

Article

Performance Evaluation of a Biological Pre-Treatment Coupled with the Down-Flow Expanded Granular Bed Reactor (DEGBR) for Treatment of Poultry Slaughterhouse Wastewater

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Abstract: Poultry slaughterhouse wastewater contains high concentrations of chemical oxygen demand (COD), total suspended solids (TSSs), fats, oil and grease (FOG), proteins and carbohydrates. It is important that the wastewater is treated to acceptable environmental discharge standards. In this study, the poultry slaughterhouse wastewater (PSW) was treated using two-stage processes consisting of a biological pre-treatment using a biodegrading agent (Eco-flushTM) coupled with a down-flow expanded granular bed reactor (DEGBR). The results showed that the biological pre-treatment was observed to be highly effective for removal of FOG, COD and TSS with a removal efficiency of $80 \pm 6.3\%$, $38 \pm 8.4\%$ and $56 \pm 7.2\%$, respectively. The DEGBR showed a stable performance in terms FOG, COD and, TSS removal, with average removal efficiencies of $89 \pm 2.8\%$, $87 \pm 9.5\%$, and $94 \pm 3.7\%$, respectively. The overall removal rate performance of the integrated system of pre-treatment and DEGBR in terms FOG, COD and TSS, was $97 \pm 0.8\%$, $92 \pm 6.3\%$ and $97 \pm 1.2\%$. Furthermore, the average volatile fatty acid/alkalinity (VFA/Alkalinity) ratio of 0.2 was reported, which indicated that the DEGBR was stable throughout the operation.

Keywords: chemical oxygen demand (COD); fats oil and grease (FOG); total suspended solids (TSSs); poultry slaughterhouse wastewater (PSW); down-flow expanded granular bed reactor (DEGBR)

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1. Introduction

The meat processing industry is one of the largest consumers of freshwater in the agricultural sector [1]. Poultry slaughterhouse industries use a considerable amount of portable water during processing of live birds to consumable meat, which results in the production of high volumes of poultry slaughterhouse wastewater (PSW) [2]. The rising demand of poultry produce requires an increase in poultry slaughterhouses' ability to treat high volumes of PSW [3]. Ordinarily, poultry slaughterhouse effluent is difficult to treat, and it also requires multiple treatment steps before it can safely be discharged to the environment, due to the high amount of organic matter, FOG and nutrients contained therein [4]. The challenge is that the organic matter, toxic pollutants and large particles in raw PSW are not only toxic to the environment, but also cause clogging of bioreactors [5]. As per the South African department of Agriculture, Forestry and Fisheries (2014) report, the production of poultry meat has been one of the leading purchased items among South Africans, which significantly contributes to nearly 20% of the overall meat production in

South Africa (SA) in the past decade. The nature of the activities associated with clean water usage during washing and poultry product processing culminates in the release of a tremendous volume of wastewater [6]. The vast generation of PSW also presents itself as a potential threat to the ecosystem, as well as human health. Therefore, the implication of untreated organic substances, such as blood and feathers, would result in the eutrophication of receiving surface water, thereby causing environmental pollution and a health hazard on people exposed to it [7]. Ordinarily, poultry slaughterhouse processes involve a series of processing steps ranging from the slaughtering of live birds to the conversion of the live birds to edible meat fit for human consumption [1]. The latter processing steps require large volumes of freshwater, and this contributes to the pollution of the freshwater sources if the PSW is released untreated. The high volume of water utilized in such facilities stems from various processing steps requiring potable water because of the need to provide safe products [8]. To treat the PSW, suitable bioreactors are needed.

Most reactors have operational challenges which range from clogging, longer processing time and lower efficiencies [9]. However, one of the most successful and effective process equipment used in the treatment of wastewater is the static granular bed reactor (SGBR) [10]. In the SGBR, PSW is fed at the top of the reactor where it flows downward via a bed of anaerobic granules. However, some problems may be encountered during the operation of the SGBR, including clogging and sludge wash-out, which are caused by high and excessive concentrations of biomass growth [7]. The excessive concentration of biomass growth generally leads to lower COD removal efficiencies. An important aspect that can assist in the treatment of PSW is the use of a novel bioremediation agent called Eco-flush™ in the pre-treatment step, which is followed by the down-flow expanded granular bed reactor (DEGBR). The DEGBR was designed to mitigate the challenges experienced by the SGBR [11]. The DEGBR is a modified adaptation of the SGBR that is fitted with a recycle stream on the side of the reactor, which is used for recycling effluent from the bottom of the reactor to the top [11]. This is meant to prevent issues such as flow channeling, to improve the distribution of the feed to the biomass, and to implement intermittent fluidization whenever it is required using a top-down recycling strategy.

This study seeks to evaluate the performance of an integrated system containing a biological pre-treatment coupled with a DEGBR for the treatment of PSW in order to achieve high removal efficiencies of COD, TSS and FOG. Furthermore, the performance of the system was then compared to other biological systems used in previous studies for the treatment of PSW.

2. Materials and Methods

2.1. Experimental Set-Up

The equipment and design layout of the lab-scale plant entailed a pre-treatment process which consisted of a 20 L mixing tank and 20 L holding tank which had a magnetic stirrer. Ordinarily, the stirrer operated at a maximum of 100 revolutions per minute (RPM). The purpose of the stirrer was to create a homogenous PSW to ensure consistency in the process. The effluent produced was then screened and pumped by a peristaltic pump to the DEGBR anaerobic digester. The DEGBR was made of polyvinyl chloride (PVC) with a volume of 2 L capacity, 0.62 m height and 0.065 m in diameter as illustrated in Figure 1. At the bottom of the DEGBR, pumice stones were fitted as an underdrain, which rested on a 2 mm stainless sieve mesh. The pumice stones aided as a sludge retaining medium and also allowed the attachments of bacteria to grow as shown in Figure 2. In addition, a recycle stream was fitted on the side of the reactor with the aim of preventing clogging of the bioreactor. Biogas was collected at the top of the DEGBR into 500 mL plastic storage bag.



Figure 1. Photograph of the modified down-flow expanded granular bed reactor (DEGBR) (source: D.N. Dlamini).

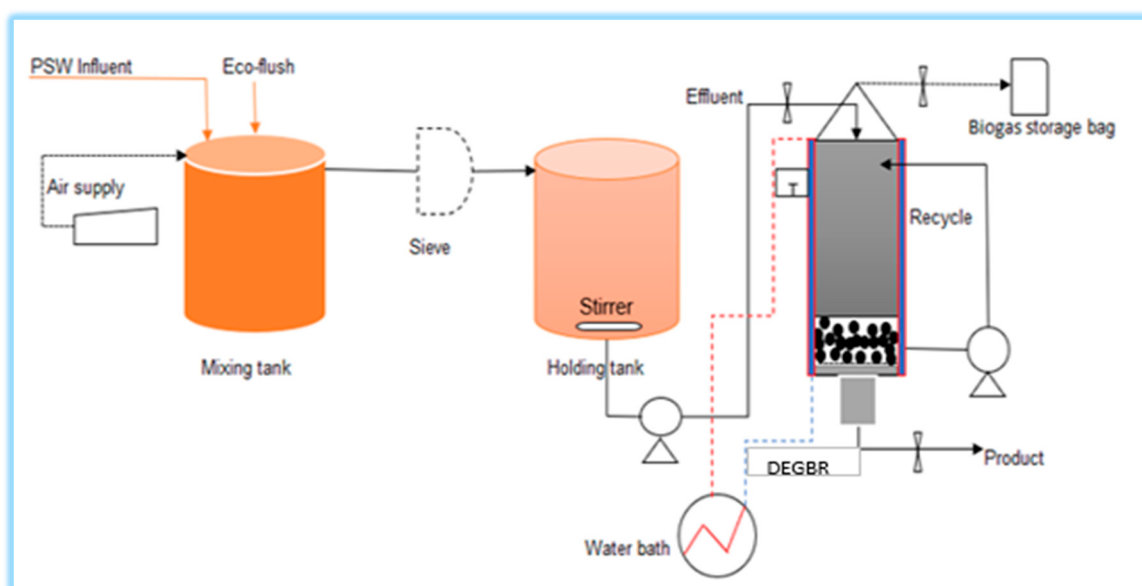


Figure 2. Schematic illustration of the modified DEGBR.

