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# Organic removal rates and biogas production of an upflow anaerobic sludge blanket reactor treating sugarcane molasses

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ABSTRACT

Response surface methodology was used to develop an empirical model for the optimisation of feed carbon to nitrogen ratio and temperature of an upflow anaerobic sludge blanket reactor treating sugarcane molasses. The developed quadratic models were found to be statistically significant (Prob > F values: 0.0747 and 0.0495) and there was a good correlation between predicted and actual values ($R^2$ values: 0.80 and 0.65). The results indicated that carbon to nitrogen ratio and temperature interactions had a significant effect on biogas production and organic removal rate. Within the range of parameters tested, the optimal biogas production (0.832 L/L.d) and organic removal rates (77.7%) were achieved at a temperature of 38 °C and a total organic carbon to nitrogen ratio of 22:1 (wt/wt). The reactor exhibited the potential to generate energy through biogas formation with a rich methane content of 66.5 vol%.

## 1. Introduction

The sugar industry contributes to the development of the economy in many countries, but arguably has a negative effect on the environment. The conversion of natural habitats for cane cultivation in coastal areas and tropical islands has led to critical environmental damage, loss of biodiversity and ecosystem services at landscape levels (Rein et al., 2011). In addition, the milling and refining operations of sugar mills produce effluents that may contain massive quantities of organic matter and sludge, which can degrade fresh water resources and deplete dissolved oxygen resulting in mortality of aquatic species if discharged to the environment without adequate treatment (Sahu et al., 2015). Sugar industry wastewater (SIWW) is generated from cleaning process pipes and equipment, washing of floors, and leakages (Rein et al., 2011). In some countries with poor environmental regulations and laws, the annual cleaning of sugar mills results in contamination of surface waters.

Combined SIWW contains high concentrations of organic matter such as proteins and sucrose, and oil and grease. The chemical oxygen demand (COD) concentration of SIWW from various countries including India, Uganda and Pakistan typically ranges between 3000 and 6000 mg/L, but values as low as 35 mg/L and as high as 7432 mg/L have been reported (Muhammad and Ghulam, 2015).

Physicochemical treatment methods such as coagulation with lime are costly and have intensive energy requirements for mixing. Anaerobic treatment of sugar industry wastewater by anaerobic digestion technology guarantees energy production whilst reducing the organic content of the wastewater. In this study (i) temperature and feed carbon to nitrogen (C/N) ratio of an upflow anaerobic sludge blanket (UASB) reactor treating sugarcane molasses was optimised in order to improve COD removal and biogas production, and (ii) an empirical model was developed to predict the performance of the UASB at a range of C/N ratios and temperatures.

## 2. Materials and methods

### *2.1. Set-up and operation of upflow anaerobic sludge blanket bioreactor*

A single-stage biotreatment process consisted of a 46 L glass UASB reactor with a 22.3 L column (internal ø = 154 mm, $h$ = 1200 mm) and a 23.8 L bulb (internal ø = 357 mm) (Fig. 1). A head space was left at the top of the bulb to allow for separation of liquids, solids and biogas. The influent reservoir consisted of six 5 L Schott Duran glass bottles, and the waste effluent was collected into a 40 L polypropylene container. The influent was fed into the bottom of the reactor via silicone tubing using a Watson-Marlow peristaltic pump (Spirax-Sargo Engineering plc, Cheltenham, England) controlled by a Yaskawa Varispeed controller (Illinois, USA) (work rate of 1.2 KW, 2.4 mm clamp size). A portion of the treated effluent was recycled via the influent stream. The system flow rate was 0.95 L/hr, while the recycle flow rate was 2.52 L/hr, giving a recycle ratio of 2.65. A Y-piece with clamps was

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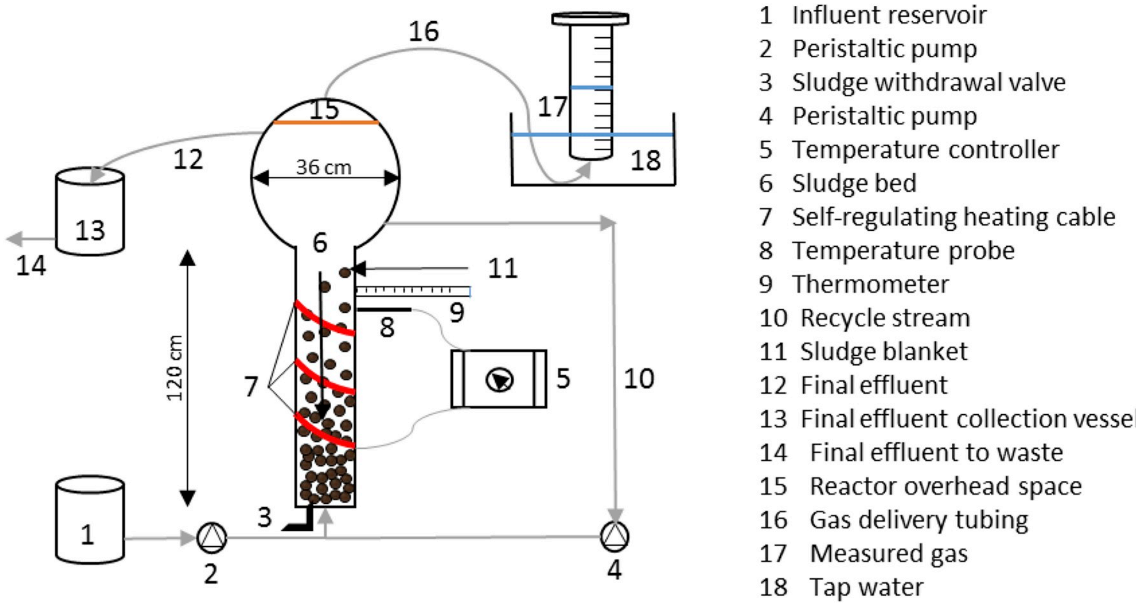


