosorption refers to the ability of certain types of microbial biomass to bind and concentrate pollutants from aqueous solutions. It is similar to chemical adsorption in that the amount of pollutants that attach to the sorbent is dependent on the kinetic equilibrium and composition on the cellular surface. It is a reversible process and can remove high concentrations of pollutants from wastewater. Unlike bioaccumulation, this process is not metabolic and does not require respiration. It is largely affected by the pH and concentration of the biomass [11].

AGS technology has been used as biosorbents in recent studies, mostly towards the use of metallic cations. Wang et al. [12] recently reviewed advances in the use of AGS as biosorbents. They reviewed the ability of granules to be used as biosorbents at various stages (fragmented, compact, fluffy, etc.) and with a diverse range of pollutants. The authors go on to describe the main isotherm models and kinetic equations that govern these systems. For this study, two key kinetic equations, and two adsorption isotherm models were adopted and compared. Table 1 displays those equations and their purpose. Comparing these parameters is integral in understanding whether AGS technology can be used as a viable biosorbent.

Table 1. Adsorption kinetics and isotherm models, along with their function [12]

Model Type	Model Name	Equation	Terms of Equation	Purpose
Kinetic	Pseudo-first order	$\log(Q_e - Q_t) = logQ_e - \frac{K_1^{'}t}{2.303}$	Q_e = biosorption capacity $K_1^{'}$ = pseudo-first order biosorption rate (1/min) Q_t = adsorbed amount (mg/L) t = time (min) $K_2^{'}$ = pseudo-second order biosorption rate (g/mg·min)	This equation assumes that the adsorption process is reversible from liquid to solid. Additionally, there is an equilibrium between the sorbent and sorbate.
	Pseudo-second order	$\frac{1}{Q_e - Q_t} = \frac{1}{Q_e} + K_2^{'}t$		This equation assumes that the rate controlling step is a chemical reaction between the adsorbent and adsorbate.
Isotherm	Langmuir	$\frac{1}{x/m} = \frac{1}{\left(x/m\right)_{max} bC_{eq}} + \frac{1}{\left(x/m\right)_{max}}$	x/m = amount of solute adsorbed per weight of adsorbent (mg/g) C_{eq} = equilibrium solution concentration of solute (mg/L) $\left(x/m\right)_{max}$ = the maximum value of x/m b = Langmuir constant (related to energy of adsorption) K and n = Freundlich constants	This isotherm assumes that a fixed number of active sites are available to adsorb compounds. It also assumes that the active sites are uniform and are only able to adsorb one molecule per active site. It does not account for any interaction between the particles being adsorbed, or mass transfer limitations.
	Freundlich	$log\left(\frac{x}{m}\right) = log(K) + \frac{1}{n}log(C_{eq})$		This isotherm does not make the monolayer assumption as the Langmuir isotherm. The underlying assumption of this model is that active sites can bond with multiple adsorbate molecules.

The purpose of the present work is to assess the ability of the AGS technology to be applied to PPW. There are several parameters analysed in varying ambient conditions to fully comprehend the potential of this biotechnology in a multitude of environments. Additionally, some biodegradation studies and biosorption studies have been conducted. To the authors best knowledge, there is no study that has delved this deeply into this area of research.

2. Materials and Methods

2.1 Experimental Setup

Aerobic granules were developed in an SBR with a large height/diameter ratio to enable the growth of strong settling microorganisms. The reactor was of diameter 89 mm with a working volume of 4.5 L, at an exchange ratio of 50%. Influent was fed via an Aalborg TPU1 pump from the bottom of the reactor at controlled time intervals. These time intervals were controlled using NOMA ON/OFF indoor timers to regulate the SBR operation. Furthermore, aeration was provided using fine microbubble diffusers located at the bottom of the reactor such that it created an upflow air velocity of 2.2 cm/s. The settling time was gradually reduced from 20 min during start-up to 8 min to achieve mature aerobic granules. The experiments were conducted both in India and Canada. The operational setup was kept consistent during the experimental runs at the Indian Institute of Technology Bombay (IIT-B).

The reactor was seeded with inoculum from the Pine Creek Wastewater Treatment Facility located in Calgary, AB for the experiments conducted in Canada. The treatment plant employs a biological nutrient removal (BNR) system, and the seed sludge had an initial mixed liquor suspended solids (MLSS) concentration of 7200 mg/L, a sludge volume index (SVI) of 180 mL/g and a mean particle size of 110 μm. In India, the reactor was seeded with sludge from the Navi Mumbai Wastewater Treatment facility, which employs SBR operation. Therefore, the resulting SVI was slightly lower that of Canada at about 114 mL/g, with an initial MLSS of 5000 mg/L.

In both systems, prior to start-up, the sludge was allowed to acclimatize by leaving it under constant aeration with synthetic feed in batch mode for 2 days; this drastically improves the settleability by washing out any weaker microorganisms.

Real PPW studies were carried out by transferring waste granules (about 800 mL) from the SBR on day 60 to a 2 L batch column. The column was inoculated with PPW in step-wise increments prior to assessing the full capacity of treatment. The reactor was run on a 24-hour cycle, with a manual decant and filling routine being applied on a daily basis. The exchange ratio was also maintained at 50%. All other operational parameters such as air flow velocity were kept identical to the SBR.

2.2 Media

Synthetic wastewater was prepared according to a previous study by Tay et al. [13]. The recipe is as follows: sodium acetate (anhydrous) 2.93 g/L, NH_4Cl 350 mg/L, K_2HPO_4 30 mg/L, KH_2PO_4 25 mg/L, 94 $CaCl_2 \cdot 2H_2O$ 30 mg/L, $MgSO_4 \cdot 7H2O$ 25 mg/L, $FeSO_4 \cdot 7H_2O$ 20 mg/L, and microelement solution at 1.0 mL/L. Additionally, tannin/lignin, purchased from Sigma-Aldrich and VWR were added to the system at varied concentrations.

Pulp mill effluent from Millar Western Forestry Products was used for studies with real PPW. Millar Western employs a total chlorine free (TCF) process, and the effluent was retrieved after primary clarification. The chemical oxygen demand (COD) and total organic carbon (TOC) of the raw effluent was roughly 6700 mg/L and 2500 mg/L respectively, with total nitrogen (TN) of 18 mg/L, and phosphorus of 20 mg/L. This yielded a COD: N: P ratio of 100:0.27:0.30, thus signifying nutrient deficient conditions. The raw tannin/lignin concentration was 450 mg/L, with a total phenols concentration of 60 mg/L. The turbidity of the wastewater was around 4200 NTU, with an initial pH of 6.4 and was tinted brown due to the tannin/lignin compounds.

2.3 Analytical Methods

All samples were filtered using a 0.45 μm membrane filter prior to any analysis. COD, ammonia-nitrogen, total phenols and total phosphate were measured using HACH kits and a spectrophotometer (DR/2800). COD incubation was performed using the USEPA certified HACH COD incubator. Additionally, TOC and TN were analysed using a Shimadzu TOC and TN Analyser (TOC-L and TNM-L units). Biomass characteristics such as MLSS, MLVSS and SVI_{30} were determined according to the standard methods [14]. For SVI_5, a 5-min settling time was employed as described by Liu et al. [15].

Tannin/lignin were measured using the tyrosine method (tannin-lignin method). In this colourimetric method, a reaction occurs between tannin/lignin, sodium carbonate and the Tanni-Ver® solution (Folin phenol reagent) which results in a blue colour. The intensity of this colour is then measured at 700 nm and the results are reported as mg/L as tannic acid. There can be interferences in this method, from other phenolic compounds such as cresol and resin acid. However, in the SBR studies, these were not added to the system. As for the real wastewater, Millar Western determined that the concentrations of these compounds were minimal as compared to the tannin/lignin concentration.

2.4 Batch Adsorption Experiments

Adsorption experiments were carried out in 40 mL vials with 5mL of fresh granules, i.e. no tannin or lignin were added to cultivate the granules (corresponding MLSS = 19.7 g/L). The rest of the mixture was a 50% tannin and 50% lignin mixture, with dilution resulting in the following concentrations: 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L and 250 mg/L. The vials were shaken in a water bath shaker for 30 minutes at 160 rpm and 25 °C. Initial and residual concentrations were measured after filtering using 0.45 µm membrane filters and the results were analysed.

2.5 Batch Biodegradation Experiments

The ability of AGS to biodegrade mixtures with 50% tannin and 50% lignin was tested using a 2L column in batch mode with 20% of the reactor volume being granular biomass (corresponding MLVSS = 4885 mg/L). The targeted concentrations of tannin/lignin were: 50 mg/L, 100 mg/L, 250 mg/L, 400 mg/L, 500 mg/L and 1000 mg/L. The medium was as follows [16]: tannin/lignin, NH_4Cl, $MgSO_4 \cdot 7H_2O$, K_2HPO_4, and KH_2PO_4 at a weight ratio of 1:0.4:0.26:3.3:2.7.

The Haldane model has been famously used in many studies to describe the biodegradation kinetics in a biological system. It is used mostly to describe the effects of substrate inhibition. The ability of AGS to degrade inhibitory substrate has been described in several studies [16–18]. The equation is as follows:

$$V = \frac{V_{max}S}{K_s + S + \frac{S^2}{K_i}}$$

where, V (g substrate/ (g VSS · day)) and V_{max} (g substrate/ (g VSS · day)) are the specific and maximum biodegradation rates of the inhibitory substrate, respectively. S (mg/L), K_s (mg/L) and K_i (mg/L) are the inhibitory substrate concentration, the half-saturation concentration and inhibition constant, respectively. The specific biodegradation rate, V = (1/X·dS/dt), where X = mass of the biomass (MLVSS concentration).

2.6 Microbial Community Analysis

Genomic DNA was extracted using a DNeasy PowerSoil Kit from QIAGEN, Inc. (MD, USA). Paired-end sequencing based on the 16S rRNA gene was performed using the Illumina MiSeq platform with primers 357wF (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′), which covered V3–V4 hypervariable regions [19] . Sequencing data was analyzed using the R packages. Sequence trimming, quality filtering and merging were done using the DADA2 Pipeline [20] , as well as subsequent OUT tabulation, chimera removal and taxonomy assignment with the latest Silva taxonomic database (Silva version 132 [21]). The Phyloseq package was used to tabulate relative abundance at various taxonomic levels [22]."

3. Results and Discussion

3.1. Sludge Properties

There are several parameters that help in distinguishing the transition from flocculant to granular sludge, namely the SVI. Additionally, particle size over 200 microns is used as a general guideline for aerobic granules [23]. Fig. 2 portrays the granules during the different operational periods. It is interesting to note that by Day 90, the granule size increased dramatically, and granules were roughly 2-5 cm in size. Granules of this size are very rarely found in other works as it is noted that granules disintegrate if they become too large [24]. However, it seems as if a slimy layer, potentially extracellular polymeric substances (EPS) could have enveloped the granules to act as a buffer to the toxic compounds. Although this has not been proven in this study, there has been some work indicating that bound EPS (B-EPS) has the ability to act as a protector against toxic pollutants [25]. In a review by Nouha et al. [26], the authors indicated that EPS was also used to remove toxic recalcitrant compounds. The primary method proposed in an article by Jia et al. [27] to aid in the removal of toxic organic compounds is through the use of enzymes present in EPS such as oxidoreductase and hydrolase, which are capable of degrading polycyclic aromatic hydrocarbons (PAHs). Additionally, within the article, the authors go into detail about the ability for EPS and PAHs to come together by hydrophobic interactions, which could also be another pathway of degradation. Fig.2f displays the interior cross-section of the granule, wherein there are four distinct layers present. An anaerobic core, followed by an anoxic and oxic layer, and the fourth layer could potentially be a B-EPS layer. Additionally, a visible colour change of the aerobic granules was observed, starting from a light tan colour and gradually darkening as tannin and lignin adsorbed onto the surface. Eventually, the granule surface was saturated with the toxic compounds thus yielding a dark brown colour.

The first set of aerobic granules were spotted within 12 days of operation. Aerobic granules were cultivated within 9 days, with an average particle size well over 200 microns. The SVI profile changed significantly from about 250 mL/g on day 10, to less than 50 mL/g from day 60 onwards. The toxic environment caused the bacterial community to experience a slight shock during the start-up of the reactor. However, the system recovered rapidly and maintained a steady biomass concentration from there on.

Granulation percentage is another method used to describe the extent of granulation in the reactor. The formula is as follows:

$$Gran.\,\% = \frac{SVI_5}{SVI_{30}} * 100$$

Upon initial inoculation with seed sludge following an acclimation phase of 2 days, the granulation percentage was 52%, and gradually increased to 90% by Day 64 and stayed consistent throughout the operational period. Fig. 1 in the supplementary material shows the profile of the granulation percentage.