2.2. Operating Conditions

Pre-Treatment and Sample Preparation

The pre-treatment was operated at ambient temperature and a mixture of a bioremediation agent known as Eco-flushTM (20 mL) and 20 L of raw PSW was aerated using air stone spargers over a period of 24 h. The Eco-flushTM is a commercial product used for the coagulation of FOG [12], which was donated for research purposes by Mavu biotechnologies (Pty) Ltd. Eco-flushTM is a hydrolysis bioremediation agent, containing microorganism isolated from the soil, grown and stored in a physiological dormant state. The microorganisms are resuscitated to produce enzymes which in turn delipidate the PSW. Eco-flushTM constituents are: enzymes, microbial consortia, organic nitrogen, potassium, organic carbon, water and organic matter. After aeration, the PSW was allowed to settle under anaerobic conditions for an extra 24 h. Overall, the Eco-flushTM has an ability to oxidize NH_3 into NO_3^- and NO_2^- and also eliminates H_2S , including odor-producing organisms. Subsequently, after settlement, the PSW was then filtered through a 53 μm sieve screen. The reason for screening was to remove larger particulate matter including feathers.

2.3. DEGBR Set-Up, Operation and Inoculation

The DEGBR, with a working volume of 2 L, was constructed with a clear polyvinyl chloride (PVC) column with internal dimensions of 0.62 m height and 0.065 m in diameter. Pumice stones were placed at the bottom of the reactor to aid as a biomass retaining aid. Pumice stones were chosen due their inertness and thus support the accumulation of beneficial bacteria, which also help cleanse the pollutants from the PSW. A mass (0.5 kg) of pumice stones was immersed in the wastewater and had an average porosity of 0.66 [11]. The DEGBR was operated at a mesophilic temperature of $\pm 37^\circ\text{C}$ sustained using a water bath. After pre-treatment, the screened product was pumped to the DEGBR for further processing using the Antech aspendose A 5.1 L/0.5 B peristaltic pump purchased from Enelsa in Turkey. Prior to this, the inoculation of the DEGBR was done using an activated sludge obtained from a SAB brewery which is located in the Western Cape, Newlands, SA. The collected activated sludge sample was collected in the form of a mixed liquor directly from a USAB [13]. Accordingly, the DEGBR was inoculated according to the following ratio: 1.6 L of raw PSW effluent in combination with 0.4 L of brewery activated sludge supplemented with a 50 mL solution of dry milk. The milk was added as an organic source to sustain the micro-organisms and help them to adapt to the new environment. Thereafter DEGBR was operated for 126 days (18 weeks), whereby the first 30 days were used to acclimatize the microbes, and the balance of the operational days was used to operate the DEGBR at a constant hydraulic retention time (HRT) of 106 h [14]. The reactor plant operated at an influent flowrate of 0.45 L/hr.

2.4. Sample and Analytical Methods

Sampling for the process was done at the feed and exit points of every stage in the process. The samples were collected using sample bottles and stored in the cold room set at -5°C before analyses. Thereafter, the collected samples of the wastewater were analyzed for Alkalinity, TSS, volatile fatty acids (VFA), COD, FOG, and pH. All the samples collected were analyzed using standard methods [15]. A Geotech Biogas 5000 portable gas analyzer manufactured by Keison Products in the United Kingdom (UK) was used to determine the gas composition emitted that was collected in the biogas storage bags.

Statistical Analysis

The analyses for each parameter measured were completed in triplicate. An average for each parameter was calculated from the measurements recorded. Averaged standard deviation ($\pm\text{SD}$) values were used to compare the concentrations and removal efficiencies for different parameters. The removal efficiency was calculated using Equation (1):

$$\eta = \frac{\text{Influent (mg/L)} - \text{Effluent (mg/L)} \times 100\%}{\text{Influent (mg/L)}} \quad (1)$$

2.5. Procedure Followed for Study

Figure 3 shows a graphical schematic summary of the study. Initially, the study underwent a brain storming session whereby a research project design of the process was developed. A proposal of the study was subsequently done and submitted to the higher degrees committee of the Cape Peninsula University of Technology (CPUT). Once the proposal was approved and ethics clearance obtained thereof, the plant was then assembled by Malutsa (Pty) Ltd. (Western Cape, Wellington Industrial Park, South Africa).

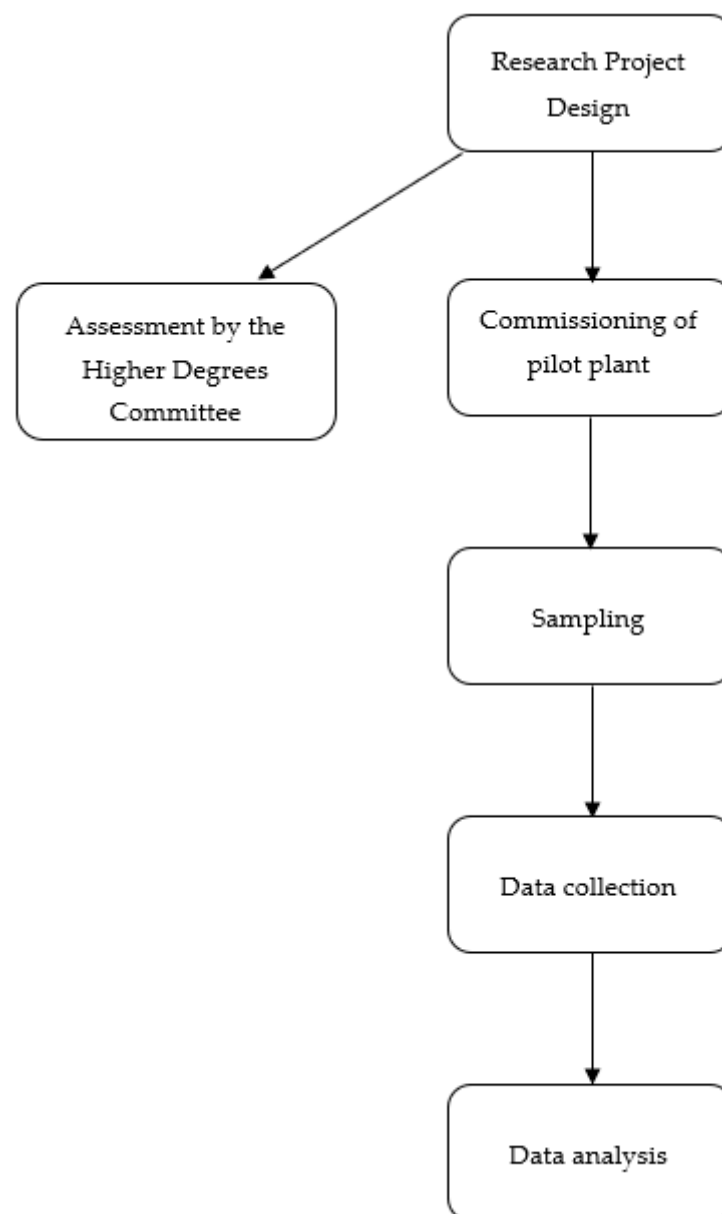


Figure 3. Graphical representation of the study.

3. Results

3.1. Biological Pretreatment Performance

Figure 4 provides an insight into the performance of the pre-treatment system with respect to COD, TSS and FOG removal. The graphs in the first row illustrate the variation of the concentration of each water quality parameter at the inlet of the system, while the graphs in the second row provide the wastewater quality parameters at the outlet of the treatment unit, and finally the graphs in the last row provide the variation of the removal percentages for each indicated parameter. It was noticed from the COD and FOG concentrations at the inlet of the pre-treatment system that some values were outliers. To evaluate this, the inlet and outlet values of each parameter were box plotted with the aim of identifying potential outliers in each distribution.

