

# Optimization of the COD Removal Efficiency for a Static Granular Bed Reactor Treating Poultry Slaughterhouse Wastewater

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## Abstract

In this study, the efficiency of an anaerobic treatment system for wastewater from a South African poultry slaughterhouse was evaluated using a lab-scale static granular bed reactor (SGBR). The down-flow SGBR (2 L) was operated continuously for 138 days under mesophilic conditions (35-37 °C), at hydraulic retention times (HRTs) ranging from 24 to 96 h and average organic loading rates (OLRs) of 0.78 to 5.74 g COD/Lday. The SGBR achieved an average chemical oxygen demand (COD) removal efficiency of 80% and the maximum COD removal achieved was 95%, at an HRT of 24 h and average OLR of 5.74 g COD/Lday. The optimization of the SGBR, with regard to a suitable HRT and OLR, was determined using response surface methodology (RSM). The optimal SGBR performance with regard to the maximum COD removal efficiency was predicted for an OLR of 12.49 g COD/Lday and a HRT of 24 h, resulting in a 95.5% COD removal efficiency. The model R<sup>2</sup> of 0.9638 indicated that the model is a good fit and is suitable to predict the COD removal efficiency for the SGBR.

## Keywords

Anaerobic digestion; Chemical oxygen demand; Optimization; Poultry slaughterhouse wastewater; Response surface methodology; Static Granular Bed Reactor

## INTRODUCTION

The SGBR is a relatively new high-rate anaerobic reactor developed with the purpose of simplifying the design, operation, and maintenance of high-rate anaerobic reactors whilst still maintaining high-quality influent treatment and maximizing biogas production (Ellis, 2008; Evans, 2004). The design of the SGBR is based on the up-flow anaerobic sludge blanket (UASB) reactor design; however, the distinguishing feature of the SGBR is its down-flow configuration which eases the separation of the wastewater, solids, and biogas. Unlike other down-flow anaerobic reactors, such as the anaerobic filter (AF), the SGBR uses highly active anaerobic granules (Debik & Coskun, 2009; Evans, 2004). In addition, the down-flow configuration of the SGBR enables the accommodation of higher suspended solid concentrations in comparison to up-flow anaerobic reactors, such as the UASB reactor and expanded granular sludge bed (EGSB) reactor, which

frequently experience solids washout, i.e. the loss of granular biomass due to the high up-flow velocities used. There are bioreactor conditions which must be monitored and maintained to ensure that anaerobic and aerobic bioreactors operate optimally and more importantly to avoid process inhibition and system failure. Firstly, the micro-organisms used must have sufficient time for the biodegradation of organic matter and the sorption of unreactive species, and secondly, the organic loading must be controlled in such a manner that overfeeding of the micro-organisms is prevented (Nayona, 2010). The HRT and OLR are two of the key operating conditions used to ensure that an appropriate balance is achieved in bioreactors treating PSW (Wellinger et al., 2013). These conditions can be optimized using statistical methods, such as RSM.