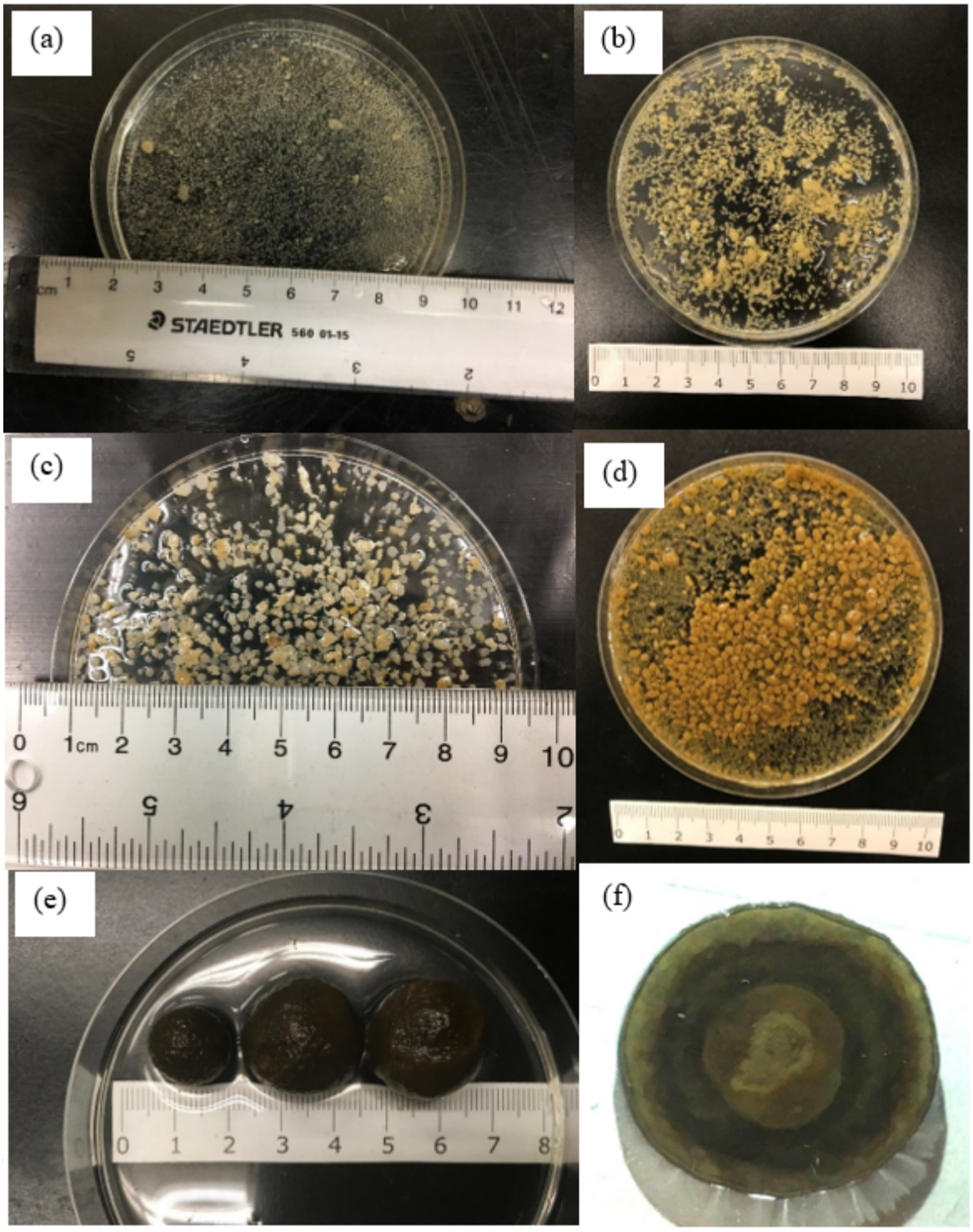


Fig. 2. Granules on (a) Day 9 (b) Day 16 (c) Day 30 (d) Day 60 (e) Day 90; (f) A cross-section of the granule on Day 90

3.2. Removal Characteristics in SBR

Removal profiles for the operational period of the SBR are shown in Fig. 3. The SBR showed promising removal with synthetic wastewater using sodium acetate as the primary carbon source, with the addition of tannin/lignin at an initial concentration of 50 mg/L. In this study, the aim was to cultivate granules in the toxic environment to create a tolerance for the compounds from raw seed sludge itself. This method has been employed in several toxic wastewaters and has proven to be effective [28,29].

The addition of tannin/lignin did not affect the removal of other organic components as seen by the COD removal efficiencies. The COD removal efficiency stayed well above 90% for the bulk of the experiments. TOC removal efficiency also remained relatively steady as the operational period continued, achieving an average removal efficiency of 94%. Sodium acetate is a readily biodegradable source, therefore the bacterial community inside the granules can easily use this carbon source as substrate. Although the removal characteristics of COD along with TOC were positive, a better indication of the performance can be garnered through analysing the degradation of tannin/lignin.

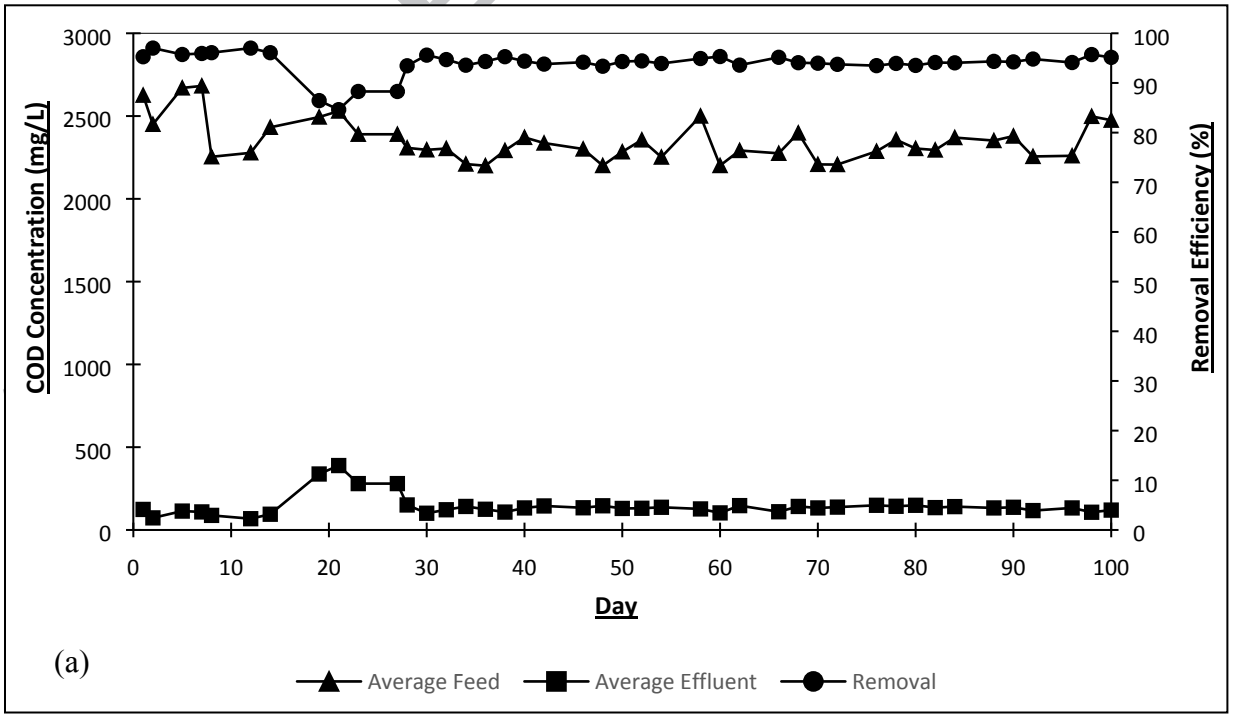
Ammonia-nitrogen removal did suffer slightly due to the increased toxicity in the environment. The average removal efficiency was 62%, which is substantially low. This is an indication of the bacterial community responding to a toxic environment with recalcitrant compounds. However, Zhang et al. [30] has suggested spiking the system with a moderate carbon source to improve the reactor performance, particularly COD and NH_4^+-N removal. Using sodium propionate along with sodium acetate as carbon sources proved to be effective in increasing the removal efficiency significantly. Therefore, to improve the removal efficiency of nitrogen, a co-metabolic pathway could be introduced as a potential remedy.

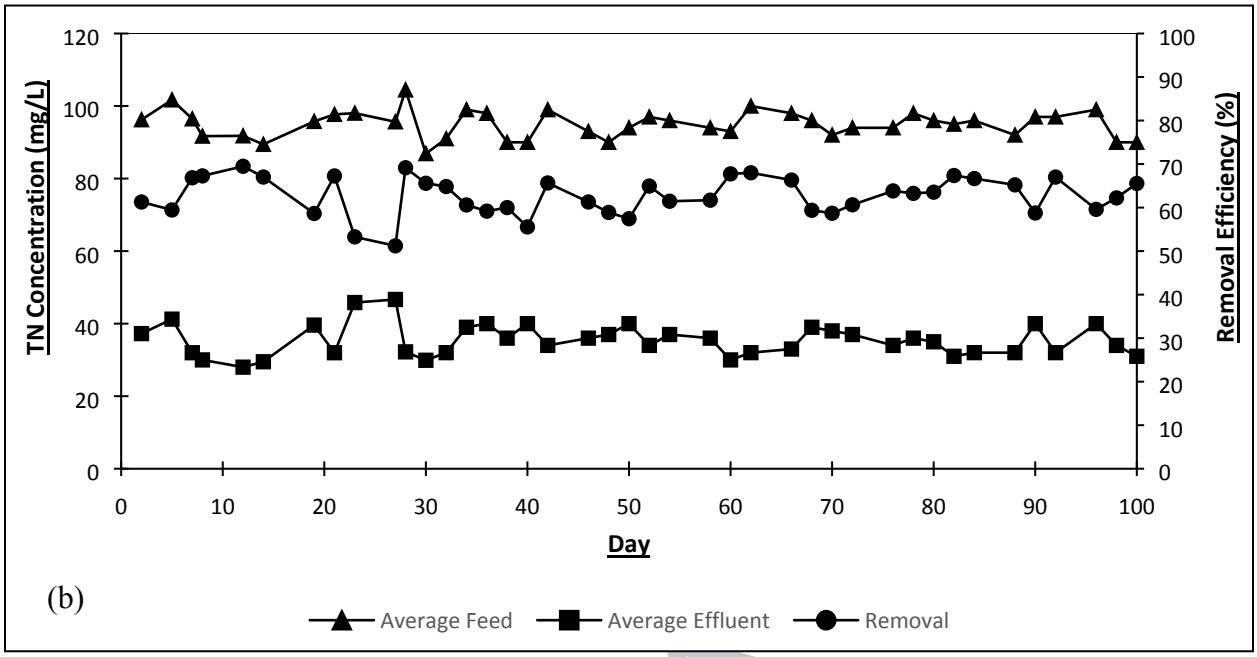
Phosphorus removal remained steady at around 50%, achieving a maximum removal of 60% during the initial stages of granulation. Toxic environments tend to inhibit the nutrient removal process. If the ratio of bacterial biomass is to be taken into consideration, Phosphorus removal could be improved by employing an anaerobic feeding cycle to induce the release of phosphorus, prior to luxury uptake during the aerobic cycle. Although, the granulation time increases as a downside of the process, it stabilizes the reactor and may have resulted in better phosphorus removal efficiencies [31]. Another method suggested by Angela et al. [32] involves the use of

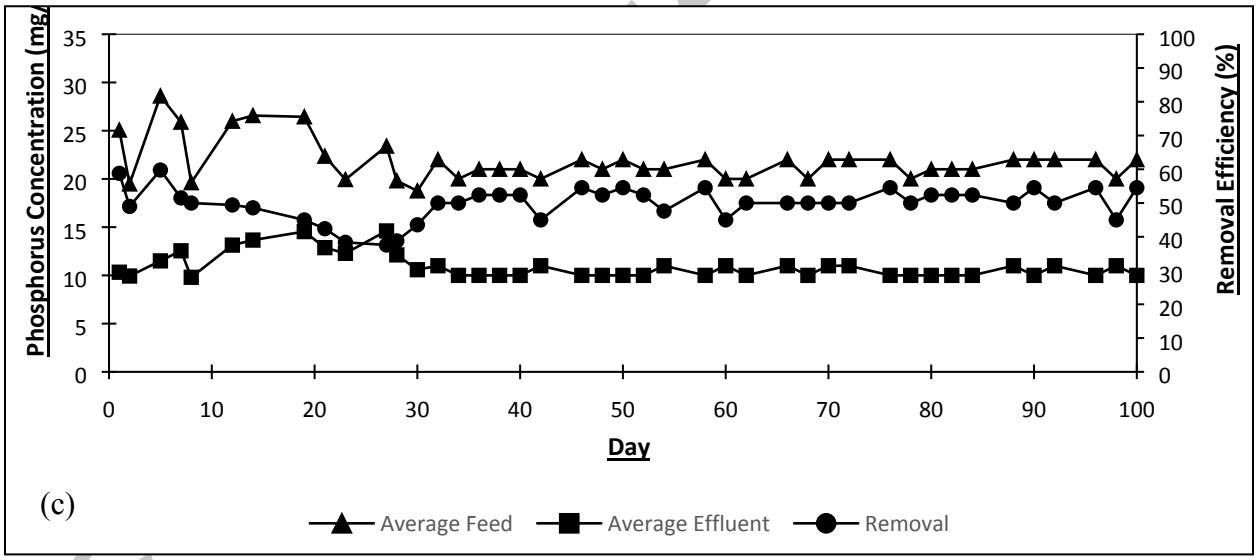
high concentrations of calcium ions to induce the precipitation of calcium hydroxyapatite ($Ca_5(HPO_4)_3OH$) within the granule under alkaline conditions. However, this method is highly dependent on the pH, temperature, ionic strength and ratio of Ca/P and introduces more complexities into the system.

A likely theory that can provide some explanation as to the removal of nitrogen and phosphorus in the wastewater is assimilation. Both nitrogen and phosphorus can be removed by assimilation of heterotrophic bacteria. For every 100 g of COD removed, one can expect between 3-5 g of nitrogen, and 0.8 g of phosphorus to be removed by the biomass. These values are calculated by knowing taking into consideration the cell yield (about 0.65) and biomass formula, $C_5H_7NO_2P_{0.074}$. Therefore, with a COD concentration of around 2000 mg/L, about 60 mg/L of nitrogen and 16 mg/L of phosphorus is removed by the biomass, roughly accounting to about 66% and 36% removal of nitrogen and phosphorus, respectively.

Tannin/lignin were removed up to 80% during the initial stages, but slowly decreased to an average removal of 70%. Biosorption was a primary removal mechanism at the onset of granulation. However, as time proceeded the adsorptive capacity decreased and therefore the removal efficiency suffered. The latter sections of this study will further identify this removal characteristic.