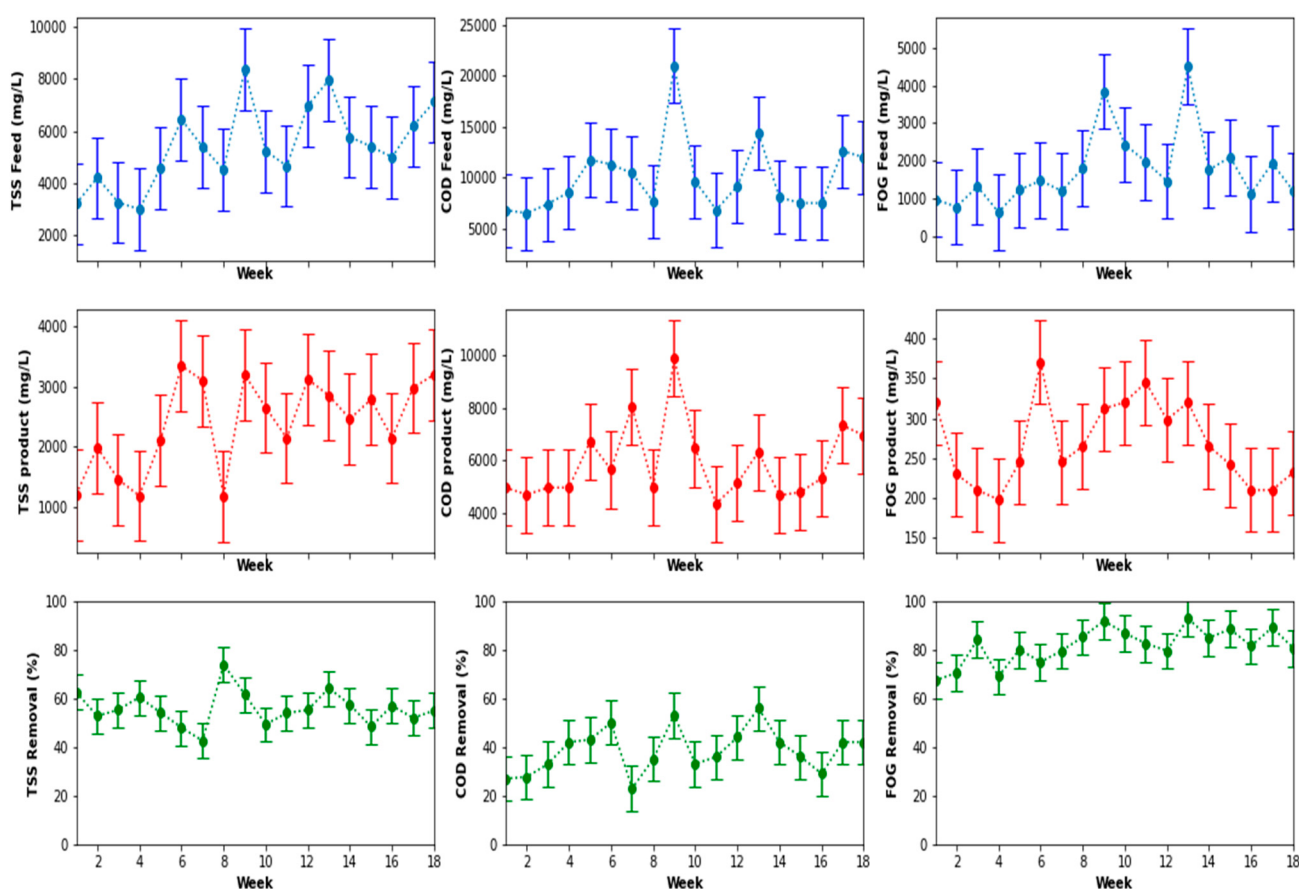


Figure 4. Performance of the pre-treatment unit before outlier replacement.

As shown in Figure 5a, the boxplot of the distribution of each parameter, assessed both at the inlet and the outlet of the pre-treatment system, indicated the presence of outliers for FOG and COD, as well as the outlet COD concentrations. For the boxplots, an interquartile rule to identify the outliers was used, with the outliers being replaced by median values which are dependent on the size of the dataset. This culminated in the boxplot in Figure 5b from which the absence of outliers can be noticed. Outliers in this experiment could originate from variations in the activities of the poultry slaughterhouse at the moment of the collection of the sample, or erroneous concentration determination of a given parameter during the experiment. These outliers were corrected for ease of data interpretation and the outcome of the experiment at each PSW treatment stage.

The performance of the pretreatment system is shown in Figure 6. The fluctuations in the quality of the feed and product of the system and the corresponding removal efficiencies after the data processing indicated FOG removal efficiency oscillating around 80%. Furthermore, Figure 6 also shows removal efficiencies of the TSS and the COD ranging between 45% to 80% for the TSS, and a variation between 25% to about 60% for the COD. These removal efficiencies fluctuated with the quality of the feed to the system. Overall, the pre-treatment system culminated in an average removal efficiency of $80 \pm 6.3\%$, $38 \pm 8.4\%$ and $56 \pm 7.2\%$ for the FOG, COD and TSS, respectively.

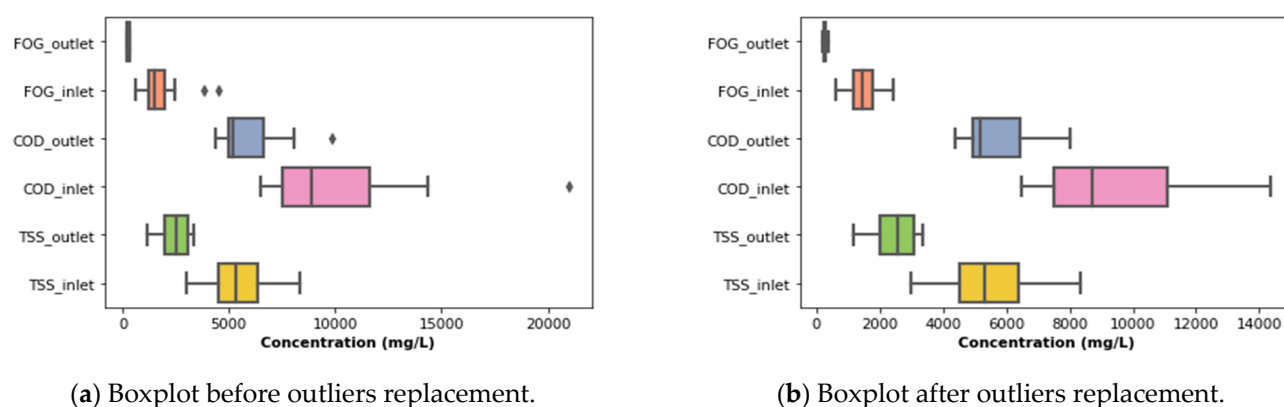


Figure 5. Boxplots of the investigated parameters during the pre-treatment stage.

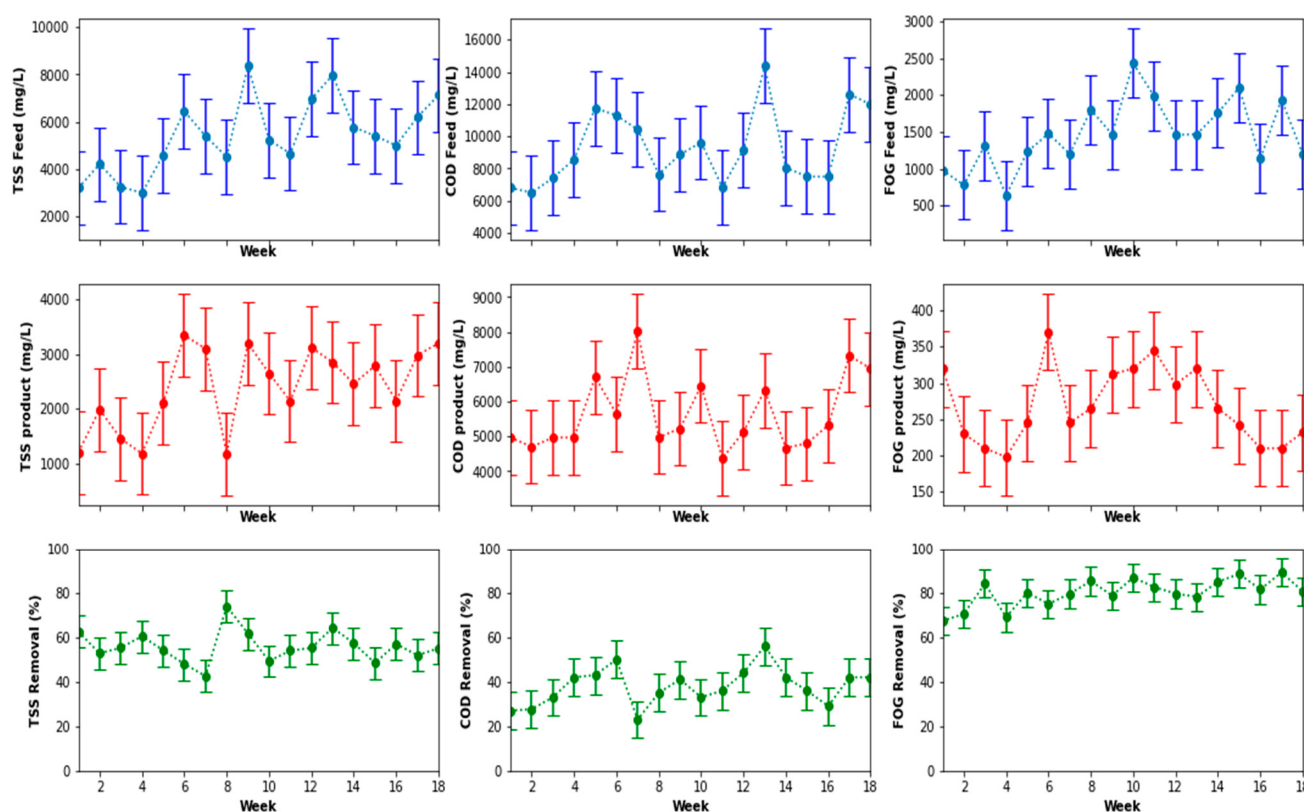


Figure 6. Performance of the pre-treatment system after outlier replacement.