**Fig. 1.** Schematic diagram of the experimental set-up of the upflow anaerobic sludge blanket reactor.

fitted at the base of the reactor to facilitate sludge withdrawal. The temperature of the UASB was controlled by a self-regulating heating cable (ELSR-H-BOT cable temperature reaches up to 210 °C) connected to an electronic temperature controller (ELTC-14) and a temperature probe (Pt100) supplied by Eltherm South Africa (Pty) Ltd. The effluent tube was formed to a U-shape to prevent escape of biogas through the effluent. A biogas delivery tube emerging from the overhead port of the UASB reactor was connected into the inverted graduated cylinder of the water displacement system used to measure biogas production. A mild steel framework fitted with wooden holders supported the reactor. The frame and the UASB were covered with black plastic bags to prevent photosynthetic algal growth.

### 2.1.1. Inoculation of reactor

Granular sludge from a full-scale UASB reactor treating brewery wastewater [South African Brewery (SAB), Newlands brewery, South Africa] and mixed liquor volatile suspended solids (MLVSS) from a full-scale activated sludge wastewater treatment plant treating domestic wastewater (Bellville wastewater works, Bellville, South Africa) was used to inoculate the UASB. The MLVSS and granular sludge were mixed at a volume ratio of 1:3 and the UASB reactor was filled with the mixed inoculum up to a volume of 20 L and made up to 46 L with synthetic SIWW with a COD concentration of 1880 mg/L. Prior to start-up, the UASB reactor was set in a recycle mode for 36 h at a re-circulation rate of 2.52 L/hr. The average characteristics of the synthetic SIWW during the experimental period are given in Table 1.

### 2.1.2. Operational parameters

The UASB was run for a period of 160 days during start-up and 57 days for the experimental runs, including the validation experiments. During the start-up phase, the hydraulic retention time (HRT) and organic loading rate (OLR) was varied twice until acceptable performance was achieved at a total organic carbon (TOC) concentration of approximately 1760 mg/L and HRT of 48.9 h. During the experimental runs, the TOC and HRT were maintained at 1760 mg/L and 48.9 h, respectively, but the C/N ratio and temperature was varied. Each independent variable was adjusted at three levels (−1, 0 to +1). A total of 11 runs, inclusive of 3 centre points, were carried out (Table 2). The statistical significance of the model terms and their interaction terms was examined using the Prob > F value and the quality of fit of the models was accepted at a Prob > F value less than 0.05.

**Table 1**
Characteristics of synthetic sugar industry wastewater ($n$ = 3).

| Parameter | Range | Average |
|---|---|---|
| pH | 10.09–10.48 | ND |
| COD (mg/L) | 4080–4150 | 4101 |
| BOD (mg/L) | 1038–1066 | 1055 |
| BOD/COD ratio | 0.25–0.26 | 0.26 |
| TOC (mg/L) | 1730–1790 | 1760 |
| TN (mg/L) | 35–77 | 55.9 |
| TP (mg/L) | 16.0–16.5 | 16.4 |
| SO42- (mg/L) | 140–132 | 138 |
| Alkalinity (mg/L) | 3903–4233 | 4123 |

ND = Not determined; COD = Chemical oxygen demand; BOD = Biological oxygen demand; TOC = Total organic carbon; TN = Total nitrogen; TP = Total phosphorus; $SO_4^{2-}$ = Sulfate.

In order to determine the experimental error and validate the reproducibility of the polynomial model fitted using linear regression analysis, the centre point (31.5 °C, 36 mgTOC/mgN) corresponding to the mid-level (zero level) was repeated three times (Beszedes et al., 2011).