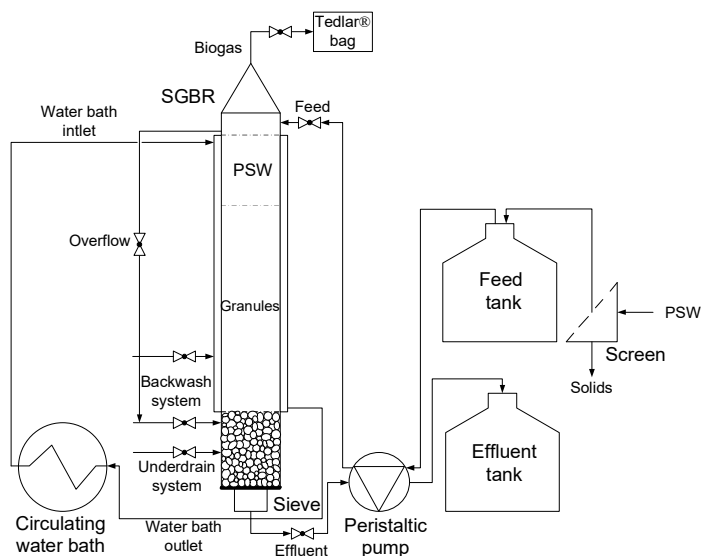
The SGBR has been extensively used for the treatment of wastewaters from different industries, including poultry slaughterhouse wastewater (PSW) (Chong et al., 2012; Park et al., 2012). According to the literature reviewed, mainly laboratory and pilot-scale SGBRs operating at ambient temperature (i.e. approximately 25°C), have been researched. The pilot-scale SGBR systems are being used for wastewater treatment with the intention of studying the effect of the HRT, solids retention time (SRT), and OLR. The data collected from such pilot-scale studies are to be used for the design of full-scale SGBR systems and the commercialization of this new technology (Park et al., 2012). Variations of the SGBR operating conditions, i.e. temperature, OLR and HRT, have proven to have relatively minimal effect on the stability of the SGBR and do not significantly affect the effluent quality (Ellis, 2008). Therefore, the aim of this study was to: 1) determine the feasibility of treating PSW using a SGBR and 2) optimize the SGBR with regard to the HRTs and OLRs using RSM.

## MATERIALS AND METHODS

### *PSW characterization, SGBR setup and inoculation*

PSW was sourced from a poultry product processing plant located in the Western Cape Province (South Africa). The fresh PSW samples were stored in 25 L polyvinyl chloride (PVC) containers and were refrigerated at 4 °C. The characteristics of the PSW used in this study, specifications of the PSW and anaerobic granules used for the SGBR inoculation are listed in Table 1. A lab-scale down-flow SGBR, made from a cylindrical glass column, with an active volume of 2 L (height and inner diameter of 0.62 m and 0.065 m, respectively), was used in this study. A perforated influent tube was positioned at the top of the SGBR to evenly distribute the influent. A second perforated tube was positioned in the centre of the underdrain bed as part of a backwashing system, which also had an overflow line. Pumice stones (average diameter of 5-20 mm) were used as the underdrain bed to prevent the washout of the anaerobic granules. A stainless-steel sieve (pore size: 2 mm) was fixed to the bottom of the SGBR to retain the pumice stones, which occupied a volume of 0.3 L. The SGBR system consisted of a 5 L feed storage tank, a 5 L effluent storage tank, polyvinyl chloride (PVC) and glass fittings, silicone tubing, a MINIPULS® Evolution peristaltic pump (Gilson, USA), and a circulating water bath were used to supply warm water to the jacket (Figure 1). The SGBR was inoculated with 0.4 L anaerobic granules obtained from a full-scale mesophilic up-flow anaerobic sludge blanket (UASB) reactor operated at a local brewery plant (Newlands, South Africa) and fed with 1.6 L of PSW. A dry milk solution of 0.01 L (1427±65 mg COD/L) prepared with potable water (50% (v/v)) was used to facilitate the acclimatization of the anaerobic granules. The specifications of the anaerobic granules and PSW used are presented in Table 1B and C. The SGBR feed was prepared by filtering the untreated PSW through a mesh screen (pore size: 2 mm) to remove floating solids, which could potentially clog tubing and cause operational challenges. The filtered PSW was then diluted (50% and 25% (v/v)) with potable water to prevent shock loading of the SGBR during the start-up period. After the acclimatization period (48 h), the HRT was set to 2.3 days (55 h) to initiate the start-up OLR of 1.18 g COD/L.day. A start-up OLR of approximately 1 g COD/L.day is recommended for SGBRs based on previous studies (Park et al., 2012; Lim, 2008). The HRT was maintained at 55 h and the SGBR was operated in a continuous

mode under steady state conditions, throughout the start-up period (28 days). As the performance of the SGBR stabilized with regard to the percentage COD removal, the HRT was varied stepwise to simulate variations in the feed flow rate and thus the OLR so as to adequately assess the performance of the SGBR in treating PSW. The SGBR was maintained at mesophilic temperature (i.e. 35-37 °C) throughout the 138 days of continuous, steady-state operation.