3.1.1. FOG Removal

The results of this study illustrated that the supplementation of 20 mL of the Eco-flush™ for every 20 L of PSW highly influenced FOG removal. Using Eco-flush™ as a FOG reduction agent was indicative of the bacterial consortia present in it, which aided in the biological degradation of the pollutants presents in the effluent. Since the raw PSW was first aerated through sparging, which supplies dissolved oxygen to the wastewater and the microorganisms, this resulted in the proliferation of the bacterial community that subsequently degraded the organic material and pollutants in the wastewater. Basically, the microbes decoupled bonds in the organic contaminants and further coagulated less soluble matter found in the raw PSW. At an average FOG removal efficiency of $80 \pm 6.3\%$ (Table 1), the results depicted a pre-treatment unit that performed exceptionally well under the above set design and operational conditions. This study proved to perform well as compared to another study whereby an evaluation of a biosurfactant, obtained from cultures grown

on cassava wastewater were used in the pre-treatment of PSW, yielding an oil and grease removal efficiency of 70% [16].

Table 1. Raw and pre-treated poultry slaughterhouse wastewater (PSW) characteristics average range.

Parameter	Inlet Feed (mg/L)	Product (mg/L)	Efficiency (η) (%)
COD	9946	5896	39
TSS	5399	2392	56
FOG	1765	269	80

3.1.2. COD Removal

As for COD removal, the effect of biological pre-treatment using Eco-flushTM indicated that the process also effectively reduced the organic matter load present in the raw PSW; albeit, with a lowly COD removal efficiency of $38 \pm 8.4\%$. However, this was done under ambient temperature conditions in which the biological pre-treatment can be proven to be a challenge because of the high concentration of organic matter present in the PSW. However, with an increased organic loading rate, the total removal of COD contributing constituents in the PSW can increase proportionally. As shown in Table 1, a COD product discharge of 5896 mg/L and COD removal efficiency of $38 \pm 8.4\%$ showed that there was a need of a subsequent treatment stage that would further reduce the COD to prescribed discharge standard of wastewater (5000 mg/L) as per the City of Cape Town by-laws, 2013 [17]. Overall, this study proved to be more efficient as compared to a similar study whereby the biological pre-treatment of poultry product processing wastewater removed only 24% tCOD in an aerated equalization tank [18].

3.1.3. TSS Removal

In reference to Table 1, the high values obtained at the inlet feed were attributed to solids' presence and entrapment in the animal fat which is normally observed during pick processing times [19]. Furthermore, it was established that the presence of high carcass debris, might have been a contributing factor to the organic matter found in the raw PSW culminating in the high concentrations of TSS due to high FOG [20]. However, when Eco-flushTM was added, some FOG hydrolysis ensued, which consequently resulted in the break-down of hydrocarbons, some of which were hypothesized to be converted into useful nutrients in the form of soluble fatty acids [21] for the bacterial population, releasing some solids which can be removed through filtration or a screening device. For this study a 53 μm screen was used, thus a significant portion of large particles of less soluble matter were filtered out as this will assist in the reduction of particles that could clog the DEGBR in the subsequent stage [5,18].

Overall, the biological pre-treatment unit reduced COD from 9946 to 5896 mg/L, TSS from 5399 to 2392 mg/L and FOG from 1765 to 269 mg/L, respectively. A clear indication of the aesthetics of the raw PSW in comparison to the pre-treated PSW samples is shown in Figure 7, suggesting an initial satisfactory performance of the biological pre-treatment stage.

3.2. The Performance of the DEGBR

The product of the pre-treatment system was continuously fed to the DEGBR with the intention of further reducing the concentration of the contaminants present in the PSW. The fluctuation of the concentration of the COD, TSS and FOG during the experiments for both the inlet and the outlet of the DEGBR, including their corresponding removal efficiencies, are provided in Figure 8. Following the same methodology as the data processed for the pre-treatment system, the evaluation of the presence of outliers was undertaken.



Figure 7. Photographs of the raw PSW in comparison to pre-treated PSW.

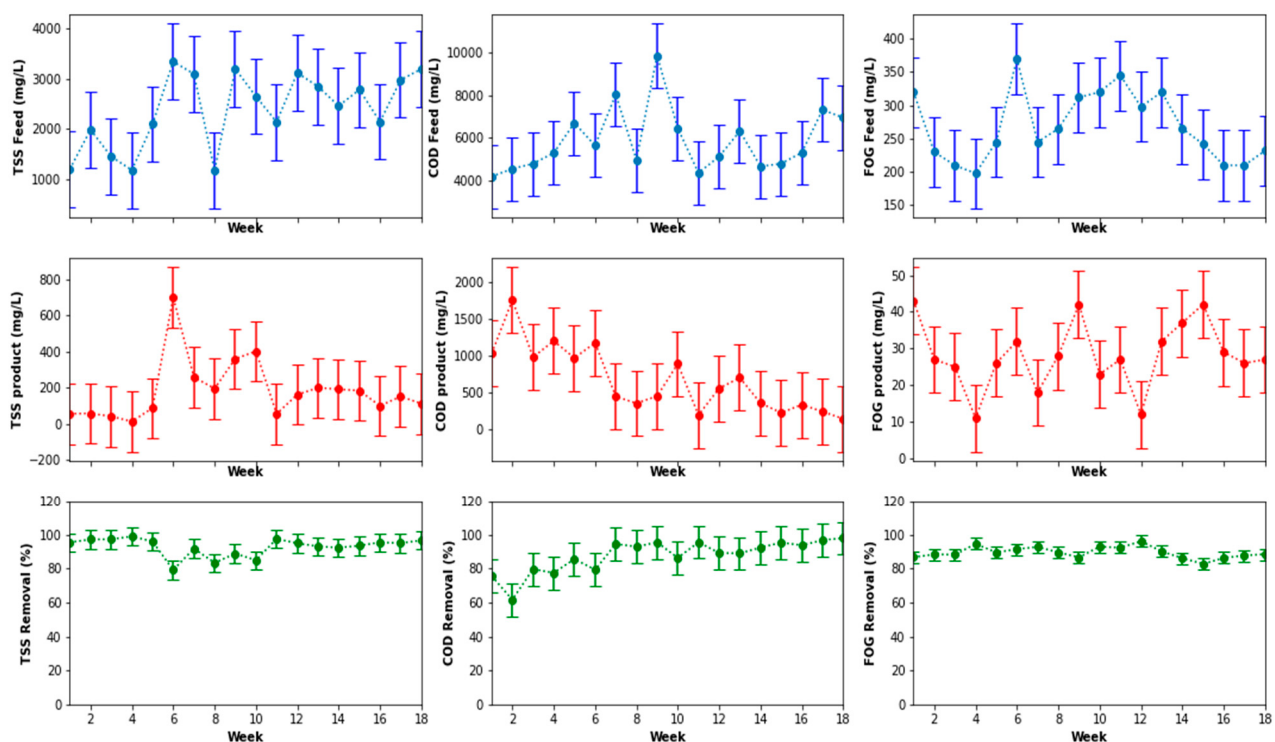


Figure 8. Performance of the DEGBR before outliers replacement.

From Figure 9a, it can be observed that the distribution of the inlet COD and TSS had an outlier each. These outliers were replaced with the median value of each affected distribution, as depicted in Figure 9b. From the latter, it can be observed that the data processing step addressed the challenges associated with the presence of outliers in the listed distribution and led to a further analysis of the performance of the DEGBR, as illustrated in Figure 10 indicating how the fluctuations varied for the concentration of TSS, FOG and COD at the inlet and outlet of the DEGBR, including their corresponding removal efficiencies.