The coefficients of the second-order polynomial model (Equation (1)) were determined accordingly by calculating the process' responses (COD removal efficiency and biogas production rate):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{i,1}^{k} \sum_{i=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Where $\beta_o$ is the constant term, $k$ is the number of independent variables, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are the linear, quadratic and interaction effect coefficients of the terms. $x_i$ and $x_j$ are the coded independent variables. $\varepsilon$ is the residual of the experiment or random error. The significance and reliability of the independent variables and their interactions were evaluated by analysis of variance (ANOVA) and regression analysis processed by Design-Expert® Software Version 10 (Stat-Ease, Inc., Minneapolis, USA). The quality of fit of the second-order polynomial model was determined using the Prob > F test, determination coefficient ($R^2$) and lack of fit (LOF) test.

**Table 2**
Design matrix of central composite design, responses and analysis of variance for reduced quadratic models.

| Run order | Run No | Input factors | | Responses | | ANOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (A) Temperature (°C) | (B) C/N ratio (mgTOC/mgN) | COD removal (%) | BP (L/L.d) | Model & Terms | Sum of squares | DF | Mean square | F-value | Prob > F |
| | | 25 | 36 | 77.7 | 0.74 | | | 5 | 1.76 | | |
| 3 | 2 | 25 | 50 | 77.7 | 0.64 | A | 0.60 | 1 | 0.60 | 1.40 | 0.2906 |
| 9 | 3 | 31.5 | 36 | 77.2 | 0.67 | B | 1.93 | 1 | 1.93 | 4.47 | 0.0882 |
| 7 | 4 | 31.5 | 22 | 79.1 | 0.78 | AB | 3.24 | 1 | 3.24 | 7.51 | 0.0407 |
| 11 | 5 | 31.5 | 36 | 76.8 | 0.64 | $A^2$ | 0.97 | 1 | 0.97 | 2.25 | 0.1942 |
| 1 | 6 | 25 | 22 | 76.9 | 0.66 | $B^2$ | 2.70 | 1 | 2.70 | 6.25 | 0.0545 |
| 6 | 7 | 38 | 36 | 75.8 | 0.8 | Residual | 2.16 | 5 | 0.43 | | |
| 8 | 8 | 31.5 | 50 | 77.7 | 0.86 | Lack of Fit | 1.91 | 3 | 0.64 | 5.16 | 0.1666 |
| 10 | 9 | 31.5 | 36 | 76.5 | 0.59 | Pure Error | 0.25 | 2 | 0.12 | | |
| 2 | 10 | 38 | 22 | 78.7 | 1.06 | $R^2 = 0.80$ | | | | | |
| 4 | 11 | 38 | 50 | 75.9 | 0.87 | [$R^2_{Adj} = 0.61$; $R^2_{Pred} = -0.66$] | | | | | |
| | | | | | | Biogas production (L/L.d) (model) | 0.12 | 3 | 0.041 | 4.37 | 0.0495 |
| | | | | | | A | 0.079 | 1 | 0.079 | 8.39 | 0.0231 |
| | | | | | | B | 2.817E-003 | 1 | 2.817E-003 | 0.30 | 0.6022 |
| | | | | | | $B^2$ | 0.042 | 1 | 0.042 | 4.41 | 0.0739 |
| | | | | | | Residual | 0.066 | 7 | 9.457E-003 | | |
| | | | | | | Lack of Fit | 0.063 | 5 | 0.013 | 7.71 | 0.1188 |
| | | | | | | Pure Error | 3.267E-003 | 2 | 1.633E-003 | | |
| | | | | | | $R^2 = 0.65$ | | | | | |
| | | | | | | [$R^2_{Adj} = 0.50$; $R^2_{Pred} = 0.06$] | | | | | |

### 2.2. Analytical methods

#### 2.2.1. Influent and effluent analyses

Triplicate influent and effluent samples (~30 ml) were collected in sterile containers over a cycle period of 3–10 days during start-up and at the end of each run during the experimental phase. Samples were analysed immediately after collection.

The chemical parameters of the influent and effluent samples were determined using a Merck (Darmstadt, Germany) Spectroquant® Pharo 100 instrument and Merck cell tests for COD, volatile organic acids (VOA), (TOC), total nitrogen (TN) and alkalinity/acid capacity (cat nos: 14541, 14555, 01763, 14537, and 01758, respectively).

The pH was determined using a pH 700 probe and instrument (Eutech Instruments, Stanger, South Africa) according to the manufacturer's instructions.

#### 2.2.2. Measurement of biogas and methane content

A water displacement system was used to quantify the rate of biogas generation at steady state by taking triplicate measurements of the volume of water displaced in an inverted cylinder over the period of 1 h. The % of methane, carbon dioxide and molecular oxygen in the biogas was also determined after each change in operational parameters during the experimental phase using a Geotech Biogas 5000 instrument (Goodspeed environmental services, Everton, South Africa) according to the manufacturers' instructions.