**Figure 1** Schematic diagram of the lab-scale SGBR system

**Table 1** A) Characteristics of the untreated PSW used in this study, B) Specifications of the PSW and C) anaerobic granules used for the SGBR inoculation

Parameter (Unit)	A	B	C
	Values	Values	Values
Chemical oxygen demand (COD) mg/L	5216	2800 ±36	-
pH at 25 °C	5.6	7.5	7.5
Conductivity at 25 °C mS/m	159	-	-
Ammonia (as N) mg/L	25.2	215	-
Ortho-phosphate (PO <sub>4</sub> <sup>3-</sup> ) mg/L	24.3	31	-
Nitrate (NO <sub>3</sub> <sup>-</sup> ) mg/L	1.0	1.9	-
Total suspended solids (TSS) mg/L	1580	675	42867
Volatile suspended solids (VSS) mg/L	1533	1625	51391
Volatile fatty acids (VFA) mg/L	421	-	-

**Table 2** Summary of SGBR operating conditions

Operating time (days)	HRT (h)	Flow rate (Q) (L/h)	Average OLR (g COD/Lday)	Dilution (v/v) (%) [number of days]
28	55	0.0364	1.18	50 [28]
29	96	0.0208	0.78	50 [15] 75 [8] None [6]
27	48	0.0364	1.96	None
28	36	0.0486	4.10	None
26	24	0.0729	5.74	None

### ***Response surface methodology***

RSM was used for the optimization of the COD removal efficiency for the SGBR treating PSW. A central composite design (CCD) was used to determine the optimum operating conditions, i.e. hydraulic retention time (HRT) (B) and organic loading rate (OLR) (A), and the interaction between these independent variables, with the COD removal efficiency (%) being the response variable. The OLR and HRT were chosen based on their direct impact on the COD removal efficiency and the chosen ranges were based on the operating conditions used for the SGBR in this study, i.e. conditions reported in Debik & Coskun (2009) and Basitere et al. (2017). Design Expert® 10.0.3 statistical software (Stat-Ease, Inc., USA) was used for the experimental design. A two-factor (i.e. HRT and OLR), two-level (i.e. low (-1) and high (+1)) CCD was applied, with a total of 15 experimental runs generated. This corresponded to 0.73 (min) to 12.5 (max) and 1 (min) to 4 (max) for OLRs and HRTs. Analysis of variance (ANOVA) was used to evaluate the significance of the response surface quadratic model, the individual variables and their interactions (Bustillo-Lecompte & Mehrvar, 2017).

### ***Sampling and analysis***

Feed and effluent samples were collected at 24 h intervals (i.e. Mondays, Wednesdays, and Fridays) and analysed in triplicate. The COD analysis samples were prepared using Merck COD solutions (solution A: Cat. No. 1.14538.0065 and 1.14679.049; solution B: Cat. No. 1.14539.0495 and 1.14680.1495), digested in a preheated Spectroquant TR420 Thermoreactor, and measured using the Merck Spectroquant Nova60. A weekly composite sample of the feed and effluent were sent to a South African National Accreditation System (SANAS) accredited lab (Scientific Services, South Africa), for confirmation of the COD results.