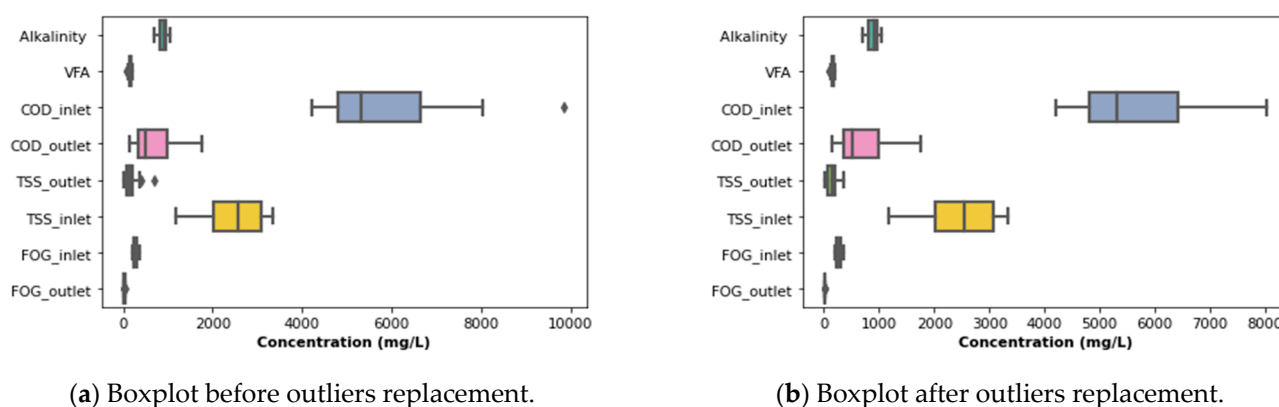


Figure 9. Boxplots of the investigated parameters associated with DEGBR performance.

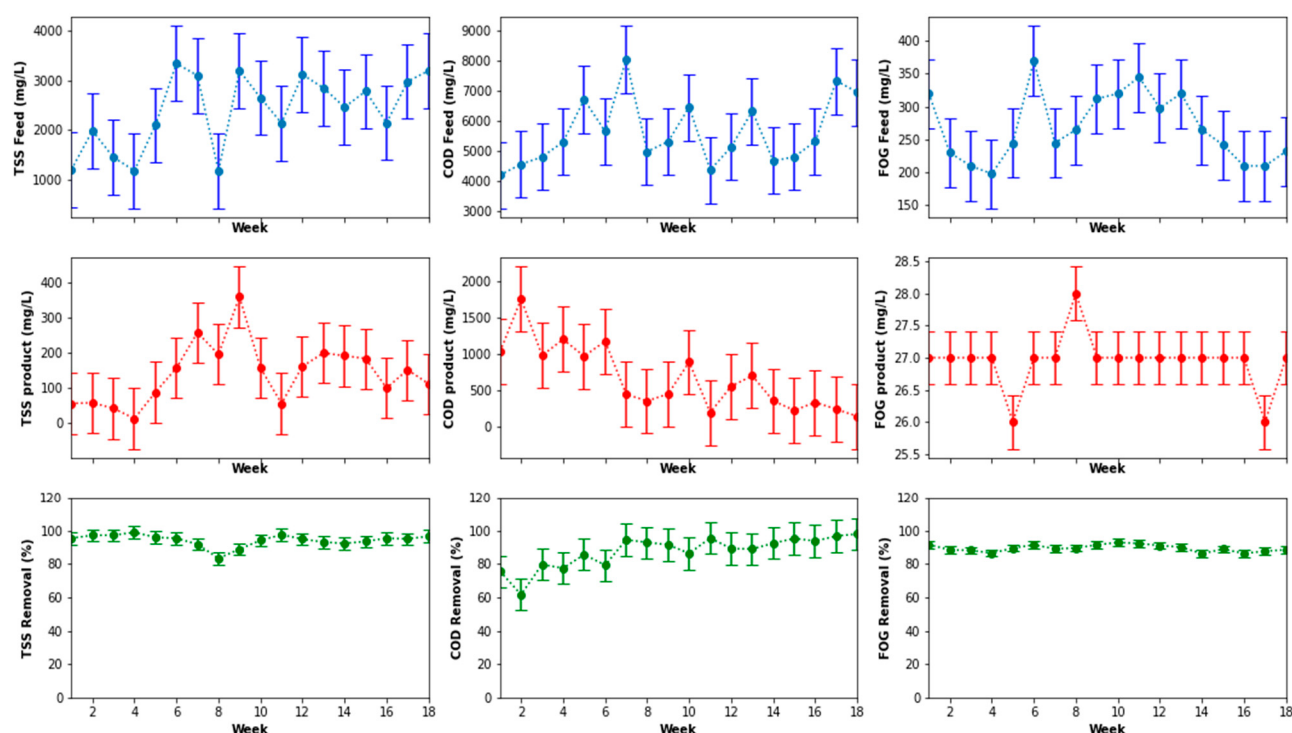


Figure 10. Performance of DEGBR after outliers replacement.

Overall, the average removal efficiencies were $89 \pm 2.8\%$ for the FOG, $87 \pm 9.5\%$ for the COD and $94 \pm 3.7\%$ for the TSS. The concentration of these contaminants was below the required discharge standards prescribed by City of Cape Town by-laws (2013) [17] and supported the recourse undertaken suggested an efficient process system for the treatment of PSW or a wastewater having similar characteristics. The DEGBR designed for this study performed well in comparison to the EGSB used in another study, whereby it was indicated that the performance was attributed to a rapid start-up of the EGSB, in which brewery wastewater sludge was used in the treatment of slaughterhouse wastewater. It was determined that the sludge could develop faster within a period of 10 days in the EGSB reactor, with few detached sludge granules, achieving removal efficiencies of up to 72.9% of COD at an operational hydraulic retention time (HRT) of 12.1 h [22].

It was also observed that the performance of the DEGBR was high even at the beginning of the process, whereby the sludge micro-organisms were still acclimatizing to the new type of wastewater they were processing (i.e., PSW). This was due to the low organic loading rate used for the system throughout the experiment, which was sustained by the pre-treatment step used prior to the supply of the wastewater to the DEGBR system that

significantly reduced the organic load. The management of this organic load to the DEGBR with respect to the FOG, COD, and TSS is illustrated in Figures 11–13, respectively. From these figures, it can be observed that the removal efficiencies of the DEGBR remained high throughout the experiment despite various fluctuations of the organic loading rate (OLR). This demonstrates the suitability of the DEGBR for the treatment of such wastewater and highlights the importance of a good pre-treatment step prior to feeding the wastewater to the anaerobic reactor. Moreover, the highest average COD removal efficiency of 92.8% was achieved in the fifteenth to eighteenth weeks, while operating at a HRT of 106 h. This could have been attributed to by the fact that the DEGBR was operating at its optimum because of an increased number of active microbes in the stabilized process. It can be said that the DEGBR performed better in terms of COD removal (Figure 12) as compared to a study of the treatment of PSW in an up-flow anaerobic bioreactor under a low up-flow velocity, which yielded a COD removal of only 70% [23]. Similarly, the DEGBR study proved to perform better than EGSB, which yielded a low COD removal efficiency of only 60% [24].

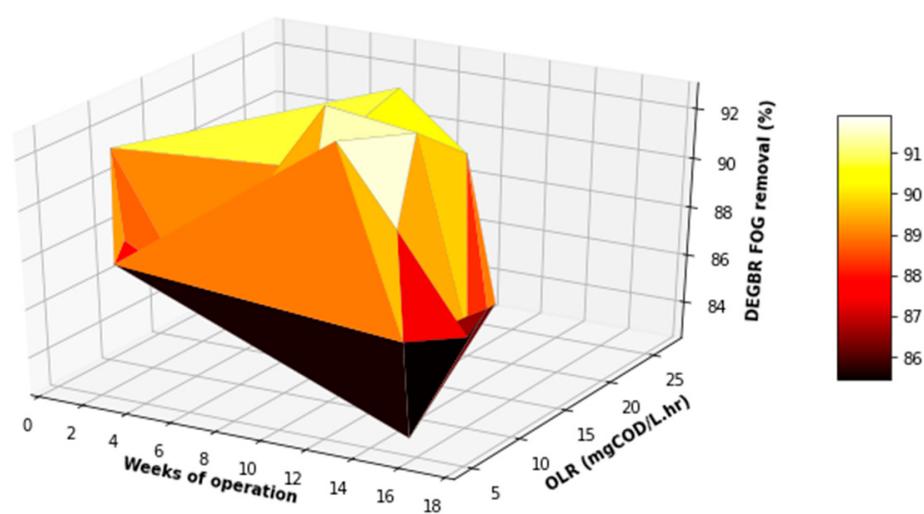


Figure 11. Performance of the DEGBR with respect to FOG removal under varying OLRs.