## 3. Results and discussion

### 3.1. Start-up of the UASB reactor

The start-up of the UASB reactor took place in four phases (Fig. 2). In each phase, adjustments were made to attempt to improve the efficiency of the system. Due to spontaneous pre-fermentation of the molasses in the holding tank caused by bacterial contamination, flies, and by atmospheric deposition, the inlet COD concentration and the OLR during phase 1 were unstable and fluctuated between 3815 mg/L and 4202 mg/L, and 4.88 kgCOD/m$^3$.d and 2.45 kgCOD/m$^3$.d, respectively. The spontaneous pre-fermentation process of the feed yielded organic acids which depleted the alkalinity of the feed and caused uncontrollable pH variations in the reactor. As a result, VOAs in the effluent accumulated to 323 mgAAE/L and 433 mgAAE/L, respectively. The COD removal efficiency showed an increasing trend from 20.1% to 81.2%, with some fluctuations between 71.4% and 61.5% (Fig. 2).

On the 65th day after start-up (Phase 2), the OLR was reduced from 2.45 to 1.86 kgCOD/m$^3$.d, and the buffering capacity of the system was increased by adding sodium bicarbonate ($NaHCO_3$) and sodium hydroxide (NaOH) to the feed such that the COD/alkalinity ratio of the feed was 0.9–1. This resulted in a drop in the effluent VOA concentration to 84–98 mgAAE/L (range) and the COD removal efficiency increased to reach a maximum of 84% on day 107. On the 124$^{th}$ to the 138$^{th}$ day after start-up (Phase 3), the HRT of the reactor was gradually reduced from 48.90 h to 14.05 h, and the OLR was increased from 1.86 to 6.49 kgCOD/m$^3$.d. During this period, the effluent COD concentration increased from 821 mg/L to 2935 mg/L, and the COD removal efficiency dropped from 78.3 to 22.8%. At an HRT of 14.05 h and corresponding upflow velocity of 0.176 m/h, the reactor contents became 'sour' and VOAs in the effluent stream accumulated to 499 mgAAE/L (Fig. 2). There was also total upliftment of the sludge bed and excessive biomass washout. It was deduced that the reactor was shocked as a result of high OLR.

On day 139, a second start-up was initiated. To prevent pre-fermentation and acidification, all influent was sterilised by autoclaving in 5 L Schott bottles and the influent was fed to the UASB from the Schott bottles via autoclaved tubing. In order to allow the microorganisms to acclimatise to the substrate, a low concentration of substrate of 1880 mgCOD/L was applied and gradually increased stepwise to 3890 mgCOD/L. The corresponding OLR increased from 0.92 to 1.91 kgCOD/m$^3$.d (Phase 4) (Fig. 2). The alkalinity of the feed was increased to approximately 4100 mg$CaCO_3$/L by adding sodium carbonate and sodium hydroxide (COD/alkalinity = 0.9–1) and the problem of pH shock and souring caused by rapid acidification was mitigated. The second start-up was rapid, and the reactor stabilised within 21 days. The concentration of VOAs in the effluent remained between 228 and 250 mgAAE/L and the COD removal efficiency increased from 60.4 to 73.6% and did not change by > 5% for two consecutive weeks. This indicated that the reactor was operating at 'steady state' and the experimental phase was initiated after day 160 (Fig. 2).

