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Application of response surface methodology to optimize the COD removal efficiency of an EGSB reactor treating poultry slaughterhouse wastewater

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Abstract

The poultry slaughterhouse industry consumes a large volume of potable water for bird processing and equipment cleaning, which culminates in the generation of high strength poultry slaughterhouse wastewater (PSW). The wastewater contains high concentrations of organic matter, suspended solids, nitrogen and nutrients. Most poultry slaughterhouses in South Africa (SA) discharge their wastewater into the municipal sewer system after primary treatment. Due to its high strength, PSW does not meet SA's industrial discharge standards. Discharge of untreated PSW to the environment raises environmental health concerns due to pollution of local rivers and fresh water sources, leading to odour generation and the spread of diseases. Thus, the development of a suitable wastewater treatment process for safe PSW discharge to the environment is a necessity. In this study, a biological PSW treatment process using an Expanded Granular Sludge Bed (EGSB) was evaluated. Response surface methodology coupled with central composite design was used to optimize the performance of the EGSB reactor. The dependant variable used for optimization was chemical oxygen demand (COD) removal as a function of two independent variables, hydraulic retention time (HRT) and organic loading rate (OLR). The interactions between HRT, OLR and COD removal were analysed, and a two factorial (2FI) regression was determined as suitable for COD removal modelling. The optimum COD removal of 93% was achieved at an OLR of 2 g-COD/L/d and HRT of 4.8 days. The model correlation coefficient (R^2) of 0.980 indicates that it is a good fit and is suitable for predicting the EGSB's COD removal efficiency.

Key words: chemical oxygen demand (COD), optimization, poultry slaughterhouse wastewater (PSW), response surface methodology (RSM)

INTRODUCTION

The agricultural sector uses a large quantity of freshwater, with global average usage exceeding 70% of all surface water usage (Bustillo-Lecompte & Mehrvar 2015). The extent of water use in the agricultural sector poses environmental challenges as it normally yields effluent that is discharged untreated, culminating in receiving water source pollution (Bustillo-Lecompte & Mehrvar 2015), further exacerbating environmental pollution. Some agricultural sector industries, such as poultry processing facilities (slaughterhouses), generate large volumes of wastewater with the potential to pollute freshwater sources if not treated appropriately prior to discharge (Gerber *et al.* 2007). This is a common challenge facing the poultry industry globally due to an increase in demand for poultry products (Avula *et al.* 2009). Generally, poultry processing plants consume about 26.5 L/bird of potable

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water during primary and secondary processing of live birds to meat (Yordanov 2010). Most of the water is used for scalding, defeathering, evisceration and equipment sanitation including the slaughtering facility. Roughly 2 to 5% of total proteins, including carcass debris and fats, oil and grease (FOG) from the carcass, are lost to the wastewater stream, resulting in high strength wastewater with high biological oxygen demand (BOD_5) (Avula *et al.* 2009) and chemical oxygen demand (COD), compared to domestic wastewater (Zhang *et al.* 1997). This indicates the need for intensive treatment prior to discharge to the environment (Avula *et al.* 2009), i.e. into receiving bodies such as rivers and lagoons. Due to increasingly stringent regulation, globally and nationally, coupled with water supply insecurity, including water scarcity in South Africa (SA), poultry product processing industries are required to develop advanced wastewater treatment systems to treat and re-use their wastewater. The treatment will benefit poultry processing plants by reducing both potable water demand and the volume of wastewater generated (Avula *et al.* 2009).

Microbial wastewater treatment technologies such anaerobic digestion (AD) can play a vital role in remedying the environmental concerns posed by PSW generation. AD is considered the most appropriate treatment technology suitable and available for PSW, even in remote regions of SA. This type of technology has been used to treat industrial wastewaters such as paper mill effluent, and textile, soft drink and domestic wastewaters (Lim 2009). Historically, AD has been considered appropriate for treating wastewater in large-scale operations. Currently, it remains the preferred method in the food waste industry due to numerous advantages such as low energy consumption, reduced production of waste biological solids, low nutrient and chemical requirements, high COD reduction, and pathogen deactivation even at high organic loading rates, as well as the production of biogas, which can be burnt to generate heat and electricity, or refined into renewable natural gas, for bottling and transformation into other fuels (Bustillo-Lecompte & Mehrvar 2015). However, AD has some disadvantages such as being sensitive to pollutants, which affect the metabolic functions of organisms in the sludge biomass, odour production during operation, an elongated start-up procedure that can be difficult to stabilize for semi-skilled operators, and potential requirement for post-treatment of the resulting treated wastewater, i.e. tertiary treatment for the effluent from the AD process to meet regulatory discharge standards (Lim 2009; Harris & McCabe 2015).