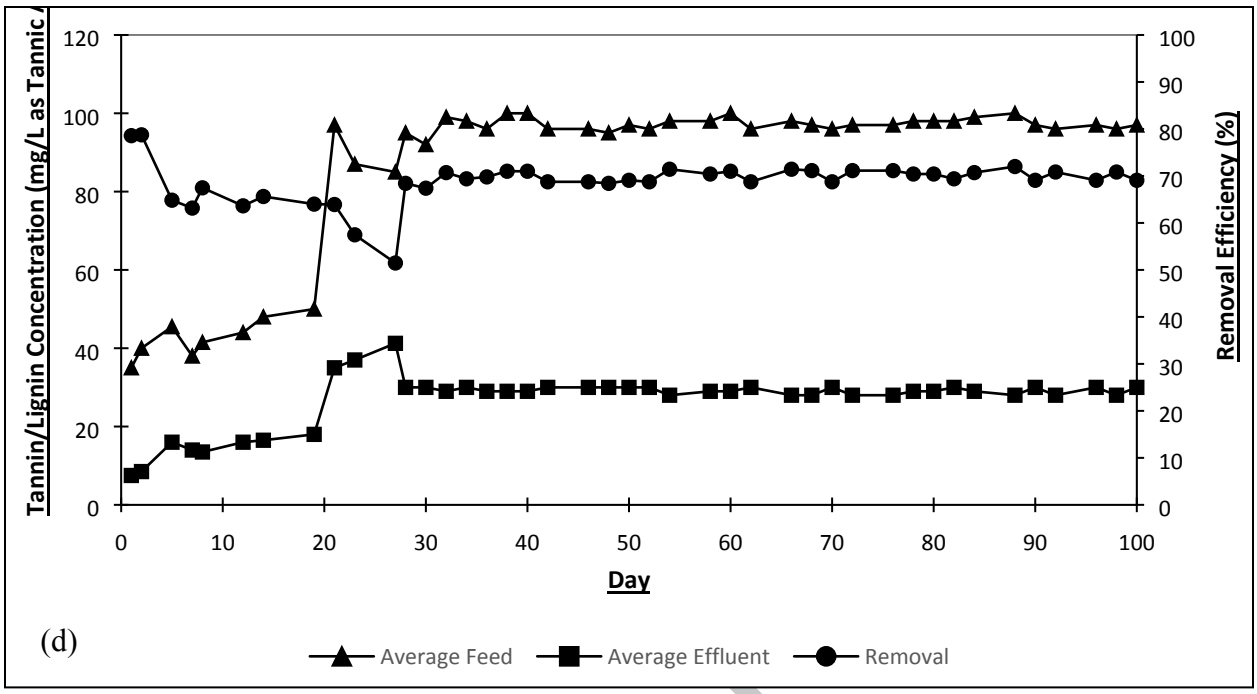


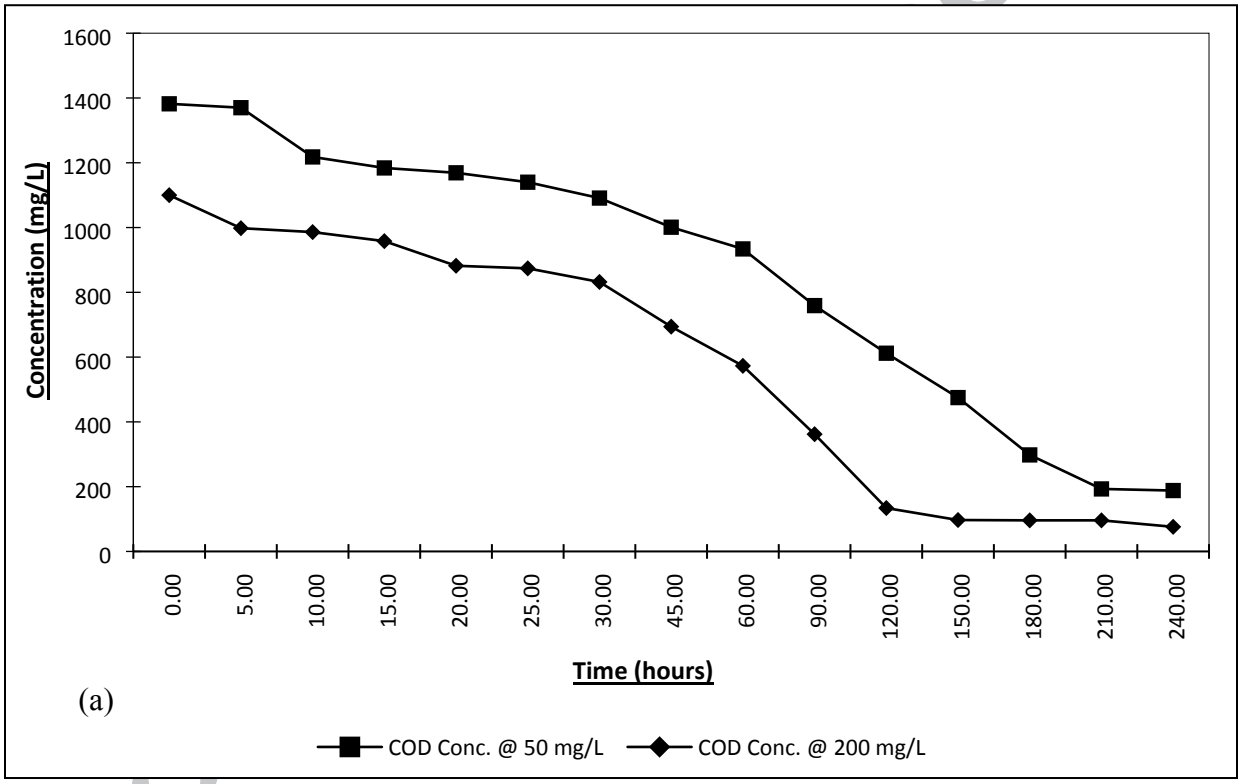
Fig. 3. Removal profiles for (a) COD (b) TN (c) Phosphorus (d) Tannin/lignin

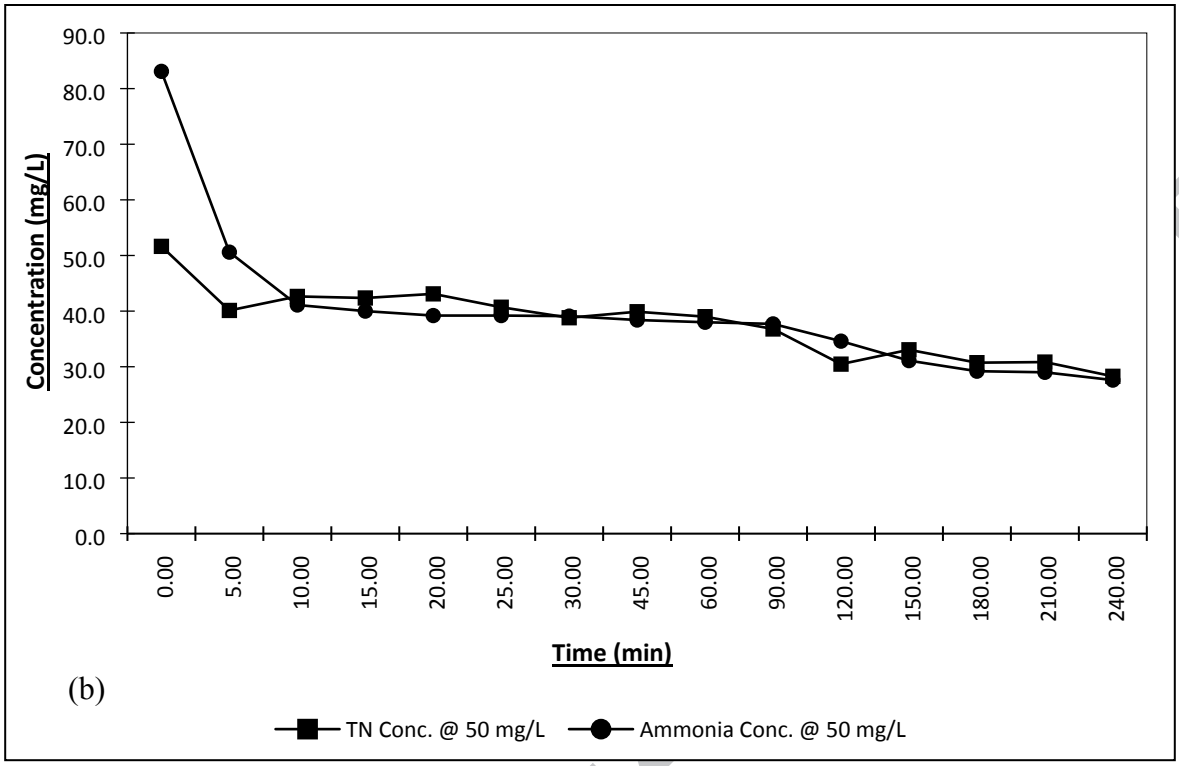
Fig. 4 displays the removal characteristics of different compounds in a single cycle. It is observed that initially, the COD removal drastically improved in both scenarios (50 mg/L and 200 mg/L tannin/lignin) due to the volumetric exchange ratio of the SBR reactor. Thereafter, a steady decrease was observed. The profile at 200 mg/L of tannin/lignin displayed substantial o organic content removal. The system was already well acclimated to the toxic environment at this point, therefore the removal efficiency was slightly better.

Both the ammonia-nitrogen and TN removal in a single cycle displayed similar profiles. Similarly, the phosphorus removal decreased from 49.2 mg/L at t=0, to 38.9 within the effluent. As mentioned previously, the nutrient removal could be further improved if an anaerobic cycle were to be introduced. The cycle performance would have exhibited a rising trend during this phase, and then a rapid decrease during the aerobic phase. In both scenarios, the removal efficiency at 200 mg/L of tannin/lignin was slightly better than at 50 mg/L. This is again attributed to the fact that the bacterial community responsible for nutrient degradation was slightly more acclimatized to its environment. In both cases, the nutrient amount removed was mostly attributed to the growth requirement of heterotrophic microorganisms capable of degrading COD and tannin/lignin. There was very minimal development of phosphorus

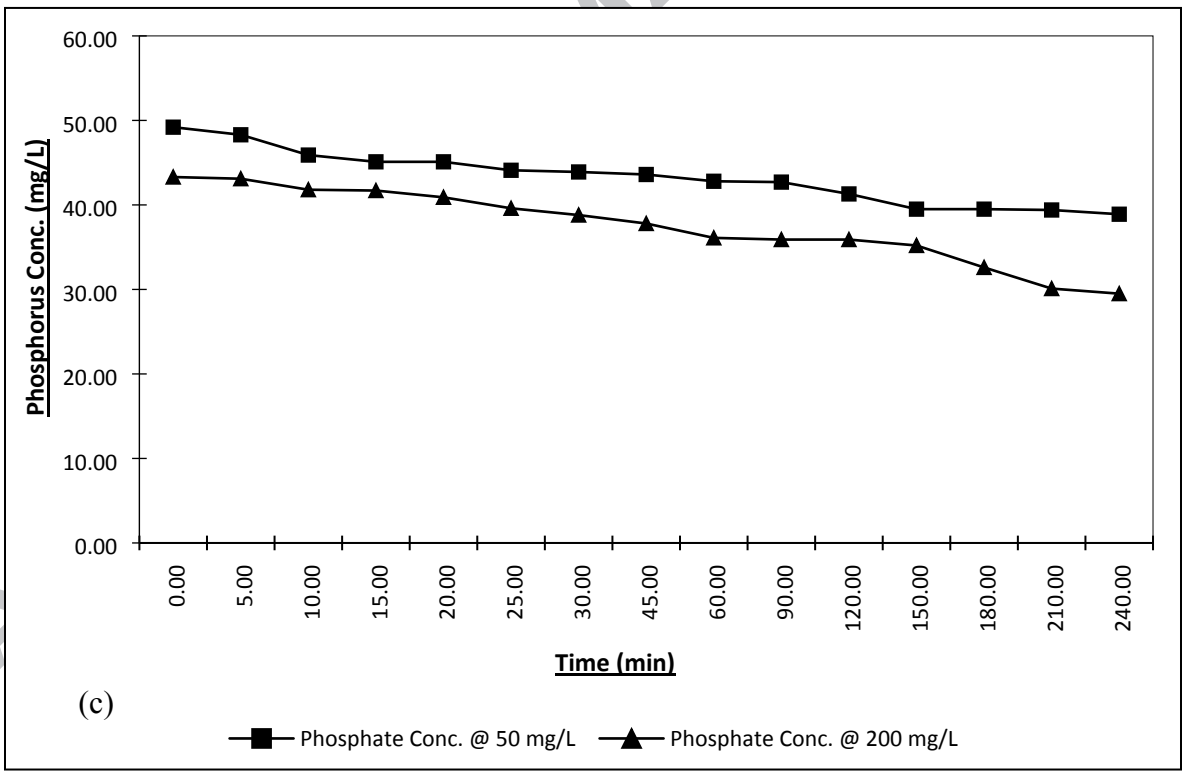
accumulating organisms (PAOs) or glycogen accumulating organisms (GAOs) which would have further improved the removal [33].

The most important parameter to delve into is the tannin/lignin concentration profile. At t=0, there was a substantial decrease in the concentration of tannin/lignin at 200 mg/L as compared to 50 mg/L. This is most likely attributed to biosorption within the first few minutes of the cycle. Biosorption was more prevalent at 200 mg/L because the MLSS concentration was higher during this time; therefore, the adsorption capacity of the sludge was higher. The latter sections of this paper will delve into the biosorption potential of AGS in more detail.