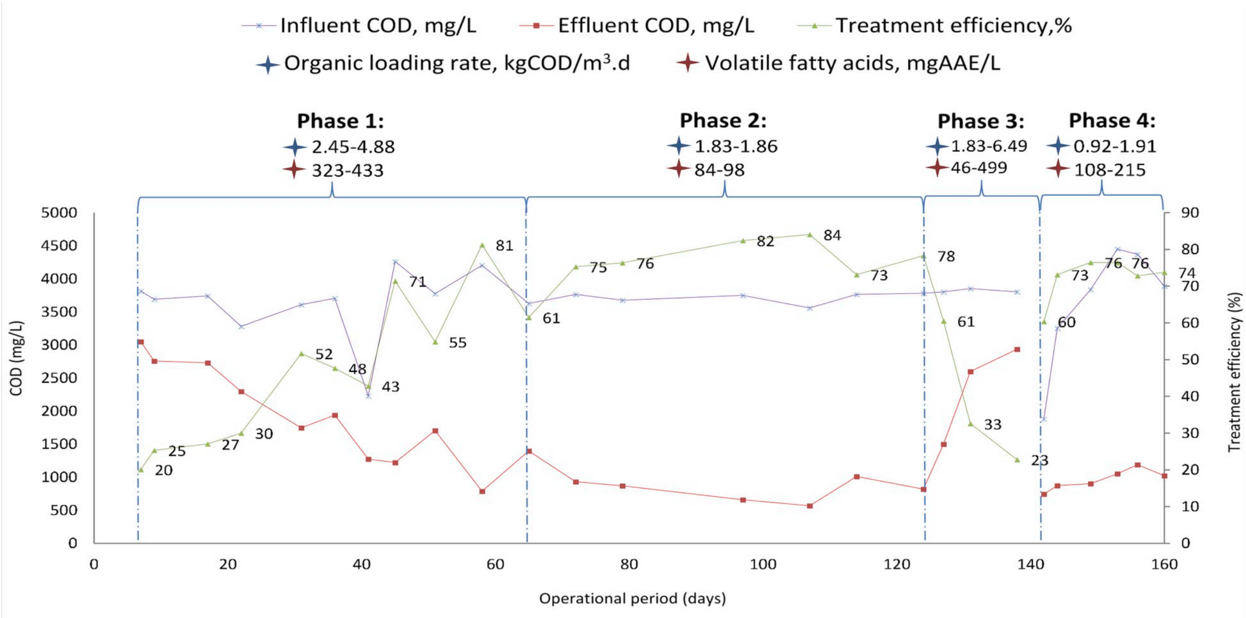


**Fig. 2.** Influent and effluent COD and removal (treatment) efficiency, and the applied organic loading rates and effluent volatile fatty acid concentration during the four phases of the start-up of the UASB. The removal efficiency at each sampling period is shown on the graph.

### *3.2. Statistical analysis of model by central composite design*

Multiple regression analysis using CCD was used to develop empirical models for predicting the responses of COD removal efficiency and biogas production rate (Table 2). In terms of coded factors these were:

$$COD\ removal\ (\%) = 77.05 - 0.32A - 0.90AB - 0.62A^2 + 1.03B^2 \quad \text{Model 1}$$

$$Biogas\ production\ (L/L.\ d) = 0.69 + 0.12A - 0.022B + 0.12B^2 \quad \text{Model 2}$$

Where, A is temperature in °C and B is C/N ratio in mg TOC/mg N. The analysis of variance (ANOVA) for the reduced quadratic models of COD removal and biogas production is summarised in Table 2. The $R^2$ value for COD removal model was 0.8 and 0.65 for biogas production model indicating that 80% of the variations in COD removal efficiency and 65% of the variations in biogas production rate were explained by the models. The difference between the predicted and adjusted $R^2$ values ($R^2_{\text{pred}}$ and $R^2_{\text{Adj}}$) for both models was greater > 0.2 indicating a block effect or potential problems with the models. In addition, the negative $R^2_{\text{pred}}$ (Equation (1), Table 2) suggested that the overall mean may be a better predictor of the response than the model itself.

The result obtained using the model for COD removal was not significant (Prob > F value = 0.0747), while that obtained using biogas production model was (Prob > F value = 0.0495). The LOF result for model 1 and model 2 was insignificant, with Prob > F values of 0.166 and 0.118 indicating that there was only 16.6% and 11.8% chance that LOF could occur due to noise. Ideally a model should fit the experimental data and should the LOF value should be insignificant (i.e. Prob > F greater than 0.1) (Mourabet et al., 2017). The linear and quadratic terms of temperature (A and $A^2$) in model 1 and C/N ratio (B and $B^2$) in model 2 were statistically insignificant with Prob > F values of 0.2906, 0.1942, 0.6022 and 0.0739, respectively. The linear interaction term of temperature and C/N ratio (AB) in model 1 was significant with a Prob > F value of 0.0407, indicating that they had a significant effect on COD removal efficiency.

### *3.3. Performance of the UASB during the operational period*

The effluent COD concentration, COD removal efficiency, effluent VOA concentration, alkalinity, pH and biogas production were used to assess the performance and stability of the UASB (Fig. 3).

The lowest effluent COD concentration obtained was 855 mg/L at a temperature of 25 °C and C/N ratio of 50 mgTOC/mgTN (Fig. 3A). The corresponding COD removal efficiency was 77.6%. The residual COD could be attributed to the low biodegradability potential of the SIWW used in this study (BOD/COD = 0.26) (Table 1).

The VOA concentration, alkalinity, and VOA/alkalinity ratio of the treated effluent ranged from 204 to 236 mgAAE/L, 4387–5223 $mgCaCO_3/L$, and 0.043–0.046, respectively (Fig. 4B). These parameters are excellent indicators of process stability in anaerobic reactors because under unstable conditions, accumulation of VOAs may deplete total alkalinity and cause souring of reactor contents (Yasar and Tbinda, 2010). In this context "souring" refers to a situation when acidogenesis occurs at a higher rate than utilisation of VOAs by methanogens. This typically leads to accumulation of volatile organic acids (VOA)/volatile fatty acids (VFA). A VOA/alkalinity > 0.8 indicates suppression of methanogens and potential process failure, while a ratio < 0.4 is indicative of process stability (Collaghan et al., 2002; Hampannavar and Shivayogimath, 2010). Furthermore, the pH of the effluent remained between 7.2 and 7.7 (Fig. 4C) which was within the optimal range (6–8) for methanogenesis and indicates good microbial activity.

A maximum biogas production rate of 1.1 L/L.d was obtained at a C/N ratio and temperature of 22.8 mg TOC/mg N and 38 °C. The methane content of the biogas ranged between 65.8% and 70.7% (Fig. 4D) which is within the standard range of methane composition (55–75%) in biogas and therefore renders it fit to be used for electricity generation and industrial heating applications (Yasar and Tbinda, 2010).