## **RESULTS AND DISCUSSION**

### ***SGBR performance: COD Removal***

During the 138 days of operation, the COD removal efficiencies exceeded 80%, with the intermittent performance reduction on several days being attributed to backwashing operations. Similarly, the low initial COD removal efficiencies (average 74.1±3.6%) observed during the start-up period could be as a result of reduced FOG hydrolysis and suspended solids interaction with the anaerobic granules which contributed to an increase in soluble COD non-biodegradation. Del Nery et al. (2007) reported 65% COD and 85% soluble COD removal efficiencies for the treatment of PSW in a UASB at an average OLR of 1.64 g COD/L.day. The average COD removal efficiencies of 78±4%, 79±5%, 86±5% and 85±5%, for the corresponding HRTs of 96, 48, 36 and 24 h, respectively, was attributed to the increased functionality of the biomass with increasing bioreactor operation time, thus the SGBRs ability to effectively tolerate increasing OLRs as the biomass matured. Several studies using the SGBR to treat a variety of wastewaters showed a similar trend (Debik & Coskun, 2009; Lim & Fox, 2011; Park et al., 2012; Oh et al., 2015). Despite fluctuations in the COD of the feed, an OLR of up to 12.5 g COD/Lday, and step-wise decreases in the HRT, culminated in the COD removal efficiencies remaining relatively consistent. Overall, the SGBR design used in this study demonstrated a stable performance with COD removal efficiencies greater than 95% observed towards the end of the study at the 24 h HRT. Comparatively, the SGBR is known to produce COD removal efficiencies exceeding 90% for a wide range of HRTs, i.e. 9 to 55 h and OLRs i.e. 0.63 to 9.72 g COD/Lday, under varying temperature conditions (Debik & Coskun, 2009; Lim & Fox, 2011; Park et al., 2012; Oh et al., 2015; Basitere et al., 2017), which is indicative of the robustness of the SGBR. Whilst it is evident that the HRT and OLR affects the COD removal efficiency, in this study neither an increase nor decrease in the HRT or OLR had long lasting impact nor adverse effects on the overall biological performance of the SGBR in terms of its COD removal efficiency. This indicated that the high biomass concentration in the SGBR was sufficient to maintain the required high removal efficiencies for a wide range of HRTs and OLRs. Debik &

Coskun (2009) operated a SGBR at HRTs of 48 and 36 h for the treatment of PSW; and achieved COD removal efficiencies higher than 90% at OLRs ranging between 2.53 and 4.97 g COD/Lday. Similarly, Basitere et al. (2017) reported COD, TSS and FOG removal efficiencies of 93%, 95% and 90%, respectively, when operating a SGBR for PSW treatment at HRTs of 55 and 40 h with average OLRs of 1.01 and 3.14 g COD/Lday.

### ***Optimization of the COD removal efficiency for the SGBR***

Response surface methodology (RSM) was used for the optimization of the SGBR operating conditions through the development of a quadratic model used to predict the COD removal efficiency of the SGBR for the treatment of PSW. The hydraulic retention time (HRT) and organic loading rate (OLR) were selected as the two independent variables which were evaluated in order to determine their effect on the COD removal efficiency. SGBRs treating slaughterhouse wastewaters are capable of operating under HRTs ranging from 8 to 96 h (0.33 to 4 days) (Oh, 2012; Mach, 2004) and OLRs as high as 12.76 g COD/Lday (Park et al., 2012). The chosen ranges used were based on the operating conditions used for the SGBR in this study, i.e. conditions reported in Debik & Coskun (2009) and Basitere et al. (2017). The SGBR system used in this study was able to consistently reduce the organic matter content of the PSW, resulting in an overall COD removal efficiency of 80% for HRTs of between 1 to 4 days (24 h to 96 h) and an average OLR of 2.75 g COD/L.day. Table 3 shows the central composite design (CCD) of the independent variables used to optimize the COD removal efficiency. The predicted results from the CCD indicated that an OLR of 12.49 g COD/Lday (A) and a HRT (B) of 1 day were the optimum conditions for attaining the maximum COD removal efficiency (95.5%) for the SGBR used for the treatment of the PSW. The overall results suggest that the COD removal efficiency attained by the SGBR increased as the organic strength of the PSW increased and the HRT was decreased. Conversely, Basitere et al. (2017) reported a decrease in the COD removal efficiencies of an SGBR treating PSW when the OLR increased subsequent to a decrease in the HRT from 55 h to 48 h. The interaction between the operating conditions, i.e. HRT and OLR, and the COD removal efficiency was determined using a polynomial regression. Table 4 summarizes the analysis of variance (ANOVA) results for the quadratic model used to predict the COD removal efficiency of the SGBR. Eq. 2 represents the resultant quadratic model which best fit the regression results.