(b)



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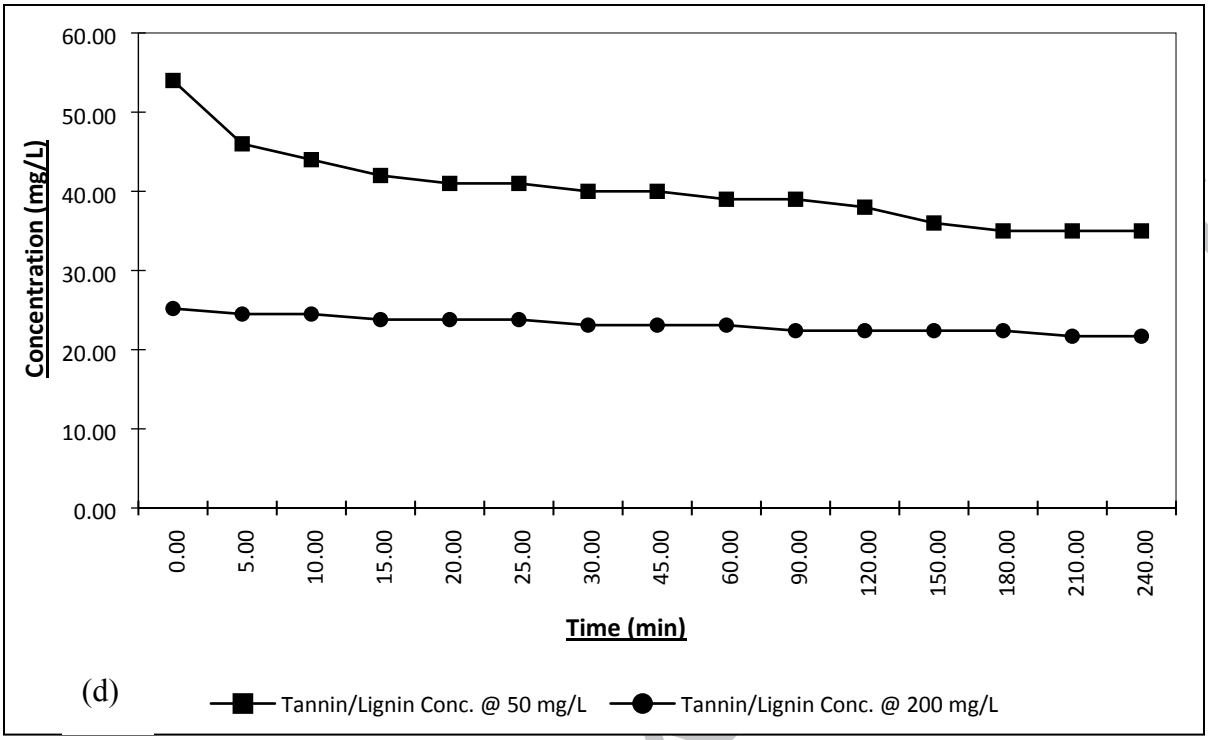


Fig. 4. Removal of (a) COD (b) TN and ammonia-nitrogen (c) phosphorus (d) tannin/lignin at 50 mg/L of tannin/lignin and 200 mg/L

3.3 Removal Characteristics in SBR in Different Temperature Environments

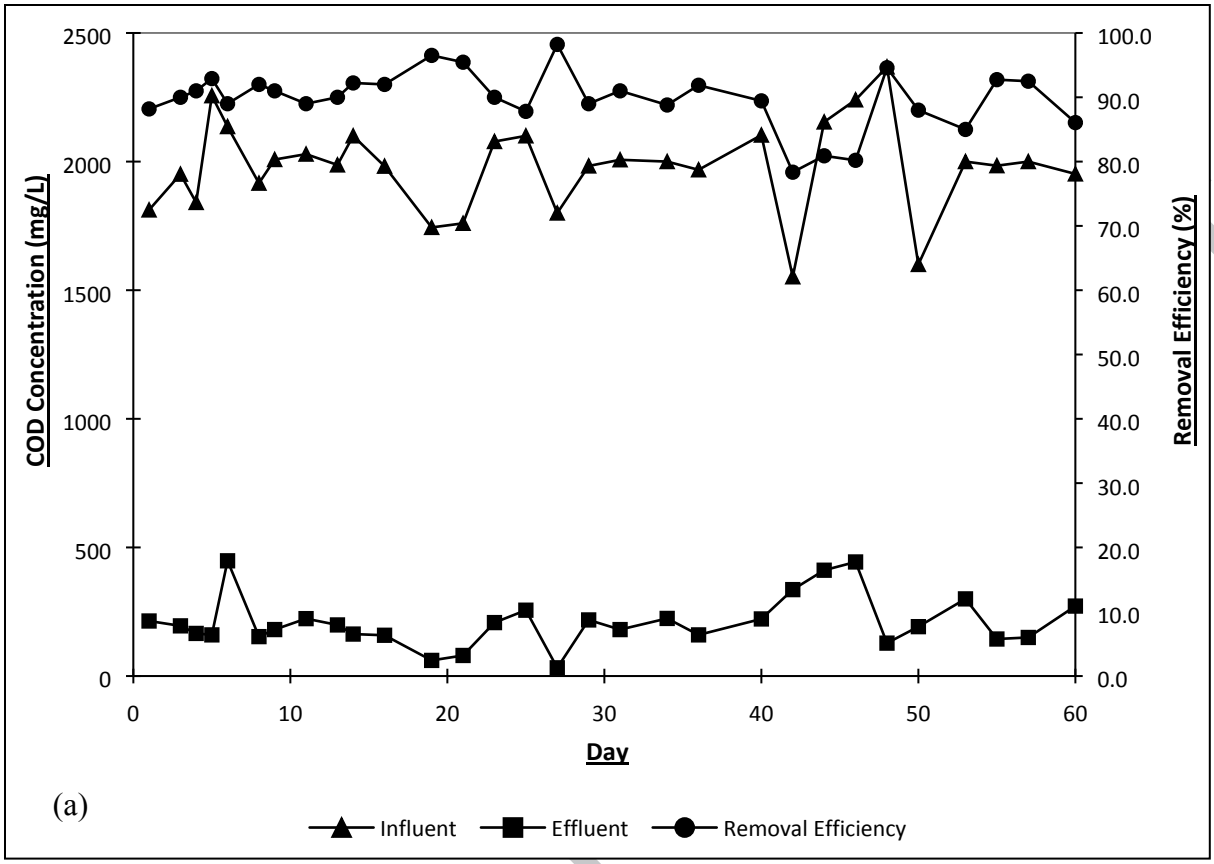
Experiments were conducted at the Indian Institute of Technology – Bombay (IIT-B) to test the effects of a different temperature environment for the treatment of tannin/lignin. The temperature range during the period of operation was between 35 – 39 °C. The humidity during this time period was around 84% - 89%, which was significantly higher than Calgary, Canada where the temperature range during the experimental run was around 20 – 22 °C, but the humidity was much lower, ranging from 31 – 33%. Additionally, the bacterial community in the seed sludge of these environments are quite different as the abundance of certain species capable of degrading phenolics and other toxic compounds will be higher in tropical environments. *Pseudomonas* grows well at 37 °C while *Flavobacterium* is most active at temperatures above 30 °C [34]. Both of these species are capable of degrading phenolic compounds [35].

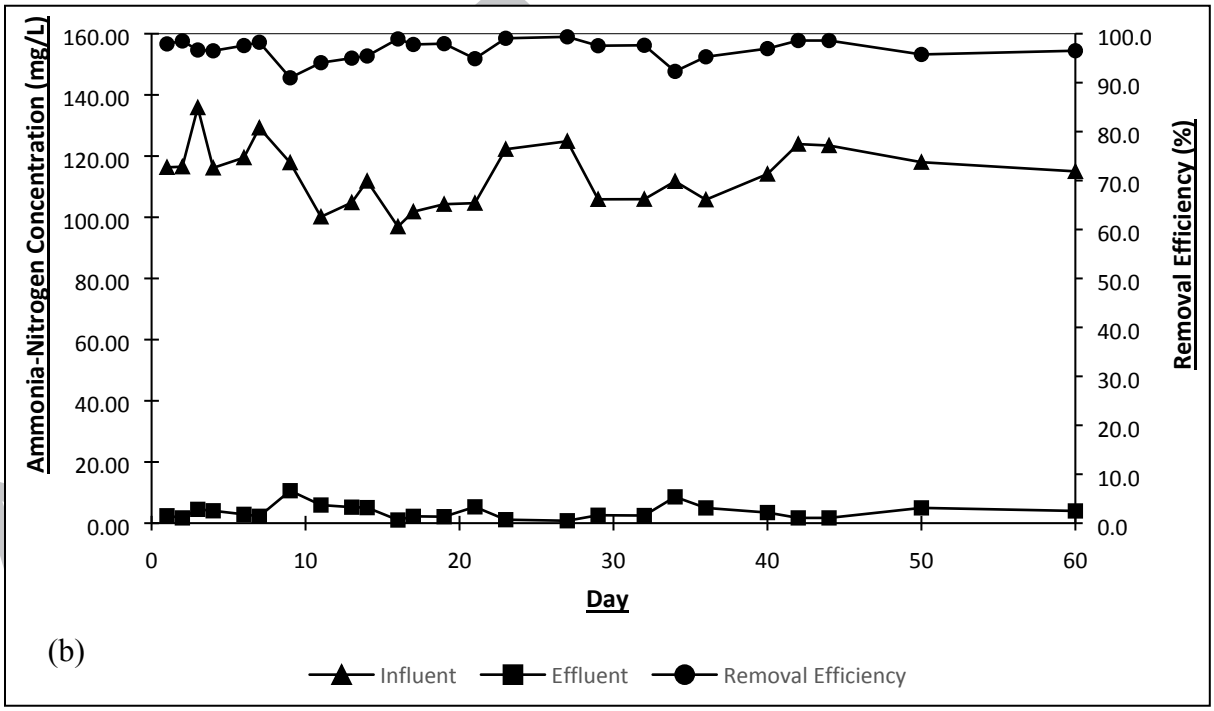
The seed sludge was obtained from a full-scale SBR plant, and upon initial inoculation to the system, the sludge exhibited moderate settling characteristics. However once high shear stress conditions were introduced, the system experienced major washout decreasing the MLSS in the reactor to about 3500 mg/L. The reactor also experienced the growth of fungus in the system

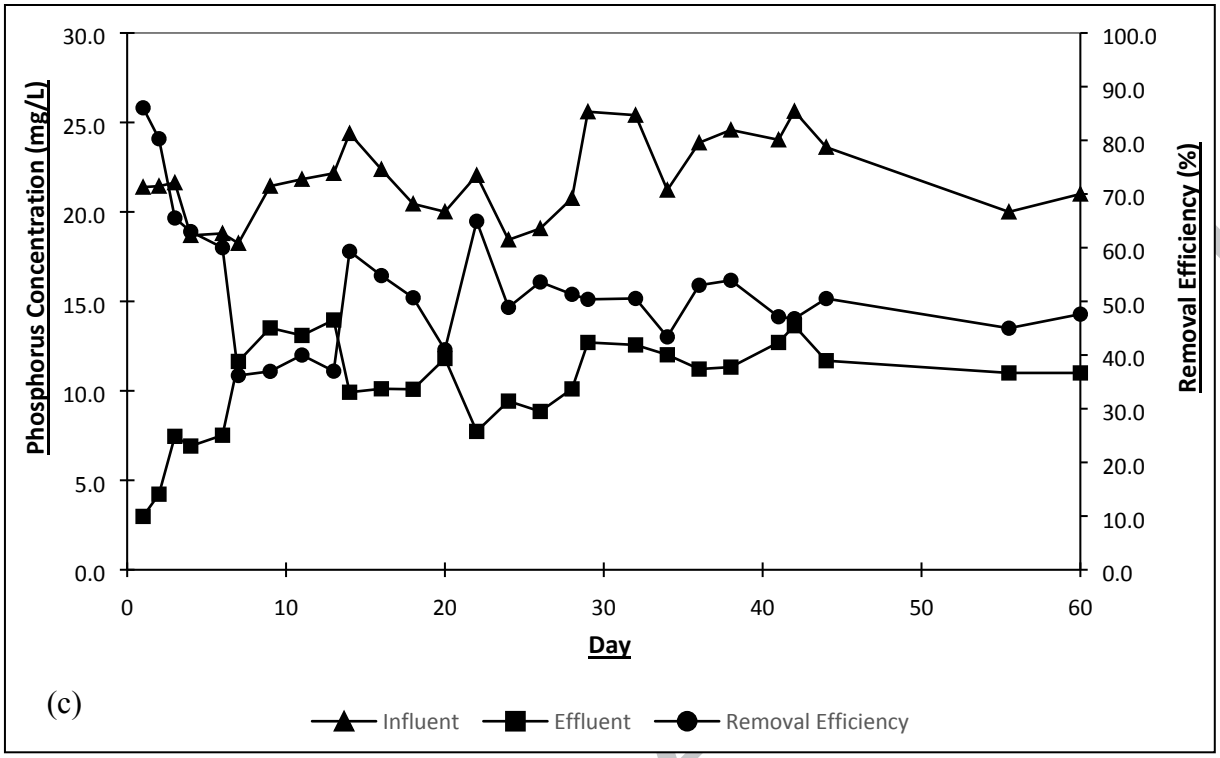
within the first 3 days of operation. The humidity in the environment lead to the growth of fungus in the reactor, and at first it seemed to hinder the system. In fact, the MLSS dipped down to 2300 mg/L. However, the reactor was left untouched and soon enough there was a drastic recovery with the MLSS reaching 12,000 mg/L within two weeks. It was also at this stage that granulation started occurring. There is a vast amount of literature on the symbiotic relationship between different fungal species and AGS [36,37].

Tannin/lignin were added to the system once steady-state conditions of the granular reactor had been achieved. The influent concentration started from roughly 20 mg/L of tannin/lignin, and eventually reached a concentration of 130 mg/L. The removal efficiency during this time was astonishing, achieving removals of 82% at a concentration of 130 mg/L. This signifies that the reactor was more successful in tropical environments as the community present within the granules was abundant in tannin/lignin degrading microorganisms. Additionally, although fungi tend to provide a structural backbone for aerobic granules, it is also a common species used towards the degradation of tannin/lignin. Wu et al. [38] used white-rot fungi to degrade lignin up to 71 % at a concentration of 4.6 g/L. This may explain the ability of the granular sludge in Mumbai to remove these toxic compounds more effectively.

During the operational period, the COD removal efficiency averaged 90% at an initial concentration of 2000 mg/L, while the ammonia-nitrogen and phosphorus removal efficiencies averaged 97% and 52% respectively. The profiles of COD, ammonia-nitrogen, phosphorus and tannin/lignin removal are shown in Fig. 5.