Generally, AD is robust and stable if the system's operation is well understood (Lim 2009). Furthermore, the process can play a vital role in waste management and the reduction of greenhouse gas emissions (Harris & McCabe 2015), while the digestate can be used as value-added, organic fertilizer for soil amendment (Lim 2009). Traditional anaerobic treatment systems such as digesters, contact process systems and lagoons, have been successfully applied to treat food processing wastewaters (Basitere *et al.* 2017; Rinquest *et al.* 2019). These systems have been used to reduce COD and BOD_5 concentrations, stabilizing the wastewater treatment system through sludge retention. Further, anaerobic treatment systems such as up-flow anaerobic sludge blankets (UASB) popularized the use of granulated anaerobic biomass, a significant improvement in wastewater treatment from traditional systems (Avula *et al.* 2009). Further development and design of AD systems resulted in systems such as the Expanded Granular Sludge Bed reactor (EGSB), a modified form of UASB, demonstrating the capability of propagated sludge granules to increase contact effectively between the wastewater and anaerobic granules (Avula *et al.* 2009). The recirculation stream improves things further because the increased up-flow velocity causes granular-bed expansion, leading to sustainably and substantially improved bioreactor performance. (UASB and EGSB reactor performance both also depend on a well-designed gas-liquid-solids separation system, separating the biogas from the wastewater and biomass).

The main objective of this study was to develop a systematic approach for the optimization of COD removal efficiency in an EGSB reactor treating PSW, using response surface methodology (RSM). The RSM software coupled with central composite design (CCD) (Stat-Ease Inc. Minneapolis, USA), combines mathematical and statistical tools used to model and optimize processing units, e.g., EGSB used

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for PSW treatment. RSM is used widely to evaluate the relative significance of operational parameters in the presence of complex interactions – e.g. in the food, environmental biotechnology, and enzyme production industries (Sathian *et al.* 2014). It has also been used and adapted in the chemical industry since its development in the 1950s (Ngongang 2016), and in the design and optimization of biological processes (Bashir *et al.* 2010). The optimization process using RSM depends on approximation of the response by a polynomial equation within a specified range (Bashir *et al.* 2010; Osman *et al.* 2014), with the quality of the polynomial expressed by the coefficient of determination (R^2).

The RSM employed using CCD, which is part of Design-Expert® 6.0.10 (Stat-Ease Inc. Minneapolis USA), designs a set of experiments to generate efficient and optimum bioprocess conditions. The software uses experimental data and depends on predetermined operational parameter ranges to optimise process efficiency. Zinatizadeh *et al.* (2011) used RSM to design dairy wastewater treatment experiments with a sequencing batch reactor, while Sathian *et al.* (2014) used one to treat textile dye wastewater for a study in which RSM was used to optimize parameters such as air flow rate, solid retention time (SRT) and cycle period. In this study, RSM (+ CCD) was employed to optimize COD removal efficiency as a function of two independent variables – hydraulic retention time (HRT) and organic loading rate (OLR), and to determine the interaction between the two.

MATERIALS AND METHODS

Characteristics of PSW

The PSW used in this study came from a slaughterhouse in the Western Cape Province, SA. It contained bird faeces, urine, FOG, blood, carcases and undigested food from birds' intestines. As a result, there were high concentrations of organics and nutrients, typically measured as COD, biochemical oxygen demand (BOD_5), total suspended solids (TSS), total nitrogen and total phosphorus (TP). Table 1 summarizes the PSW's characteristics.