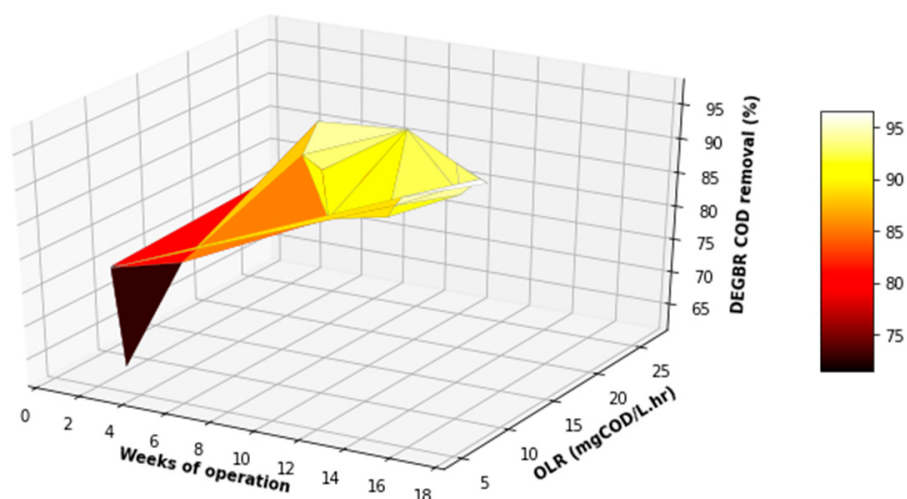


Figure 12. Performance of the DEGBR with respect to the COD removal under varying OLRs.

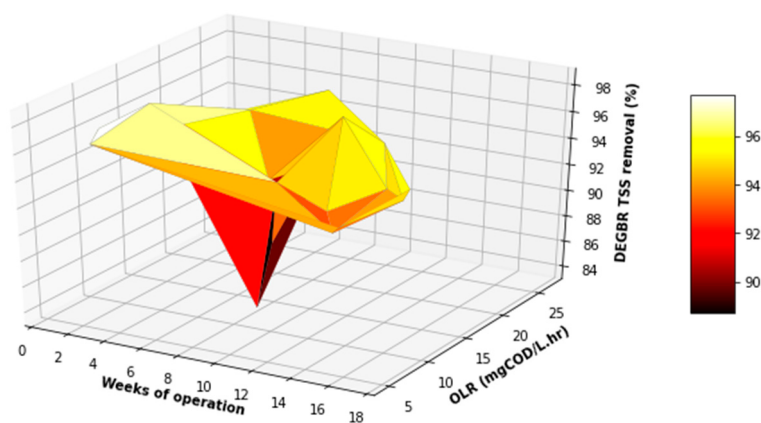


Figure 13. Performance of the DEGBR with respect to the TSS removal under varying OLRs.

3.2.1. The Effect of pH, Temperature and VFA/Alkalinity on the DEGBR Performance

Organic matter degradation happens in the DEGBR and it involves the decomposition of a large organic compounds into a simpler constituent for assimilation. The rate at which complex organic matter is degraded depends on the pH, temperature of the bioreactor, and molecular size of the pollutants including biomass activity [25]. However, there is no overall ideal operational pH, because all processes use consortia of micro-organisms, whose growth rate is differentiated at different pH values [26]. However, obviously, extreme pH can kill micro-organisms, hence there is a need to monitor and control it. This means that the operational pH is a median value which can be tolerated by most enzymes and micro-organisms. Additionally, Figure 14 depicts a variation of the pH and the temperature during the experiment reported herein, and these two parameters were also maintained within a mesophilic range (37 to 38 °C) for the temperature and a suitable pH range of 6.5 to 8.

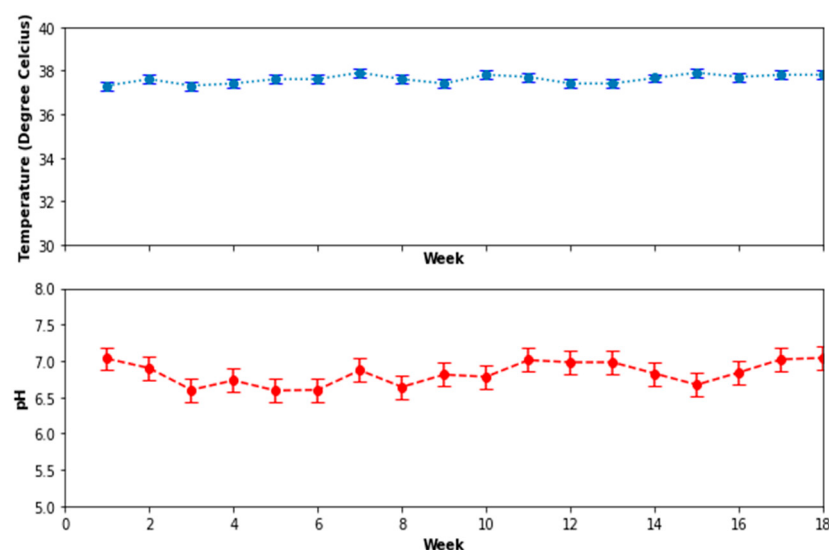


Figure 14. Variation of the pH and the temperature in the DEGBR during the processing of PSW.

However, despite the highly appraised pre-treatment step, other parameters are essential to a conducive anaerobic digestion in the DEGBR. These included the VFA/Alkalinity ratio that reflected a good acidic and alkaline balance within the bioreactor. Ideally, this ratio should be kept under 0.3 for a stable anaerobic operation [7]. A VFA/Alkalinity ratio test was performed in order to establish the progression in the digestion of the PSW and the stability of the reactor. Figure 15 shows average values of alkalinity, which ranged between 1048 to 702 mg/L, whereby the average VFA of 157.97 mg/L, and the alkalinity of 888.12 mg/L, culminated in the VFA/Alkalinity ratio of 0.2. Such a VFA/Alkalinity

ratio was satisfactory, albeit slightly low for a high-performance reactor as compared to other similar processes used for anaerobic digestion. This was the case for the DEGBR, as illustrated in Figure 15, that provides a variation of the VFA/Alkalinity ratio, including the variation of the alkalinity and VFA concentration, throughout the experiment.

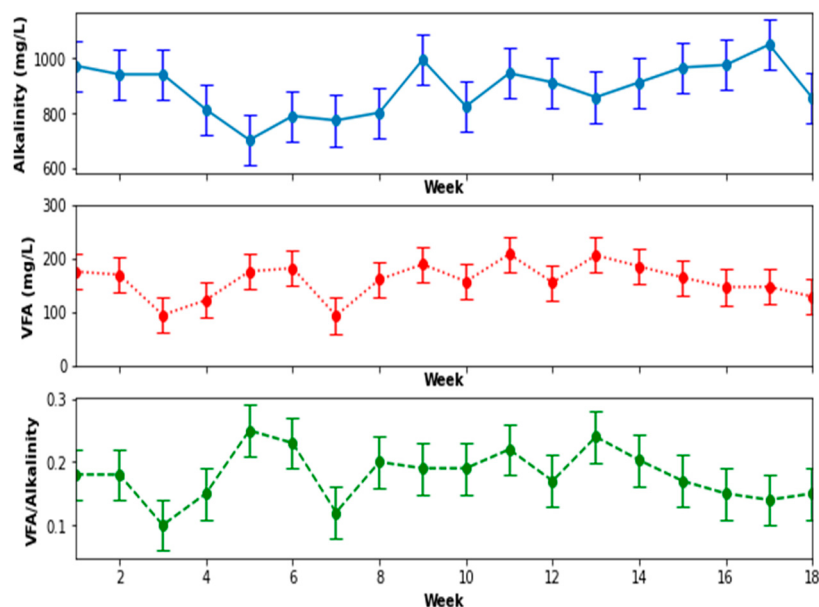


Figure 15. Variation of the VFA, Alkalinity and VFA/Alkalinity in the DEGBR.

3.2.2. Biogas Production

Biomethane gas production rate is calculated according to Equation (2) [27]:

$$K = \frac{(\sum_{t=0}^n Dt)}{n} \quad (2)$$

where K is the biomethane production rate (mL/d), t is the digestion time (d), Dt being the daily methane gas production (mL) and n refers to the day when the daily biomethane production is less than 5% of the maximum daily biomethane production for five days.