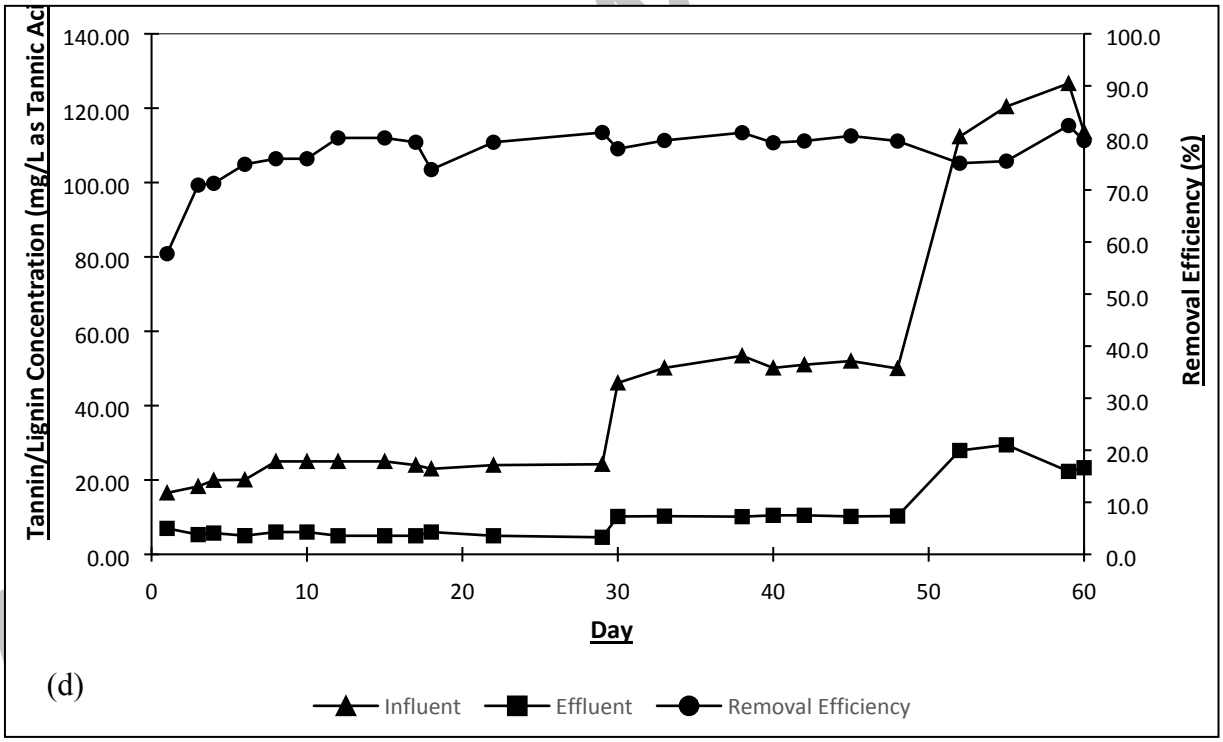


Fig. 5. Profiles of removal for (a) COD (b) ammonia-nitrogen (c) phosphorus (d) tannin/lignin for SBR in IIT-B

3.4. Treatment Efficiency with Real PPW

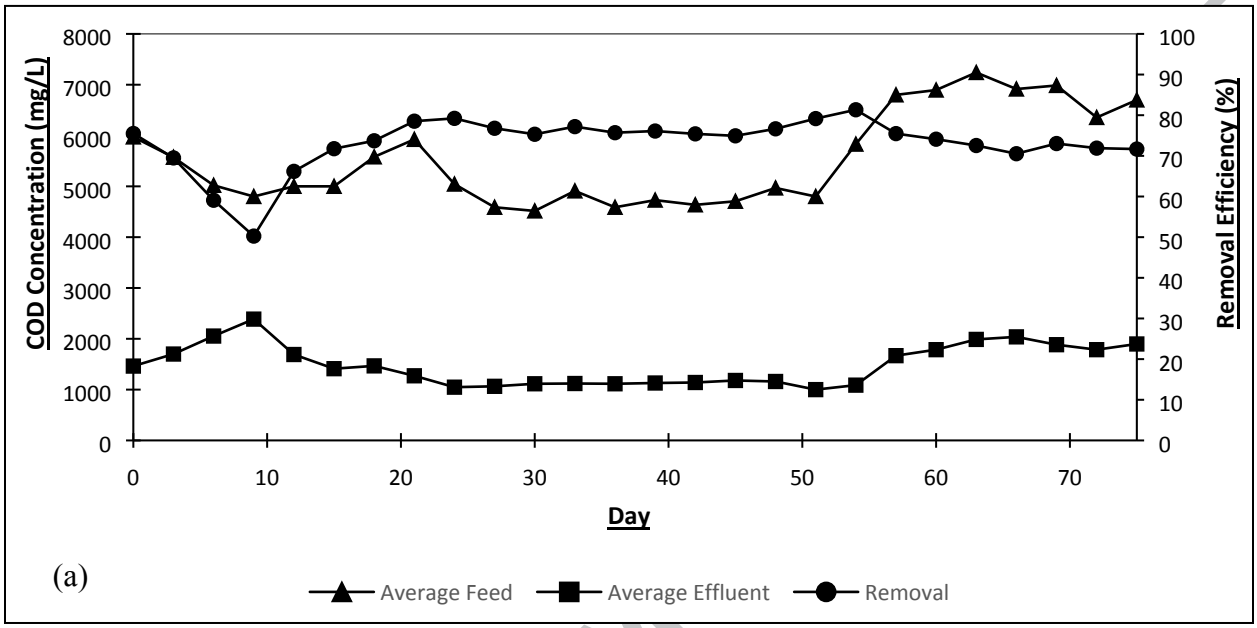
Granules cultivated in the SBR in Calgary, Canada were transferred into a 2L column for studies with real PPW. The operating conditions were kept consistent with the SBR except for the retention time, which was kept at one day. The acclimatized granules were immersed into real wastewater with no dilution. Initially, there was some disintegration of the granules as real wastewater tends to have more complexities than synthetic wastewater. However, the system did recover after a two-week operational period. After the initial washout and recovery period, the removal of tannin/lignin along with other parameters were recorded. Aerobic granules were able to survive for the duration of the experiments and removed significant amounts of organic matter, especially tannin/lignin.

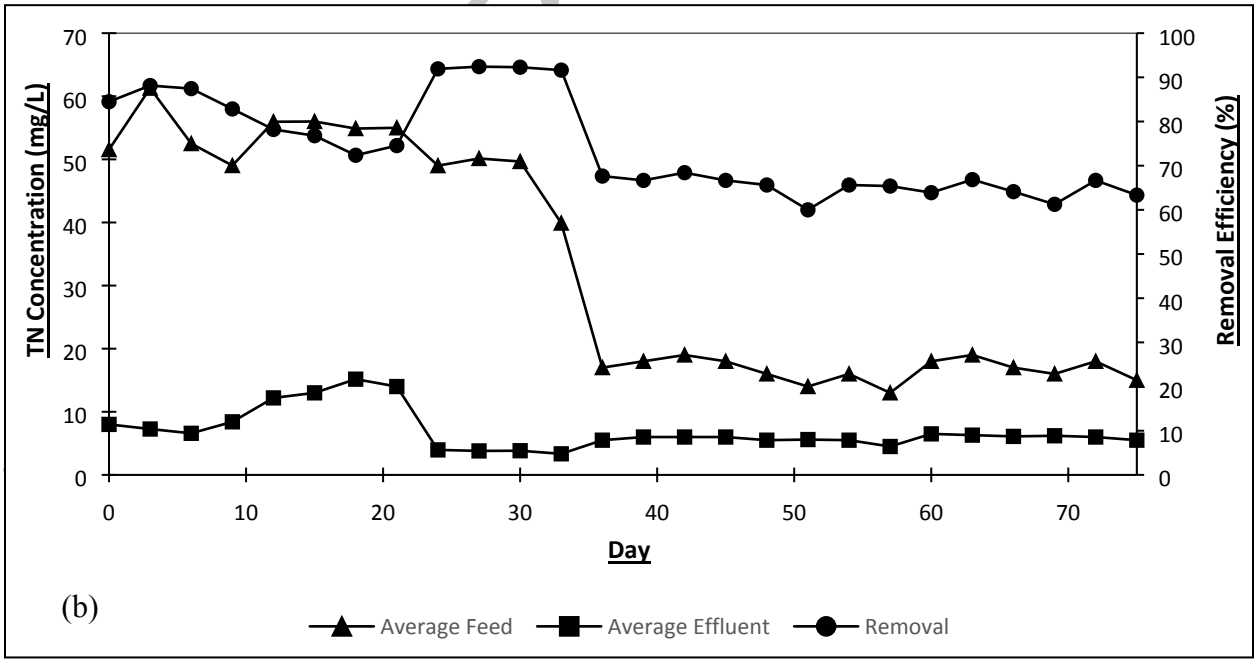
The COD and TOC removal efficiency averaged 73% and 79%, respectively. COD removal with activated sludge for pulp mill effluent ranges anywhere from 57 – 71% [1]. This is consistent with the removal efficiencies of granular sludge. In this study, turbidity was also measured as PPW tends to be high in suspended and dissolved solids [1]. On average, the turbidity decreased by 96%, which is not surprising as aerobic granular sludge technology is known for its solid-liquid separation ability [39]. This makes this technology more attractive as it minimizes processing costs further downstream.

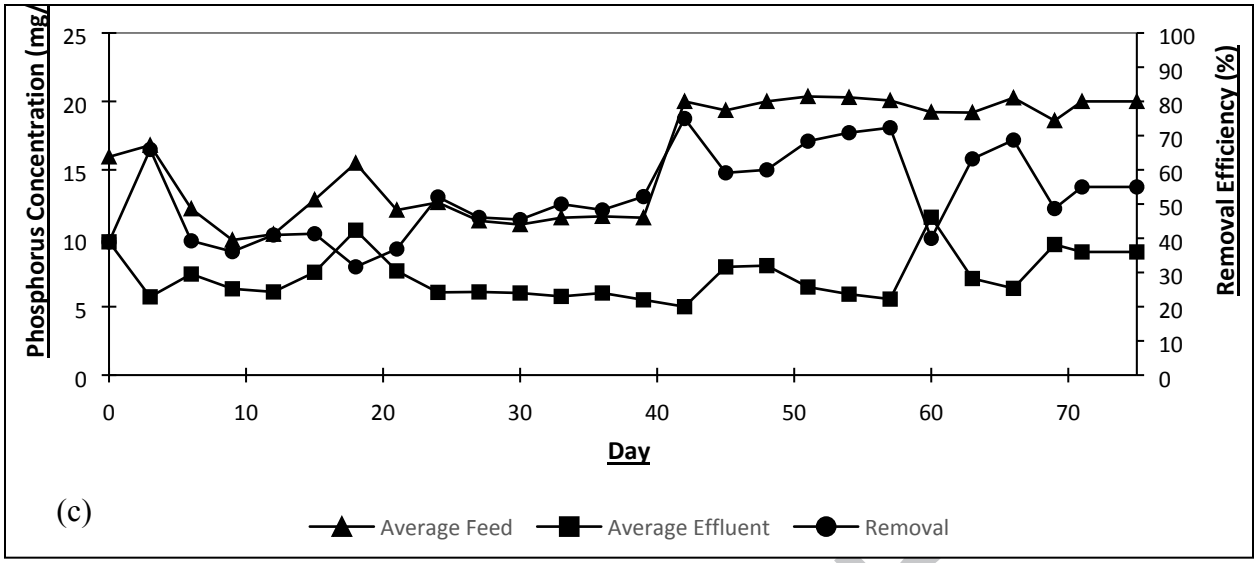
The nutrients removal potential has already been described in detail in previous sections. In real wastewater, similar scenarios were observed, wherein phosphorus removal averaged 52%. The removal of nitrogen was significantly better averaging 74%. Synthetic wastewater contained ammonium chloride as a primary nitrogen source, however in the case of real PPW, nitrogen can enter the wastewater through plant biomass. Additionally, the nitrogen sources may be bound to toxic organic compounds and are therefore more difficult to remove. Other complexities in the system may also interfere with the ability to remove nitrogen and phosphorus. Further work is required to optimize the removal potential of nutrients in PPW.

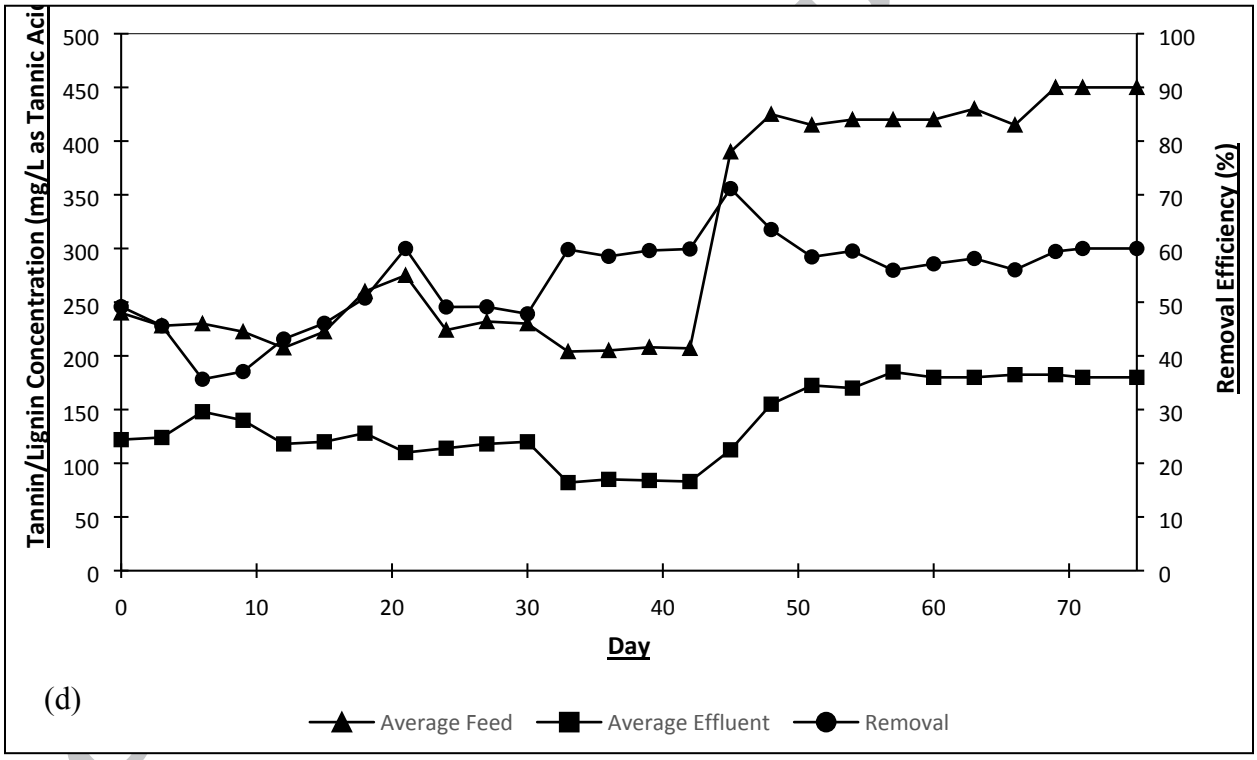
Tannin/lignin can bind with other chemicals in the cooking process, making it more diverse and complex than in synthetic wastewater [6]. Therefore, the average removal efficiency was only 54%, with the highest removal efficiency being 71%. The average phenols removal efficiency was 70%. In a study by Assalin et al. [33] , the total phenols removal was only 52.3% in a treatment system with activated sludge and ozonation [40]. Tannin/lignin removal using

activated sludge has remained quite low as the species present are incapable of degrading these compounds at such high concentrations. The profiles of COD, TN, phosphorus, tannin/lignin, phenols and turbidity removal are shown in Fig. 6.