Table 1 | Characteristics of the PSW from the Western Cape, South Africa

		PSW	
Parameter	**Unit**	**Range**	**Average**
pH	–	6.5–8.0	6.88
Alkalinity	mg-$CaCO_3$/L	0–489	225
COD	mg/L	2,133–4,137	2,903
Ammonia	mg-N/L	29–51	40
Total Phosphorus	mg/L	8–27	17
FOG	mg/L	131–684	406
TSS	mg/L	315–1,273	794
Soluble proteins	mg/L	0–368	72
Nitrate	mg-N/L	0–2,903	1,245

EGSB experimental setup and equipment

The EGSB consisted of a cylindrical glass column (Figure 1) with a working volume of 2.7 L – inner diameter 0.065 m, height 0.872 m. Ceramic marbles – average diameter 0.0157 m – were placed at the bottom of the bioreactor as packing, to retain the granular sludge in the reactor's heated section. PVC containers (5 L, $n = 2$) were used for feed and product storage. The EGSB was fed with influent at the bottom using a Gilson multi-head peristaltic pump. The effluent produced was withdrawn at the same rate. Silicon tubing

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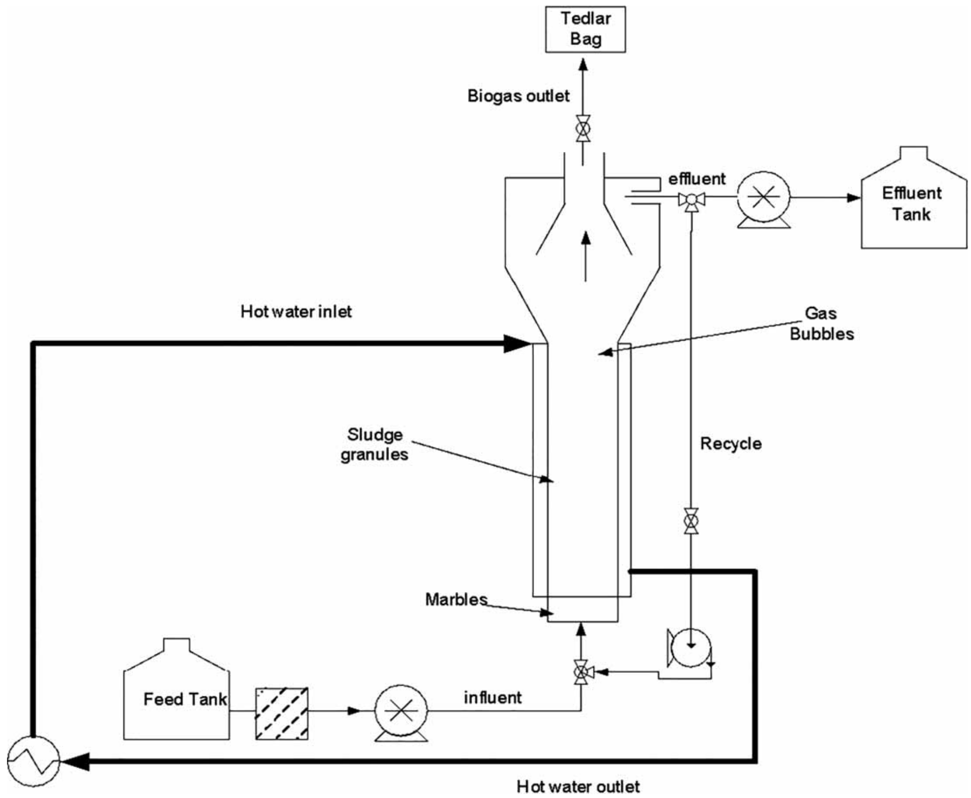

Figure 1 | Schematic illustration of the EGSB set-up treating PSW.

with internal diameter 0.8 cm was used to connect the bioreactor streams. A recycle stream connected to the feed/influent enabled sludge suspension and hydraulic mixing in the bioreactor. The bioreactor was operated at mesophilic temperature (35 to 37 °C), conditions maintained using a water jacket.