In terms of biogas production, HRT, methane content and organic removal in the treatment of authentic SIWW, the current system exhibited a distinct performance when compared to other pilot and small scale UASBs treating authentic and synthetic SIWW (Table 3). This may have been related to different start-up inocula, influent substrates, and reactor scales. In the case of scale, mixing of substrate and granules becomes less effective as the scale increases. No results about biogas production rates in pilot or full-scale UASBs treating SIWW could be found in literature, but small scale reactors (Hampannavar and Shivayogimath, 2010; Tanksali, 2013) produced biogas at a maximum of 1.6 L/L.d and 4.66 L/L.d which is greater than 1.1 L/L.d reported in this study. In addition, biogas with higher methane content has been

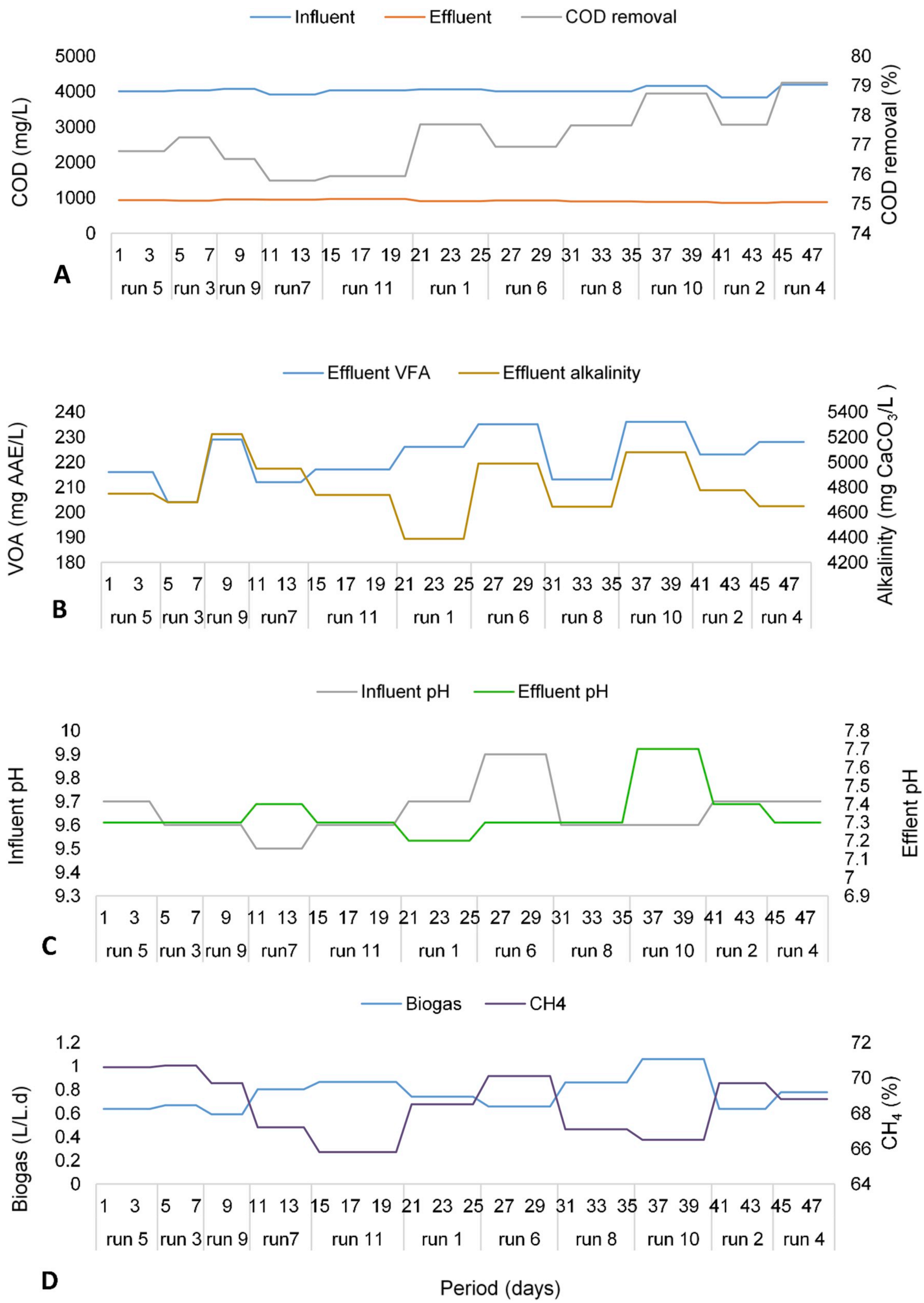


**Fig. 3.** Influent and effluent parameters during the UASB operational period: chemical oxygen demand concentration and removal efficiency (A), volatile organic acids concentration and alkalinity (B), pH (C), and biogas production (D).