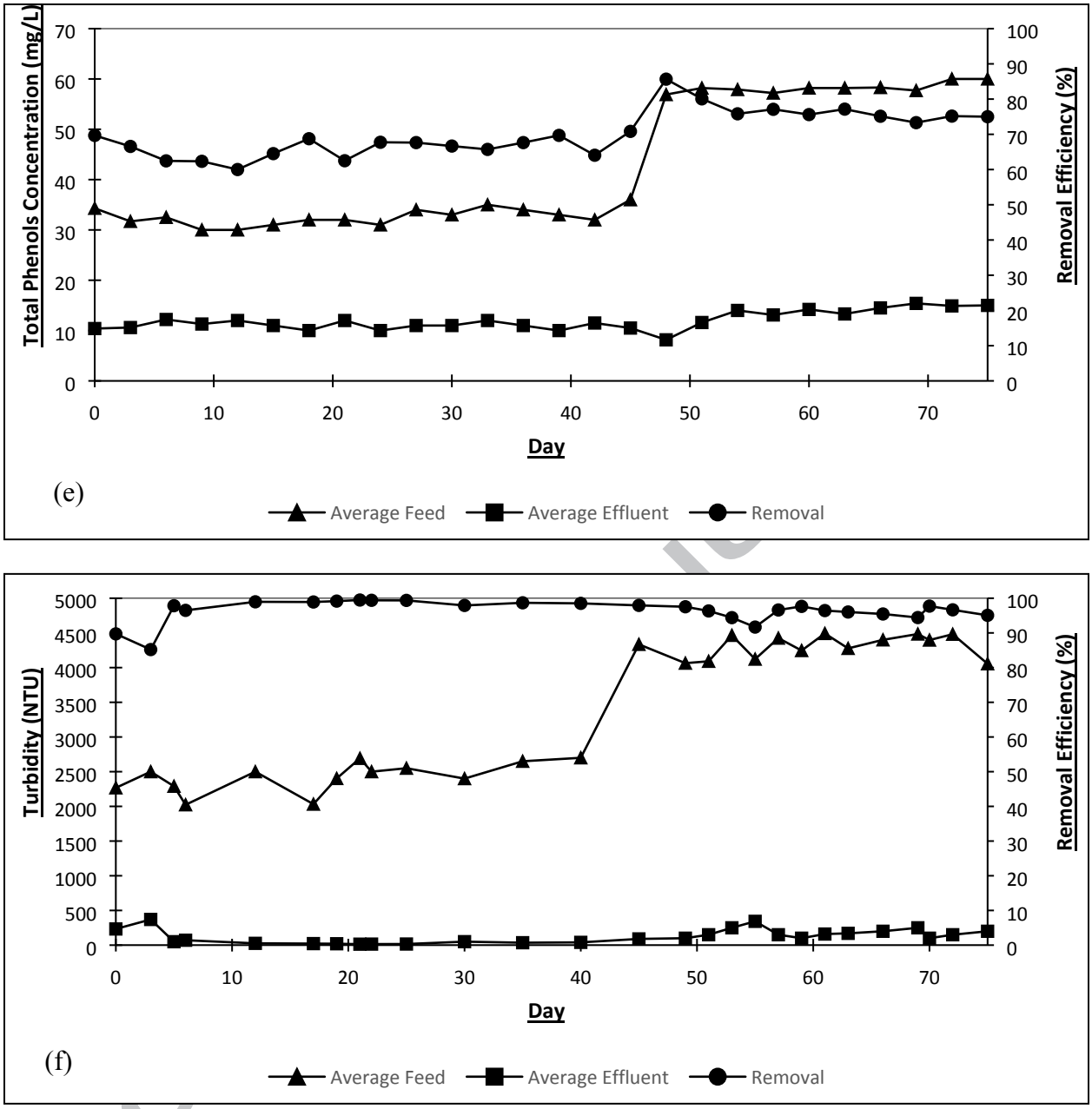
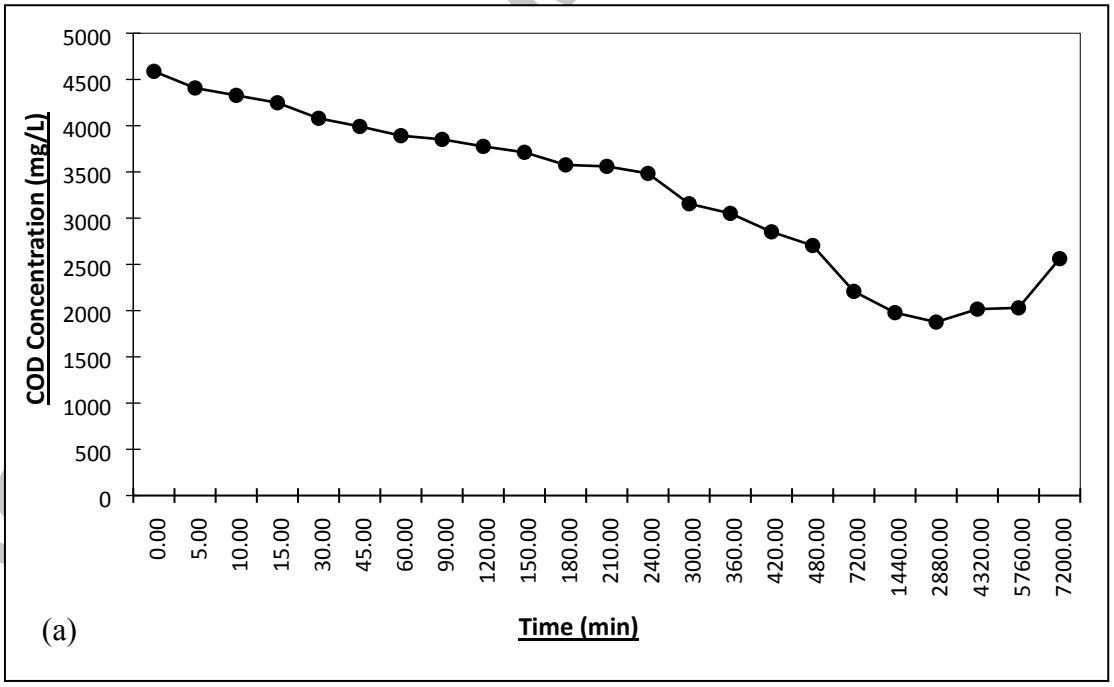
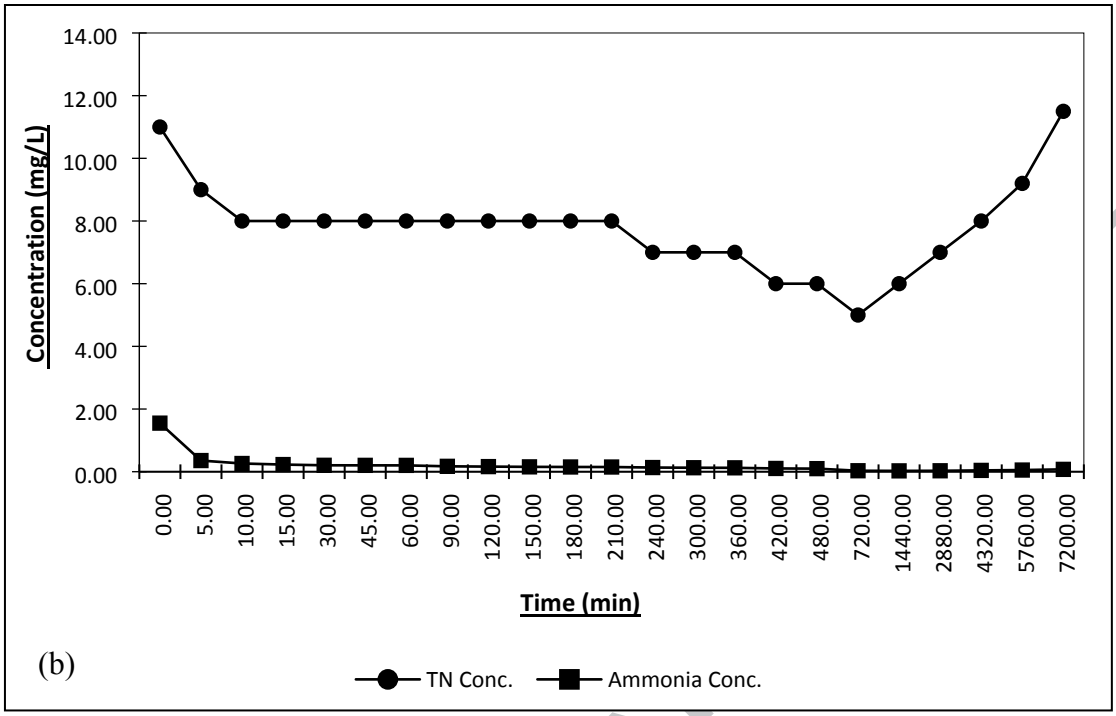


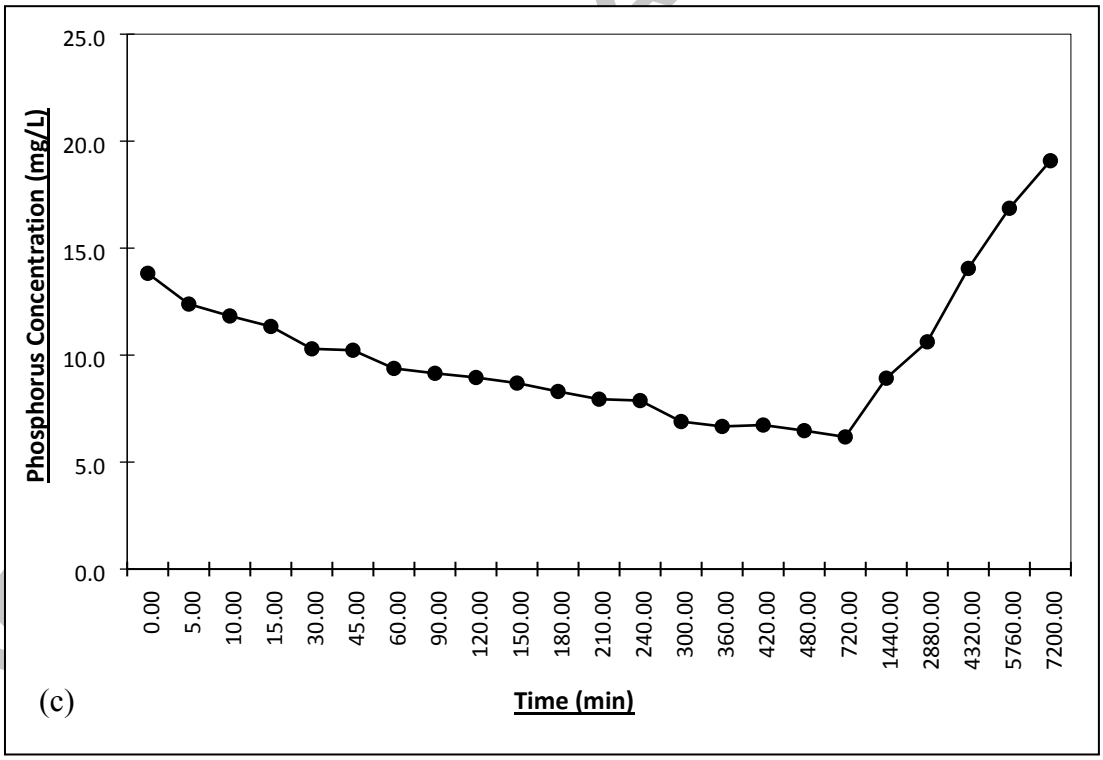
Fig. 6. Removal profiles for (a) COD (b) TN (c) phosphorus (d) tannin/lignin (e) phenols (f) turbidity

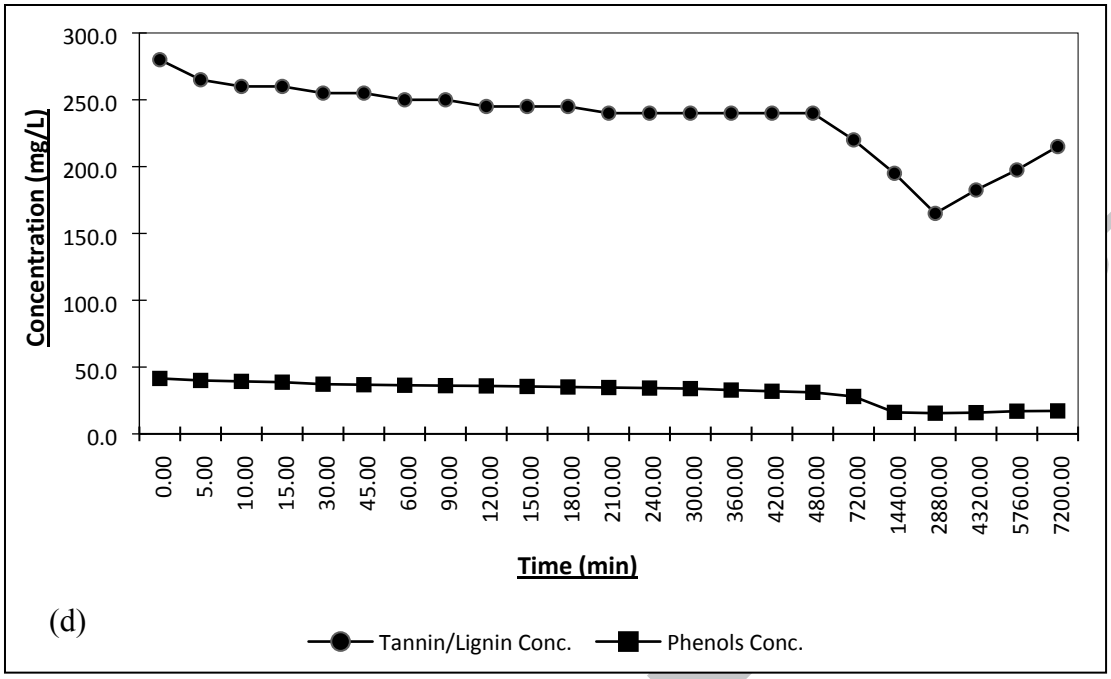
The study was further extended by looking into the removal over a longer time period. The removal profiles over a 5-day period are reported in Fig. 7. It was observed that over a 5-day period, the TOC and COD removal efficiency decreased steadily achieving a maximum removal of 80% and 75%, respectively, after 2 days of continuous operation. The ammonia-nitrogen and total nitrogen removals, along with the phosphorus profile also followed a similar trend. However, since the initial ammonia-nitrogen concentration was much less than the total nitrogen

concentration, the effects are not as visible. Tannin/lignin, along with the total phenols concentrations, also achieved its maximum degradation after 2 days of operation, with removals of 54% and 76% respectively. After 2 days, there was an increase in the concentration of all parameters except nitrogen and phosphorus, which started increasing after 0.5 days. This is attributed to granule disintegration. Biomass is usually given the formula $C_5H_7NO_2P_{0.074}$ [41], therefore when biomass disintegrates in the system, it releases these compounds back into the wastewater as they were assimilated by the biomass as part of their growth requirement. However, due to granule disintegration, the soluble components that were assimilated by the bacterial community were now released back into solution upon disintegration. Because there was no sludge wasted from the reactor (batch test), the bacterial community that disintegrated was not allowed to leave the system. Therefore, it is only natural that in a closed system, when the MLSS concentration decreased, the nutrient concentration increased because there was no further uptake of nitrogen and phosphorus for biological growth. Additionally, tannin/lignin were also released back into solution as any adsorbed compound will dissolve in solution upon disintegration of the adsorbate.