EGSB inoculation and operating conditions

The EGSB was inoculated with 0.747 L of anaerobic granular sludge from a full-scale UASB reactor operated by SABMiller PLC (Newlands Brewery, South Africa). A dry milk solution (10 mL, 50% w/v) was also prepared, and used as a feed during the EGSB's 48-hour acclimation period. The influent PSW was filtered (2 mm mesh) to remove feathers and suspended solids, which might clog the tubes. The influent was initially diluted to minimise shock loading, with dilution ratios of 50 and 30% (v/v). Undiluted PSW was used thereafter, once the bioreactor had stabilised, for long-term operation. During start-up, a 50% dilution feed was used and the EGSB's start-up HRT was 65 hours, which was reduced to 60 hours to increase mixing. The HRT was then maintained at an average of 62.5 hrs, with a constant median of 60 hrs for the trial's 43 days. The average influent OLR was 1 g-COD/L/day. Subsequently, the bioreactor was fed with 70% PSW at an HRT of between 60 and 55 hrs (average 57.5 hrs), as well as a mean HRT of 60 hrs for a total of 49 days, with an average OLR of 2 g-COD/L.d. Undiluted PSW only was fed to the reactor for a further 81 days, with HRTs ranging from 60 to 36 hrs with an interval of 12 hrs and a mean HRT of 36 hrs with an average HRT of 49.8 hours. An average OLR of 3 g-COD/L.day was maintained during this period. In total, the EGSB was operated for 173 days (Table 2).

Experimental design

The process for optimizing COD removal was designed using the RSM plus CCD as noted. The COD removal as the dependent function was optimized using two independent variables, HRT and OLR.

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Table 2 | EGSB operating conditions

Feed Dilution (%)	Days	HRT(h)	OLR (g-COD/L/day)
50	43	62.5 ± 1.77	1.05 ± 0.05
30	49	57.5 ± 1.44	1.93 ± 0.03
Undiluted	81	49.8 ± 6.00	3.11 ± 1.70

RSM was used both to optimize the parameters and determine the interaction between them. Each factor was coded at two levels – higher (+1) and lower (−1) (Table 3).

Table 3 | Factors used in the experimental design

Factors	Units	Code	Low (−1)	High (+1)
OLR	g-COD/L.day	A	1.01	4.82
HRT	Day	B	1.50	2.71

The response considered was COD removal efficiency (%). The software was used to analyse and calculate the two factorial (2FI) response coefficients. A model suitable to represent the EGSB's COD removal efficiency is shown in Eq. (1):

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where y is the response; x_i and x_j the factors; β_o a constant coefficient; β_j , β_{jj} and βi the interaction coefficients of the linear, quadratic and second order terms, respectively; k the number of factors studied and ε the error.

RESULTS AND DISCUSSION

EGSB performance predicted using RSM and CCD

The experimental COD removal results are shown in Table 4. The total numbers of experiments was 15 ($2^k + 2\,k + C_o$), where k is the number of factors ($n = 2$) and C_o comprises the centre points (seven replications) used to assess the pure error.

The interaction between HRT, OLR and COD removal as the response was analysed, and the fitness of the model, were reduced to a two factorial (2FI) regression to determine COD removal efficiency for modelling purposes. The model, based on the sum of squares, was statistically significant, and was built to fit the results. Equation (2) describes COD removal:

$$\textbf{COD removal (\%)} = \mathbf{79.21 + 15.20^{*}A + 0.72^{*}B + 17.48^{*}AB} \quad (2)$$

where A is OLR (g-COD/L/d) and B is HRT (days).

The adequacy of the proposed model was determined according to the determination coefficient (R^2), F-value and p-value (see Table 5). An R^2 of at least 0.80 is indicative of the good fit of a model 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

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Table 4 | CCD results for COD removal

	Factors		COD Removal (%)	
Run	**A (g-COD/L/d)**	**B (day)**	**Actual**	**Predicted**
1	2.71	1.17	52	51
2	2.71	1.01	48	49
3	2.5	1.44	59	60
4	2.5	1.53	58	61
5	2.29	1.85	69	68
6	2.5	1.64	63	63
7	2.5	1.78	67	65
8	2.5	2.14	72	69
9	2.5	1.93	69	67
10	2.5	2.70	78	77
11	2.5	3.50	85	87
12	2.5	3.89	93	93
13	2	4.82	91	92
14	1.5	4.29	76	78
15	1.5	4.79	80	78

Table 5 | Analysis of variance (ANOVA) of the quadratic model for COD removal

Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F value**	***P* value prob > F**	
Model	2,489.98	3	829.99	185.43	<0.0001	significant
A	294.06	1	294.06	65.70	<0.0001	
B	0.53	1	0.53	0.12	0.7372	
AB	234.87	1	234.87	52.47	<0.0001	
Residual	49.24	11	4.48			
R^2	0.980	R^2 Adj	0.975	R^2 Pred	0.964	