**Table 3** CCD of the independent variables, (A) OLR and (B) HRT

Run	Factors		COD Removal efficiency (%)	
	OLR (A) (g COD/Lday)	HRT (B) (day)	Experimental	Predicted
1	1.04	2.29	72.8	72.4
2	0.96	2.29	67.7	71.7
3	1.26	2.29	76.0	74.1
4	0.73	4	78.5	77.8
5	0.88	4	78.9	80.3
6	0.77	4	79.3	78.5
7	2.01	2	80.9	79.5
8	1.81	2	80.1	78.2
9	2.79	2	83.8	84.5
10	3.47	1.5	85.4	86.0
11	4.26	1.5	88.0	89.0
12	3.49	1.5	86.4	86.1
13	12.0	1	95.4	95.0
14	7.33	1	90.4	90.2
15	12.49	1	95.2	95.5



The adequacy of the proposed model was determined according to the determination coefficient (R<sup>2</sup>), F-value and p-value (see Table 4). An R<sup>2</sup> of at least 0.80 is indicative of the good fit of a model (Dahunsi et al., 2016). The F-value is based on the comparison between the variance related with all terms and the residual variance; whereas, the p-value refers to the probability value which is related to the F-value for all terms (Dahunsi et al., 2016). The model R<sup>2</sup>, F-value and P-value of 0.9638, 47.93 and <0.0001, respectively, obtained for this optimization study indicated that the model was suitable to predict the COD removal efficiency. The adjusted determination coefficient (R<sup>2</sup> Adj) and predicted determination coefficient (R<sup>2</sup> Pred) values obtained were 0.9437 and 0.9097, respectively. The R<sup>2</sup> values are similar to those reported in literature for the optimization process using RSM for wastewater treatment (Bustillo-Lecompte & Mehrvar, 2017). The significance of the individual variables and their interactions in the model were determined by p-values less than 0.05. According to Table 4, the A2 and B2 had an insignificant effect on the COD removal efficiency. Therefore, Eq. 2 was reduced to Eq. 3, which represents the final quadratic model equation used to estimate the COD removal efficiency. Eq. 2 correlates the experimental results and the predicted results and is thus considered as a good fit.

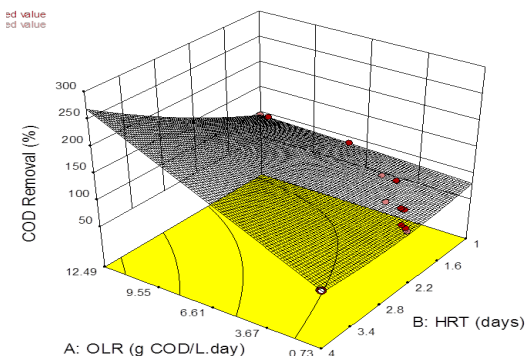
$$\text{COD Removal efficiency} = 121.64 + 51.38A + 42.57B + 44.44AB \quad (3)$$