obtained in mesophilic UASBs with lower HRTs (Gonzalez et al., 1998; Hampannavar and Shivayogimath, 2010). However, the systems were smaller and the COD removal rates were less stable. The organic removal rate compared favourably with other studies focussing on the treatment of molasses, with the exception of one pilot-scale study (Atashi et al., 2010) where 90% removal was achieved with a considerably shorter HRT, but unknown biogas production rates. UASBs have been operated at low HRT without excessive biomass washout

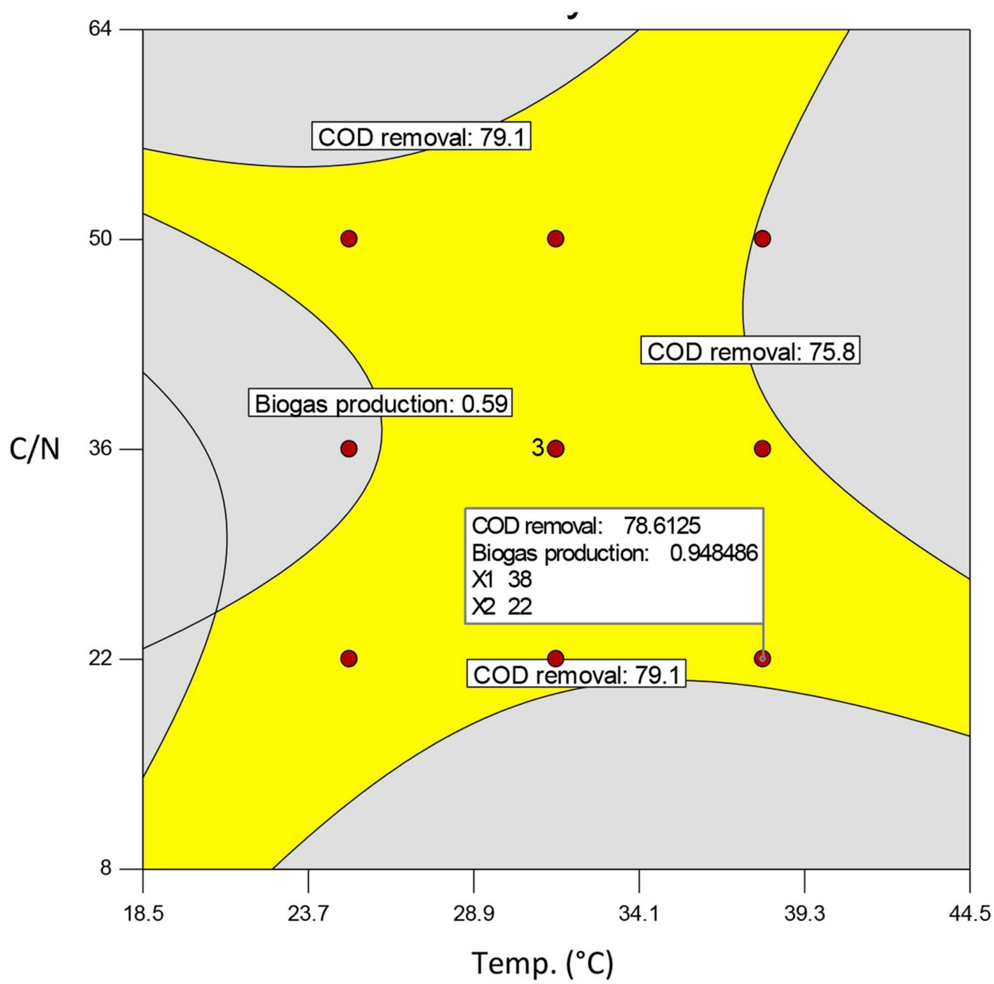


**Fig. 4.** Overlay plot showing optimal operating conditions of the UASB (C/N expressed in mgTOC/mgN).

(Atashi et al., 2010; Hampannavar and Shivayogimath, 2010) In this study, lower HRTs led to biomass washout and operational failure. It is possible that this was related to the settling characteristics of the granules and it is therefore recommended that settling tests are conducted in UASB studies.

### 3.4. Interaction of temperature and C/N ratio on UASB performance

Contour and three-dimensional (3D) response surface plots were constructed from the models and used to depict and interpret the COD removal efficiency and biogas production rate (S2) of the UASB reactor as a function of temperature and C/N ratio (Supplementary material 1).