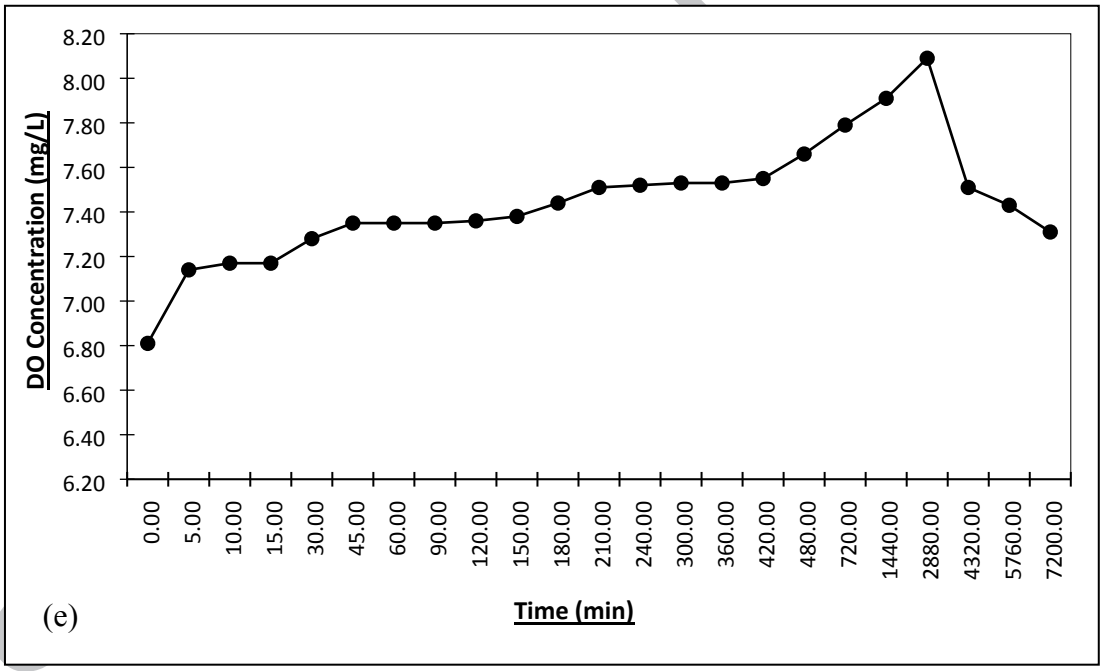


Fig. 7. Removal profile over a 5-day period of (a) COD (b) TN and ammonia-nitrogen (c) phosphorus (d) tannin/lignin and phenols (e) dissolved oxygen (DO) for real PPW

3.5. Batch Biodegradation Kinetics

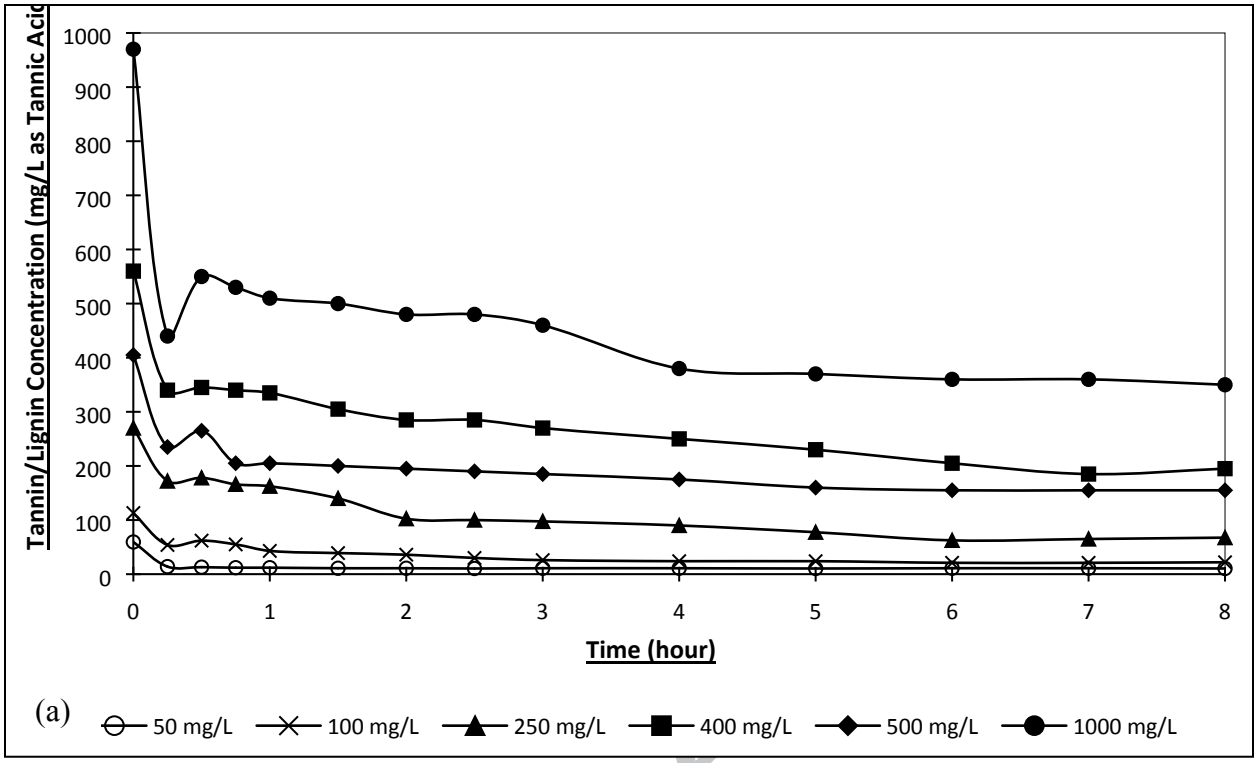
The ability of aerobic granules to degrade tannin/lignin were evaluated by monitoring the profile in batch samples over an 8-hour period. The MLSS was taken from the SBR reactor at day 55, wherein the volatile biomass concentration was 4.89 g/L. The degradation pathway is shown in Fig. 8a. The degradation profile decreased significantly within the first 30 minutes of the experiments, which is most likely attributed to biosorption onto the granule surface. Thereafter, there was a release of tannin/lignin back into solution, and the rest of the removal profile was dominated by biodegradation. Tannin/lignin was degraded more efficiently at lower initial concentrations than at higher concentrations. The experimental results were fit to the Haldane model, which describes substrate inhibition for toxic compounds, using MATLAB's Curve Fitting Tool. The resulting fit yielded $R^2 = 0.9551$ and RMSE = 1.3781×10^{-4}.

Fig. 8b shows the degradation rate versus the concentration from an initial concentration of 50 mg/L to 1000 mg/L. The specific degradation rate increased with increasing concentration up to about 270 mg/L, and then decreased gradually up to 1000 mg/L. At its peak, the maximum specific degradation rate (V_{max}) was 0.93 g tannin/lignin/ (g VSS · day). The inhibitory effects of tannin/lignin were experienced after 270 mg/L, and is signified by the inhibition constant, K_I = 27 mg/L. The half-saturation constant, K_s = 1910 mg/L. According to the Haldane model, a high K_s value and lower K_I value signifies that aerobic granules had a relatively low tolerance against tannin/lignin [42]. This can also be observed in the concentration profiles where in there was a rapid decrease in the concentration initially, followed by a gradual decrease until the end of the experiments. Therefore, it can be concluded that biodegradation may not be the most optimal form of removal for aerobic granules, and that perhaps biosorption may be more appropriate.

The main bacterial species responsible for tannin/lignin degradation are: *Pseudomonas*, *Corynebacteriaceae* and *Flavobacterium* [43]. These species have also been known to degrade other complex aromatics as well. Haldane kinetic parameters are listed in Table 2 with other phenolic compounds using a variety of biomass to compare the biodegradation ability.

Table 2. Haldane model parameters compared with other similar studies

Substrate	Concentration Range (mg/L)	Biomass Type	Haldane Model			Reference
			V_{max} (g/ (g VSS· day))	K_s (mg/L)	K_I (mg/L)	
Tannin/lignin	50 – 1000	Aerobic Granular Sludge (Mixed Culture)	0.93	1910	27	This study
p-cresol	100 – 800	Aerobic Granular Sludge (Mixed Culture)	2.97	490	210	[17]
2,4-DCP	50 – 300	Aerobic Granular Sludge (Mixed Culture)	11.472	587	19.1	[44]
PNP	10 – 300	Aerobic Granular Sludge (Mixed Culture)	0.876	17.9	89.7	[45]
Phenol	500 – 2500	*Corynebacterium* sp. DJ1 aerobic granules	15.744	33.1	1470	[42]
Kraft Lignin	50 – 15,000	*Acetoanaerobium* sp. WJDL-Y2	0.567	2206.97	8120.45	[46]



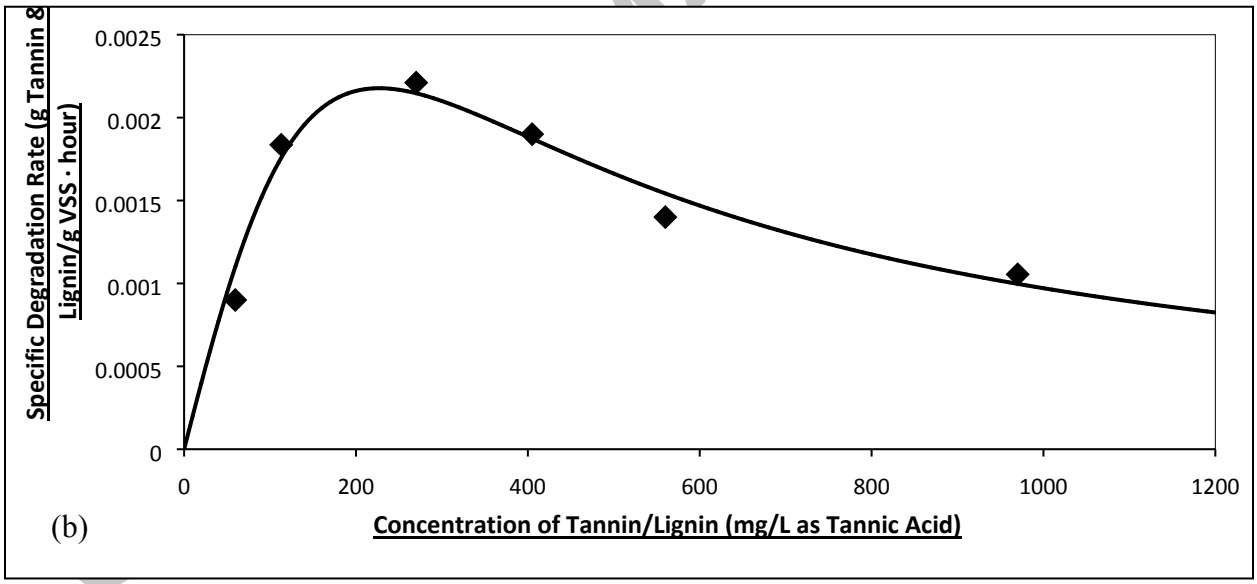


Fig. 8. (a) Degradation of tannin/lignin with time for several initial concentrations; (b) Haldane model fitted with experimental specific degradation rates

3.6 Microbial Analysis

High throughput sequencing was performed on the biomass at various times for both the synthetic and real PPW applications. *Rhodocycleceae* and *Rhodobacteraceae* remained the most prominent in all occasions. These two families are prevalent in most aerobic granular reactors as facultative anaerobic denitrifiers that promote the stabilization of the granule core [47]. Additionally, they have been linked to studies on organic toxin removal in crude oil [48] and carbamezapine degradation [49].

Burkholderiaceae species are also known for their denitrifying potential, however the family is also able to degrade various phenolic compounds. This family was detected in oil refinery wastewater that contained a variety of phenolic compounds [50].

Other species capable of degrading toxic substances are *Sphingomonadaceae* and *Xanthomonadaceae*. *Sphingomonas* spp. has been linked towards the degradation of several organic environmental contaminants such as dibenzofuran, phenanthrene, azo dyes and several other compounds [51]. The data in these studies suggests that *Sphingomonas* is essential towards the biodegradation of tannin and lignin.

The granules acclimated in real PPW were not able to develop the microorganisms required to degrade the toxic components as easily as in synthetic PPW. This indicates that perhaps there were other toxic components in real PPW aside from tannin and lignin that hindered the growth of these microorganisms. Additionally, the use of sodium acetate as a primary carbon source in synthetic PPW would allow for a co-metabolic pathway in which certain species would prosper. Further work is required to understand how to fully develop these species in real PPW (especially in SBR operation). Additionally, it is also necessary to understand the development of phosphorus-degrading bacteria in toxic environments. The results of the sequencing analysis are shown in Fig. 9.