Biogas production happens in a process called methanogenesis. However, before methanogenesis, the bacteria in the reactor converts fatty acids and alcohols into acetic acid, hydrogen, and carbon dioxide. Methanogenesis is the process whereby acetic acid, hydrogen and carbon dioxide products are degraded to produce methane and carbon dioxide [28]. Ordinarily, a suitable OLR and HRT can result in the production of methane gas [12]. Furthermore, it has been established that presence of ammonium-nitrogen (NH_4N) in the system can inhibit methane gas production [29]. It was reported in another study whereby a static granular bed reactor (SGBR) was used for the treatment of industrial wastewater that the biogas production increased with the increase in the organic loading rate [30]. For this research project, it was found that biogas production was reduced from 0.3 L CH_4 /g.COD (71%) degraded to being non-existent (0%) after 30 days from the initial start-up period, as shown in Figure 16. This was attributed to the continuous supply of facultative bacteria present in the bioremediation agent (Eco-flushTM) which was hypothesized to have had an effect of suppressing the methanogenesis process in the DEGBR.

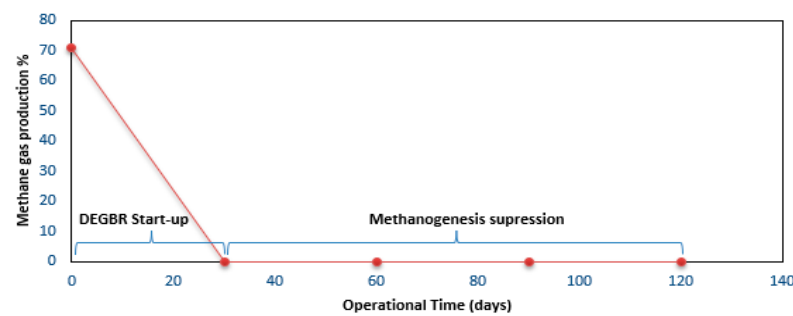


Figure 16. Biogas production during the operation of the DEGBR.

3.3. Overall Performance of the DEGBR

Figure 17 provides an overall performance of the complete system, for the removal of the COD, TSS, and FOG during the experiment, despite a slow start for the removal of COD during the first three weeks. However, the average removal efficiency was $97 \pm 0.8\%$ for the FOG and, subsequently, improved to $92 \pm 6.3\%$ for the COD and $97 \pm 1.2\%$ for the TSS. The robustness of the entire system is demonstrated by the low standard deviation of the TSS and FOG removal efficiencies, as also illustrated in Figure 17. This also demonstrates that such an arrangement can provide similar overall results for the treatment of PSW.

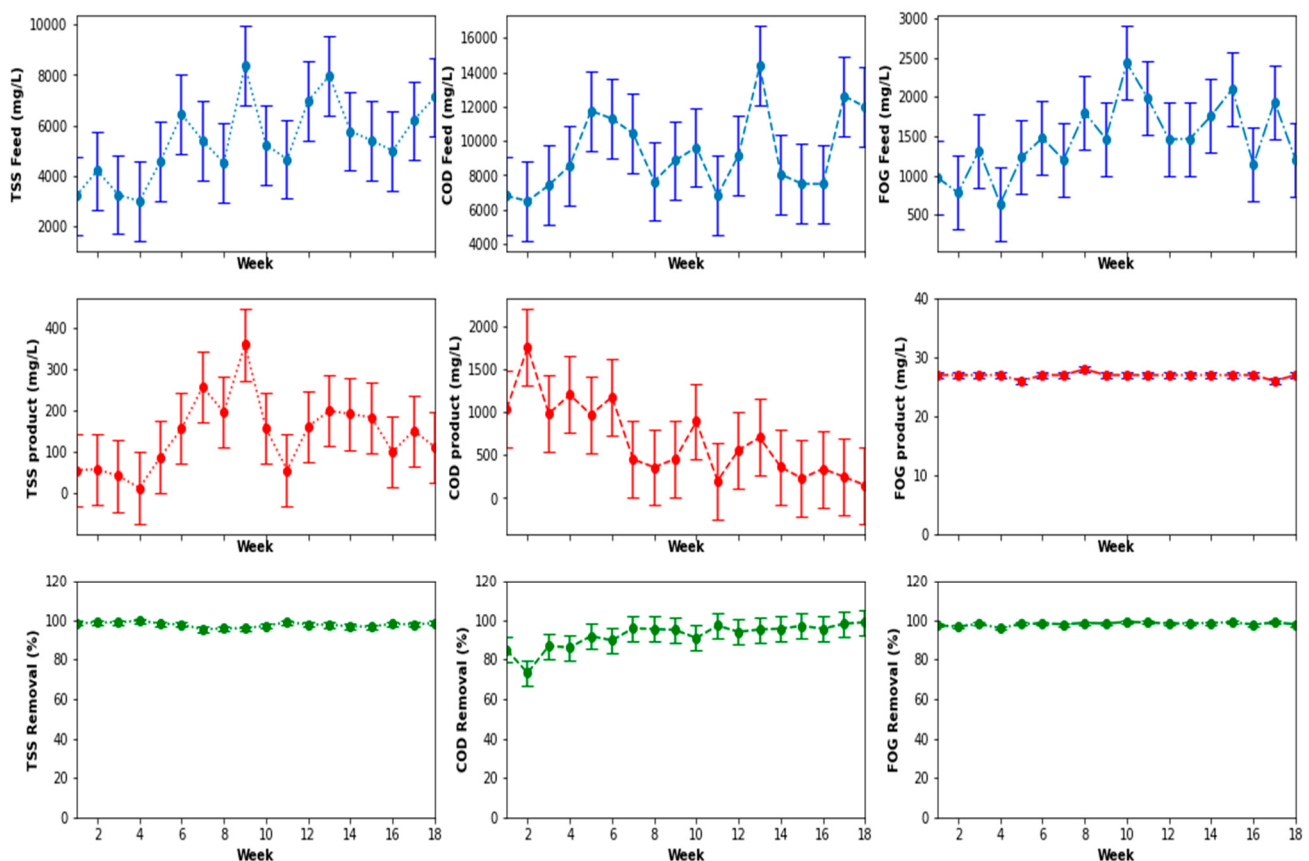


Figure 17. Overall performance of the complete system.

Table 2 below illustrates the overall performance of the biological pre-treatment of PSW coupled with the DEGBR. For overall removal efficiency results of the system, the average feed of the pre-treatment and the DEGBR product was used for the calculations. When comparing the overall performance in terms of COD removal, of the UASB and the DEGBR, the results indicated a better performance for the DEGBR in comparison with another similar study in which the treatment of PSW was undertaken, albeit the used

UASB was operated for 1228 days, yielding a removal efficiency of up to 85% for COD [14]. Additionally, the overall biological pre-treatment of PSW coupled with DEGBR of this study, performed better when compared to another similar study which ran concurrently with this study under similar conditions using a similar pre-treatment strategy for the PSW (i.e., using Eco-flush™ in the pre-treatment unit). Although, in the other study, an EGSB was used whereby it was reported that low FOG, COD and TSS removal efficiencies of about 80%, 60% and 80% were achieved [24].

Table 2. Overall performance of the biological pre-treatment coupled with DEGBR.

Parameter	Inlet Feed (mg/L)	DEGBR Product (mg/L)	Efficiency (η) (%)
COD	5896	669	92 ± 6.3
TSS	5399	185	97 ± 1.2
FOG	1765	28	97 ± 0.8

An in-Depth Analysis of Pre-Treatment Unit-DEGBR Performance Parameters

Most PSW treatment technologies normally comprise of the dissolved air floatation (DAF) system coupled with the up-flow anaerobic sludge blanket (UASB) as a primary treatment for PSW [31]. However, in most cases the latter type of anaerobic reactor usually fails to meet discharge standards because of the effluent produced from the UASB that contains high content of ammonia nitrogen and a significant amount of residual COD [32]. Another study of PSW treatment plant for high strength effluent, the DAF pre-treatment system reported FOG and COD removal efficiency of $28.0 \pm 5.6\%$ and $38.7 \pm 8.0\%$, respectively [31]. The second step of biological treatment using the UASB yielded FOG and COD removal efficiencies of about $34.3 \pm 12.5\%$ and $69.1 \pm 6.9\%$, due to the insoluble nature of lipids in the PSW, with some being purported to absorbed onto sludge granules. Since, in this study, the COD removal efficiency was a crucial parameter that was used to evaluate alternative technologies either as single units or in combination, determination of the average COD removal efficiency needed to be much higher ($92 \pm 6.3\%$) than that reported elsewhere. As such, this study proves that the designed lab-scale plant had a significant effect of reducing COD. This could be attributed by a variety of factors ranging from the use of the Ecoflush™ in the pre-treatment stage, the use of sieves/screens which eliminated larger particles in the pre-treatment units, as well as the use of a DEGBR reactor which further promoted biodegradation of organic matter, thus reducing pollutants in the wastewater. A performance attribute of $92 \pm 6.3\%$ COD removal for this study was slightly higher in comparison with other similar studies whereby the reported COD was 85% [14], refer to Figure 17 and Table 2. Furthermore, it was observed that neither an increase in ORL nor a decrease in HRT had an adverse effect on the process [7].