Figure 2 illustrates the 3-D response surface plot representing the effect of the OLR and HRT on the COD removal efficiency achieved by the SGBR during the treatment of PSW. It is evident that the SGBR efficiency is affected by both the organic and hydraulic loading rates for the reactor. Thus, a balance between the two operating conditions must be maintained in order to ensure the stable and efficient operation of the SGBR as well as to prevent reactor failure. In other words, in order to allow the micro-organisms sufficient time for the biodegradation of the organic matter (i.e. COD) present in the PSW, certain conditions must not be exceeded so as to prevent overfeeding of the micro-organisms (Nayona, 2010). The optimal SGBR performance with regard to the maximum COD removal efficiency was predicted for an OLR of 12.49 g COD/Lday and a HRT of 1 day (24 h), resulting in a 95.5% COD removal efficiency. Similarly, Muhamad et al. (2013) reported a COD removal efficiency >90% at an HRT of 1 day (24 h) for the optimization of the COD removal of a granular activated carbon sequencing batch biofilm reactor (GAC-SBBR) treating recycled paper wastewater. Ideally, it is desired that the SGBR be operated at a maximum OLR, for the shortest HRT, in order to achieve the highest COD removal efficiency without adversely impacting the SGBR performance and operation for high throughput rates of treated PSW. However, since the SGBR is a biological system there might be other factors other than the organic and hydraulic loading, which might affect the SGBR performance such as the activity of the methanogens (Oh et al., 2015); however, these were not identified and investigated in this study. Figure 2 illustrates the 3-D response surface plot representing the effect of the OLR and HRT on the COD removal efficiency achieved by the SGBR during the treatment of PSW. It is evident that the SGBR efficiency is affected by both the organic and hydraulic loading rates for the reactor. Thus, a balance between the two operating conditions must be maintained in order to ensure the stable and efficient operation of the SGBR as well as to prevent reactor failure. In other words, in order to allow the micro-organisms sufficient time for the biodegradation of the organic matter (i.e. COD) present in the PSW, certain conditions must not be exceeded so as to prevent overfeeding of the micro-organisms (Nayona, 2010). The optimal SGBR performance with regard to the maximum COD removal efficiency was predicted for an OLR of 12.49 g COD/Lday and a HRT of 1 day (24 h), resulting in a 95.5% COD removal efficiency. Similarly, Muhamad et al. (2013) reported a COD removal efficiency >90% at an HRT of 1 day (24 h) for the optimization of the COD removal of a granular activated carbon sequencing batch biofilm reactor (GAC-SBBR) treating recycled paper wastewater. Ideally, it is desired that the SGBR be operated at a maximum OLR, for the shortest HRT, in order to achieve the highest COD removal efficiency without adversely impacting the

SGBR performance and operation for high throughput rates of treated PSW. However, since the SGBR is a biological system there might be other factors other than the organic and hydraulic loading, which might affect the SGBR performance such as the activity of the methanogens (Oh et al., 2015); however, these were not identified and investigated in this study.

**Table 4** Analysis of variance (ANOVA) of the quadratic model for chemical oxygen demand (COD) removal efficiency

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value Prob>F
<b>Model</b>	818.36	5	163.67	47.93	<0.0001
<b>A</b>	75.90	1	75.90	22.22	0.0011
<b>B</b>	54.42	1	54.42	15.93	0.0031
<b>AB</b>	34.03	1	34.03	9.96	0.0116
<b>A<sup>2</sup></b>	0.058	1	0.058	0.017	0.8995
<b>B<sup>2</sup></b>	7.78	1	7.78	2.28	0.1656
<b>Residual</b>	30.74	9	3.42		
<b>R<sup>2</sup></b>	0.9638	<b>R<sup>2</sup> Adj</b>	0.9437	<b>R<sup>2</sup> Pred</b>	0.9097



**Figure 2** 3-D response surface for the COD removal efficiency as a function of variables A: OLR and B: HRT

## CONCLUSIONS

The SGBR was able to consistently reduce the organic matter content of the pre-filtered PSW throughout its 138 days of operation. The SGBR efficiently reduced the COD by 80% on average for HRTs ranging from 24 to 96 h and average OLRs of between 0.78 and 5.74 g COD/Lday. Neither an increase nor decrease in the HRT or OLR had an adverse effect on the treatment efficiency of the SGBR in terms of its biological performance. Furthermore, the SGBR successfully treated the PSW to within the industrial effluent municipal discharge limits with regard to the COD. Despite the effluent COD averaging at 3649 mg/L, which is below the permitted discharge limit of 5000 mg/L, further reduction of COD to a value below 1000 mg/L would eliminate penalties imposed on industries. Using RSM, the optimal SGBR performance with regard to the maximum COD removal efficiency was predicted for an OLR of 12.49 g COD/Lday and a HRT of 24 h (1 day), resulting in a 95.5% COD removal efficiency. The model R<sup>2</sup> of 0.9638 indicated that the model is a good fit to predict the COD removal efficiency for the SGBR. Further experiments should be done to quantitatively and qualitatively evaluate the biogas produced by the SGBR treating PSW; and the data obtained may be used to support the experimental and RSM data.

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