F-value for all terms. The model R^2, F- and P- values of 0.980, 185.43 and <0.0001, respectively, indicated that it was suitable to predict COD removal efficiency. The adjusted (R^2 Adjusted) and predicted (R^2 Predicated) determination coefficient values obtained were 0.975 and 0.964, respectively. The R^2 values are similar to those reported in literature for the optimization process using RSM for wastewater treatment (Bustillo-Lecompte & Mehrvar 2015), and those reported by Osman *et al.* (2014) when optimising COD removal from paper mill effluent using RSM plus CCD. Similarly, Sathian et al. (2014) also optimised parameters such as air flow rate and SRT in modelling COD removal in textile dye wastewater treatment. The two studies obtained R^2 values between 0.996 and 0.994, and 0.87 and 0.94, respectively. Statistically, an R^2 value exceeding 0.90 shows that the modelled results described the experimental results. Generally, the closer an R^2 value closer is to unity the better.

The relationship between HRT and OLR for optimized COD removal was plotted on a 3D graph (Figure 2). This provided the best representation of the influences of HRT and OLR on COD removal, and, as Sathian et al. (2014) noted, it is very useful in determining a system's behaviour within the known environmental parameter variations. The plot shows that the optimum OLR and HRT conditions for maximum COD removal are 2 g-COD/L.day and 4.82 days HRT, respectively, for 93% COD removal efficiency.

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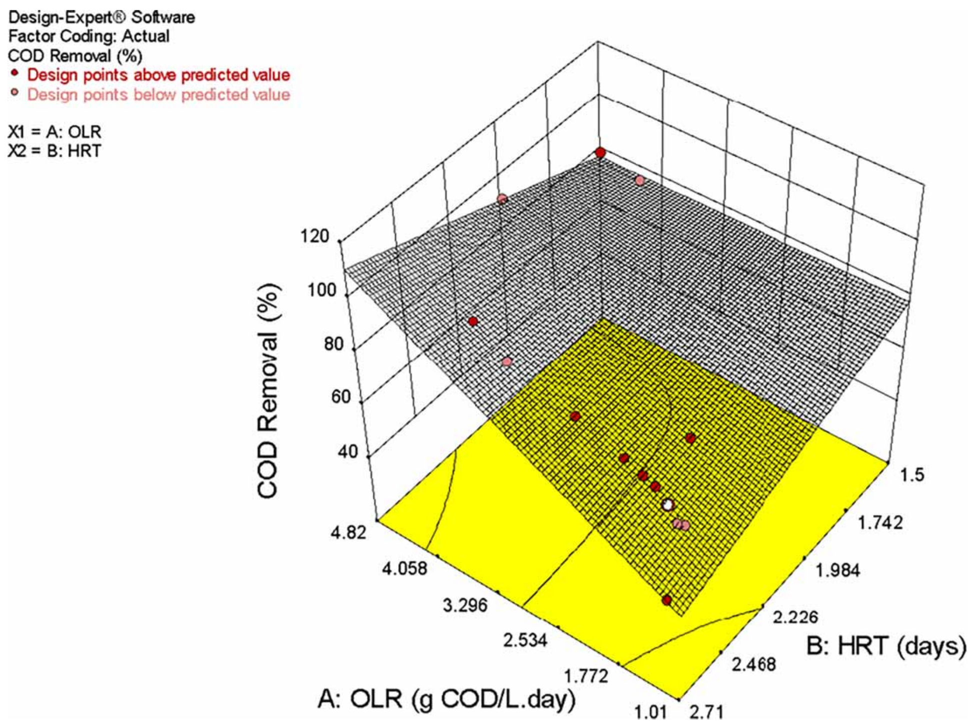

Figure 2 | 3D plot of OLR (A) and HRT (B), and their effect on EGSB's COD removal efficiency.

CONCLUSION

Optimization of the ESGB's COD removal efficiency as a function of the independent variables HRT and OLR was conducted using RSM. The ANOVA results yielded a *P*-value below 0.001 for the model developed, with R^2 being 0.98, when the experimental and modelled results were compared. This indicated that the (2FI) model's COD removal predictions were statistically significant. The optimum OLR and HRT conditions for the EGSB in this study were determined as 2 g-COD/L.day and 4.82 days respectively, with maximum COD removal of 93%. The study shows that the EGSB reactor could remove high concentrations of COD from PSW when operated under optimum conditions.

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