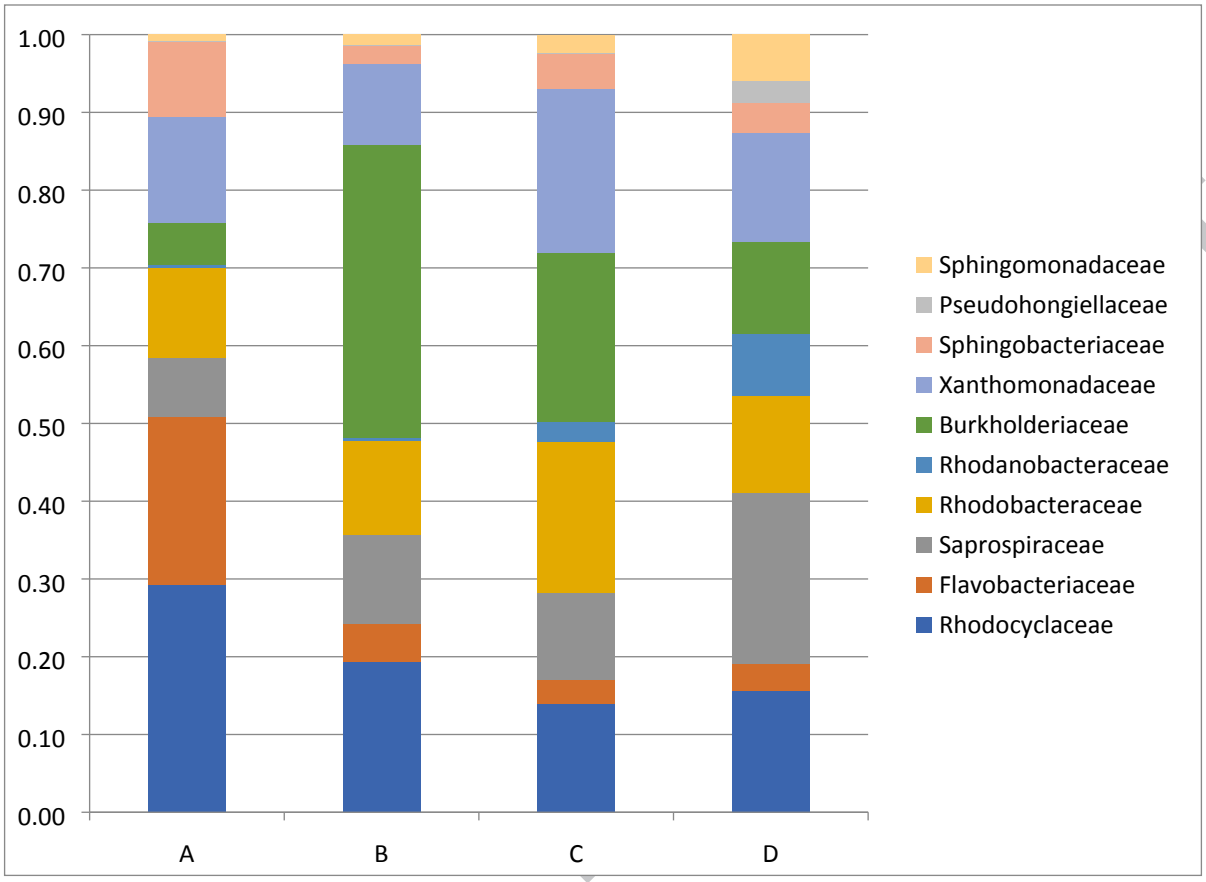


Fig. 9. Microbial analysis of biomass in: (A) Synthetic PPW on day 15; (B): Synthetic PPW on day 73; (C): Real PPW on day 10; (D): Real PPW on day 65

3.7. Biosorption

The primary removal mechanism for aerobic granular sludge is biodegradation, however several authors have denoted the ability for this sludge to be used as a biosorbent instead [12]. Target sorbates have typically included heavy metals, nuclides and some other inorganic ions. In this study, the authors have deduced that the primary form of removal for tannin/lignin is biosorption, followed by biodegradation. Fig. 10 portrays the amount of tannin/lignin adsorbed as the initial concentration varies up to 250 mg/L.

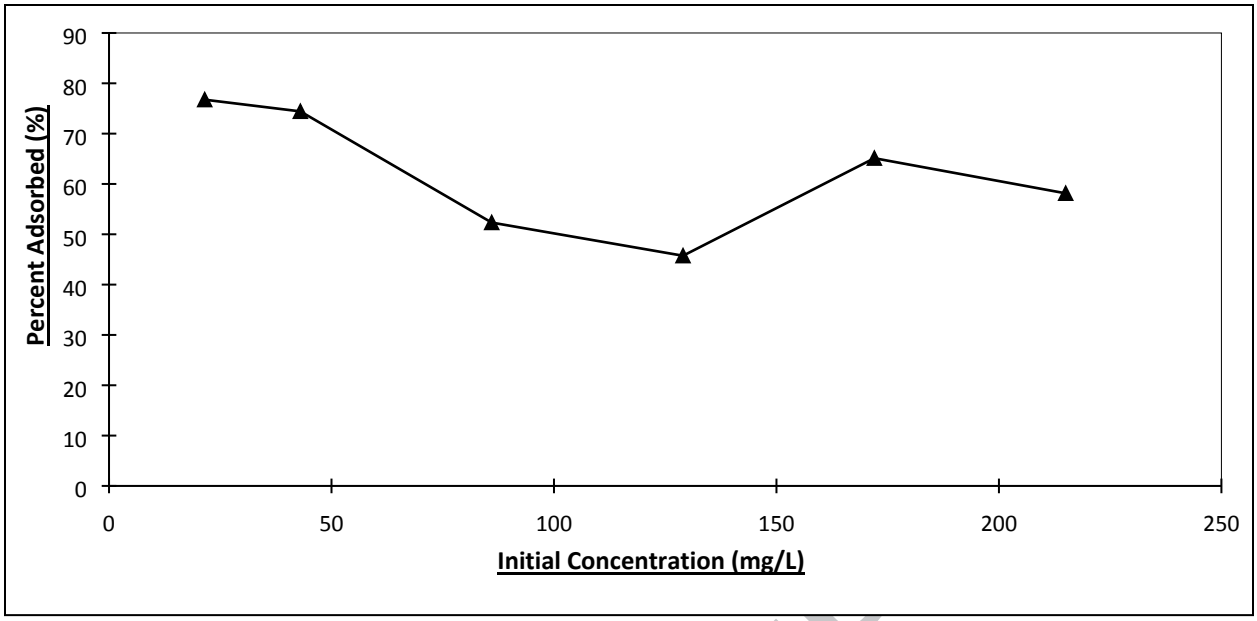


Fig. 10. Adsorbed amount represented in percentage with varying initial tannin/lignin concentrations

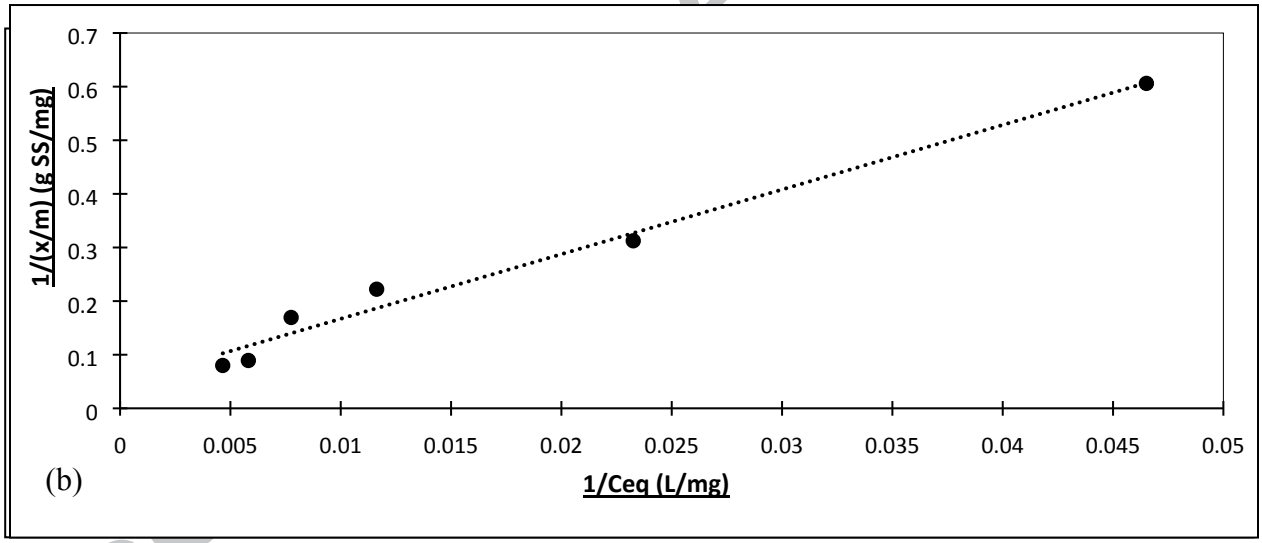


Fig. 11. (a) Langmuir and (b) Freundlich isotherm models

The adsorption profile was fitted to two basic isotherm models using MATLAB's Linear Fitting Tool: Langmuir and Freundlich. The resulting linearized fits are shown in Fig. 11. At lower concentrations of tannin/lignin, biosorption is the dominant form of degradation. At a concentration of about 20 mg/L, the granule surface adsorbed 77% of the compound. At higher concentrations, the adsorbed amount does decrease, achieving its lowest adsorption efficiency

(~45% adsorption onto the surface) at about 130 mg/L. It is at these lower concentrations that biodegradation will become more dominant as the surface capacity is exhausted.

The linearized Langmuir isotherm equation yielded the following coefficients with $R^2 = 0.9812$ and RMSE = 0.0591: $(x/m)_{max} = 21.5$ mg/g SS, $K_L = 0.00386$ L/mg. The Freundlich isotherm linearized fit, with an $R^2 = 0.9589$ and RMSE = 0.0756, yielded: n = 1.172 and K = 0.1174. According to the results of this study, the Langmuir isotherm is a better fitted biosorption model for tannin/lignin removal. Using this model, it can be determined that the maximum adsorption efficiency is 10.85 mg/g SS. For lignin removal with activated charcoal, the maximum adsorption capacity was 0.42 mg/g. Tannin removal with the same activated charcoal system yielded a maximum capacity of 0.44 mg/g [52]. One potential explanation for this phenomenon could be the EPS that provides an electrostatic force for the adsorption of these recalcitrant compounds [53]. The fourth layer observed in Fig. 2f could be a layer of EPS that formed on top of the granule serving as a helper in the biosorption process. The results from this study provide a strong indication that AGS can serve as a viable biosorbent.

3.7.1 Biosorption Kinetics

Fig. 8a can also be used to understand the biosorption kinetics of the system. After a 30 min time frame, it was observed that tannin/lignin desorbed from the sludge so that the bacterial population could start utilizing the compounds as their food source. Therefore, the R^2 values were much lower than anticipated because the biosorption kinetics model does not take into consideration the potential for desorption as well.

If the pseudo-first order kinetic equation, or the so-called Lagergren equation is used to model the biosorption, the rate constants can be calculated quite simply. There was a strong decrease in the R^2 values as the initial concentrations increased. This further confirms that at lower concentrations of tannin/lignin, biosorption is the primary form of removal. However, as the concentration increases, biodegradation becomes prevalent. The constants for both the kinetics equations are given in Table 3 and Table 4 for each initial concentration.

Table 3. Pseudo-first order adsorption kinetic parameters

Initial Concentration (mg/L)	K'_1 (1/min)	Q_e (mg/g)	R^2
59.5	0.1841	48.33	0.9972
113	0.0334	86.37	0.8795
270	0.0167	193.8	0.8894
405	0.0501	228.4	0.8772
560	0.0350	314.4	0.7806
970	0.0203	596.0	0.8069

The pseudo-second order kinetic equation fit well at lower concentrations with higher R^2 values than for the first order equation, however at higher concentrations the fit deteriorates significantly. The pseudo-first order kinetic model seemed to fit better for the AGS system, which may indicate that the reaction is reversible and that the rate determining step is not of a chemical interaction. This could also potentially indicate that perhaps the biosorption is not catalyzed by an enzyme or other biochemical reaction.

Table 4. Pseudo-second order adsorption kinetic parameters

Initial Concentration (mg/L)	K'_2 (g/mg·min)	Q_e (mg/g)	R^2
59.5	0.0167	48.9	0.9994
113	4.18E-4	80.3	0.9224
270	7.78E-5	167.4	0.9367
405	1.84E-4	218.9	0.8178
560	4.80E-5	294.4	0.8255
970	6.08E-5	534.4	0.6472

4. Conclusion

This study explored the applicability of AGS for the treatment of PPW, particularly pulp mill effluent. The following conclusions can be made from this study:

- In an SBR study in Calgary, Canada with synthetic tannin/lignin, the average COD removal efficiency was 90%, with an average tannin/lignin removal efficiency of 71%;
- In a similar SBR study carried out in Mumbai, India, the average COD removal efficiency was also about 90%, but the tannin/lignin removal efficiency was remarkable, achieving 82% removal at an initial concentration of 130 mg/L. The tropical weather conditions allowed for the development of fungi and other diverse species capable of removing these compounds at a higher capacity;
- A study on real PPW in Calgary, Canada revealed that tannin/lignin could be removed up to 54%, although the removal efficiency could be further improved by understanding the role of COD: N: P ratios to foster the growth of species capable of degrading tannin/lignin;
- Biodegradation followed the Haldane kinetic model, and yielded the following constants: V_{max} = 0.93 (g tannin/lignin/g VSS· day), K_s = 1910 mg/L, and K_i = 27 mg/L;
- Removal by biosorption achieved 58% reduction from an initial concentration of 215 mg/L. The Langmuir isotherm coefficients were as follows: $(x/m)_{max}$ = 21.5 (mg tannin/lignin/g SS), b = 0.00386 L/mg. The Freundlich isotherm coefficients were: n = 1.172, K = 0.1174;
- Biosorption kinetics revealed that the pseudo-first order kinetic model fit better to the experimental data, signifying that the interactions were not of a chemical nature.

Acknowledgements

The work presented in this paper was supported by the National Science and Engineering Council of Canada (NSERC) and the Mitacs Globalink Research Award. The authors would also like acknowledge Dr. Amritanshu Shriwastav and colleagues at IIT-B for their support, guidance and kindness in allowing this work to take place at their facility. Additionally, the authors would like to thank the services of the Centre for Health Genomics and Informatics in the Cumming School of Medicine at University of Calgary.

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