The TSS removal efficiency was another parameter used in this study to measure the performance of the system used. In another study of the biological treatment of an actual slaughterhouse wastewater (SWW) treated in an anaerobic baffled reactor (ABR), followed by an aerobic activated sludge (AS) reactor in continuous mode at laboratory scale, it was indicated that a gradual growth in the biomass led to a more stabilized TSS removal [33], as the anaerobic bed acted as a biofilter. Moreover, it was also established that an optimum minimum TSS residual is achieved when both the influent total organic carbon (TOC) concentration and feed flow rate are minimum. For this study, having an average TSS removal efficiency of the entire process being $97 \pm 1.2\%$, at an operational HRT of 106 h over 18 weeks (126 days), was commendable. This means that the longer the operational time and the lower the volumetric flow rates, the better the TSS removal efficiencies due to an improved biomass growth resulted in an acclimation process which was adequate. Moreover, increased organic loading rates (OLRs) and constant high hydraulic retention times (HRTs) resulted in a well-developed granular sludge bed in the DEGBR, which also led to enhanced TSS removal efficiencies. The high TSS removal efficiency was further attributed to the used inoculum from a brewery-activated sludge system, which had fully

acclimatized and resulted in a well-developed biomass that promoted the filtering of TSS present in the PSW [34]. Further results obtained from this study showed that, by using the Ecoflush™, solubilization of some FOG components resulted in the decoupling of TSSs, which resulted in the further filtering efficiency of the PSW in the pre-treatment stage, subsequently yielding better performance in FOG removal in the DEGBR. Comparatively, another study used for long-term PSW treatment, whereby the DAF contributed to the reduction of FOG, COD and TSS prior to the application of an UASB [19], only FOG, COD and TSS removal efficiencies of 63%, 67%, and 61%, respectively, were achieved. In another study, PSW treatment using a pilot SGBR reactor, with organic loading rates between 0.63 to 9.72 kg/m³/d and a hydraulic retention time (HRT) of 9 to 48 h, only reported removal efficiencies of COD above 90% and a TSS above 80%, respectively [35], results which were comparatively similar to the ones reported herein using a DEGBR.

Therefore, by using Ecoflush™ as a solubilization agent, it was indicated that the microorganisms present in it aided in the biological hydrolysis of some pollutants present in the PSW. Since the raw PSW was first aerated to invigorate the microorganisms in the Ecoflush™, the proliferation of bacteria in the agent resulted, which contributed to the initial degradation of organic material and pollutants. Overall, the microbes partially consumed the organic contaminants and further bound less soluble matter found in the raw PSW, which also reduced the odor of the wastewater.

Since the DEGBR ran at a stable VFA/Alkalinity ratio of 0.2, which is below 0.3, it means that there was adequate organic matter digestion in the reactor [7]. It has been established that an acidic digester or reactor is one which is above a 0.8 VFA/Alkalinity ratio; therefore, the consequence of an acidic reactor is that there could be a microbial inhibition of methane gas production [30]. However, it was found that the methanogenetic activity in the DEGBR decreased only after the addition of Ecoflush™ in the biological pre-treatment stage (i.e., as a preceding step before the DEGBR process), whereby methanogenesis was expected to ensue. This means that the supplementation of solubilizing agents in pre-treatment units for PSW might inhibit methane gas production. Overall, the DEGBR proved that there was no need of backwashing, as the recycle stream incorporated in the DEGBR design enhanced the system's performance and consistency by preventing clogging problems [9]. Table 3 further illustrates the performance of other systems similar to the DEGBR.

Table 3. Comparison of down-flow expanded granular bed reactor (DEGBR's) results to other similar wastewater treatment studies.

Technology Used	Type of Wastewater	Results	Reference
Static granular bed reactor (SGBR)	PSW	93% COD, 95% TSS, and 90% FOG	[36]
Up-flow anaerobic filter	PSW	70% tCOD, VFA/alkalinity ratio: 0.12–0.34, methane content in biogas: 46 and 56%	[23]
Expanded granular sludge bed (EGSB)	Cooking wastewater	72.9% COD	[22]
Up-flow anaerobic sludge-bed (UASB)	High fat wastewater	91.2% COD and 98.5% FOG	[37]

3.4. A Comparison of Product Parameters with the City of Cape Town Specifications

Generally, the DEGBR product samples obtained in this study were within the limit prescribed by the City of Cape Town by-laws (2013) [17]. This study reported average discharge parameters for COD, TSS and FOG of 699, 185 and 28 mg/L, respectively, in comparison with the City of Cape Town's prescribed compliance limits for COD, TSS and FOG of 5000, 1000 and 400 mg/L, respectively. Table 4 also shows that the DEGBR product samples reported an average pH and temperature values of 7.3 and 37.5 °C, which are within the prescribed discharge standards (i.e., pH range of 5.5–12 and a temperature of 0–40 °C). However, this study's treated PSW did not meet the minimum required for environmental, health and safety (EHS) guidelines values. The EHS guidelines are technical reference standards with general and industry specific examples of good international industry practice (GIIP) [38]. The product samples from this study (i.e., post the pre-

treatment and DEGBR) are shown in Figure 18. Moreover, even though the water requires further processing in order for it to reach reusable standards, treated water could be suitable to be used for other purposes such as irrigation [39].

Table 4. DEGBR's product in comparison to City of Cape Town's specifications and others.

Parameter	CCT* by-Law Limits	EHS*	DEGBR Product
COD	5000 mg/L	250 mg/L	669 mg/L
TSS	1000 mg/L	50 mg/L	185 mg/L
FOG	400 mg/L	10 mg/L	28 mg/L
pH	5.5–12	6–9	7.3
Temperature	0–40 °C	<3 ^a	37.5 °C
VFA	-	-	157.97 mg/L
Alkalinity	-	-	888.12 mg/L

CCT*—City of Cape Town: Wastewater and industrial effluent by-law, 2013. EHS*—environmental, health, and safety guidelines poultry production, world bank group, 2007. ^a At the edge of a scientifically established mixing zone which takes into account ambient water quality, receiving water use, potential receptors and assimilative capacity.



Figure 18. A comparison of raw PSW effluent and DEGBR product sample.

4. Limitations and Future Research Direction

This study was limited by a variation of slaughterhouse production peak times which resulted in the sampling of differentiated PSW with varying pollutant concentrations. Furthermore, this study does not focus on solid, sewer waste and ammonium gas generation. Moreover, the study does not focus on cost evaluation and energy usage which could be important aspects for future studies.

5. Conclusions

The study of the lab-scale plant of the biological pre-treatment of the PSW coupled with the DEGBR was successfully employed. At the pre-treatment stage, the FOG, COD and TSS removal efficiencies that were obtained were $80 \pm 6.3\%$, $38 \pm 8.4\%$, and $56 \pm 7.2\%$, respectively. Similarly, the DEGBR achieved average FOG, COD and TSS removal efficiencies of $89 \pm 2.8\%$, $87 \pm 9.5\%$ COD and $94 \pm 3.7\%$, even at high OLR of up to 8.3538 g COD/L . day with an HRT of 106 h. Moreover, the overall performance of the conjoint systems achieved a FOG, COD and TSS efficiency of $97 \pm 0.8\%$, $92 \pm 6.3\%$ and $97 \pm 1.2\%$, respectively. The DEGBR system was operating under stabilized conditions since the pH was within the optimal range of 6.6–8 and the VFA/alkalinity ratio was consistently around 0.2, albeit slightly lower than the recommended 0.3 throughout the experimental period. The average methane gas production reported during the start-up period was $0.3 \text{ L CH}_4/\text{g COD}$, which reduced as the operation ensued. This was attributed to the application of Eco-flush™ containing pre-treated wastewater feed to the DEGBR. A full-scale application of the DEGBR to treat similar high-strength wastewater treatment would provide benefits to

the slaughterhouse wastewater treatment plants, due to its simple design and operational advantages over conventional high rate anaerobic systems. Lastly, the designed lab-scale plant can be used in small and large poultry processing industries.

Author Contributions: D.N.D. is the author of the draft manuscript and was responsible for sample analysis, and initial data interpretation. M.N. checked the collated data, reanalyzed, reinterpreted the data and assisted in data presentation. M.B. and S.K.O.N. conceptualized the study, revised the manuscript and analyzed the data. E.K. designed and built the lab-scale plant under Malutsa Pty Ltd., Western Cape, Wellington Industrial Park, SA. All authors have read and agreed to the published version of the manuscript.

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