#### 3.4.1. Interaction of temperature and C/N ratio on COD removal efficiency

Within the experimental range of 22–36 mgTOC/mgN and 25–31.5 °C, as the C/N ratio decreased and the temperature increased, the COD removal efficiency increased from 76.0% and reached a maximum of 79.1%. At a constant temperature of about 38 °C, there was a reduction in COD removal efficiency from 78.7% to 74.0% with increasing C/N ratio from 22 mgTOC/mgN to 50 mgTOC/mgN. Overall, the responses were expected because higher temperatures (within mesophilic range) in bioreactors enhances hydrolysis and increases diffusion and mass transfer of soluble organics from the substrate to the biomass (Appels et al., 2008). Similarly, at high C/N ratios, the microbial growth rate slows due to nutrient (nitrogen) limitation (Karthikean and Visvanathan, 2012). It has previously been reported that co-digestion of dairy manure, chicken manure and rice straw can be achieved with C/N ratios as low as 15, but that of 20–35 is optimal for co-digestion of multiple substrates in batch mode digesters, which are in agreement with the results of this study (Dioha et al., 2013; Wang

**Table 3**
Comparison of performance parameters of UASB reactors.

| Scale | Temp. (range) | Capacity (L) | HRT (hr) | Biogas (L/L.d) | $CH_4$ (%) | COD removal (%) | Reference |
|---|---|---|---|---|---|---|---|
| Pilot | Mesophilic | 16.5 | 39–13 | NM | 79–94 | 59–91 | Gonzalez et al., 1998* |
| Small | Mesophilic | 8.4 | 48–12 | 1.6 | 71 | 80–96 | Tanksali, 2013** |
| Pilot | Thermophilic | 140 | 8–100 | NM | NM | 39–67 | Harada et al., 1996* |
| Pilot | Mesophilic | 500 | 5–6 | NM | NM | 90 | Atashi et al., 2010* |
| Small | Mesophilic | 7.95 | 6 | 4.7 | 73–82 | 89 | Hampannavar and Shivayogimath, 2010*** |
| Small | NM | 10 | 10 | NM | NM | 60–81 | Ragen et al., 2001* |
| Pilot | Mesophilic | 46 | 49 | 1.1 | 66–71 | 75–79 | **This study*** |

HRT = Hydraulic retention time; Mesophilic = 25–40 °C; Thermophilic = 40–70 °C; NM = Not mentioned *Molasses based substrate ** Sucrose based substrate *** Actual sugar industry wastewater.

**Table 4**
Predicted and actual responses.

| Solution | Temp. (°C) | C/N (mgTOC/mgTN) | COD removal (%) (model) | COD removal (%) (experiment) | Biogas (L/L.d) (model) | Biogas (L/L.d) (experiment) | Desirability |
|---|---|---|---|---|---|---|---|
| 1 | 30.9 | 50 | 77.6 | 75.4 | 0.779 | 0.691 | 0.471 |
| 2 | 38.0 | 22 | 78.6 | 77.7 | 0.948 | 0.832 | 0.806 |

et al., 2014).

*3.4.2. Interaction of temperature and C/N ratio on biogas production rate*

Within the ranges tested, the maximum biogas production rate of 0.94 L/L.d occurred at 38 °C at a C/N ratio of 36 mgTOC/mgN. As with COD removal, decreasing temperature exhibited a negative effect on biogas production. Temperatures in the margin of the mesophilic range (38–40 °C) increases hydraulic turbulence in UASBs by reducing the viscosity of the reactor contents and improves bioconversion of organic matter to biogas due to effective contact between the substrate and biomass. On the other hand, psychrophilic temperatures < 15 °C reduce the diffusion and mass transfer rates of soluble organic compounds from the bulk liquid to the biomass, causing ineffective contact between substrate and biomass which ultimately reduces the rate of substrate uptake (Lettinga et al., 2001). Temperatures and C/N ratios of 25–38 °C, and 20–35, respectively, in agreement with the results of this study, were optimal for batch anaerobic digesters treating municipal solid waste and food waste (Tanimu et al., 2014; Pavi et al., 2017).

### *3.5. Optimisation of UASB operating conditions and model validation*

Graphical representation was used to visualise and fine-tune the applied C/N ratio and temperature ranges for optimal operation of the UASB in terms of COD removal and biogas production (Fig. 4). Two optimal combinations of 38 °C and 22 mg TOC/mg N, and 30 °C and 50 mg TOC/mg N, were suggested by the model (Table 4), with the latter giving the highest total desirability (0.806). The corresponding COD removal efficiency and biogas production rate under the optimal temperature and C/N ratio was 78.6% and 0.948 L/L.d, respectively. The quadratic models were validated by running the predicted optimal conditions shown in Table 4. The experimental values did not differ significantly from the predicted values (Table 4), therefore the quadratic models for COD removal efficiency and biogas production rate were deemed adequate.

## 4. Conclusions

The results showed that the developed empirical models for predicting COD removal efficiency and biogas production have a good correlation between predicted and actual responses with $R^2$ values of 0.80 and 0.65. The UASB managed to significantly reduce the organic load of diluted sugarcane molasses over a range of temperatures and feed carbon to nitrogen ratios while recovering energy through biogas formation. This system therefore exhibits the potential to reduce the environmental impact of SIWW.

## Declarations of interest

Non.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sajce.2019.01.002.

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