Performance Enhancement of *Kality* Wastewater Treatment Plant's Up Flow Anaerobic Sludge Blanket Reactor Using Surface Response Methods

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ABSTRACT

The main aim of this study was to examine and optimize the performance of up flow anaerobic sludge blanket reactor (UASB) using the surface response method-central composite design (RSM-CCD). The influences of several operational parameters were considered, including temperature (0 to 30°C), organic loading rate (OLR) (1 to 3 kg $COD/m^{3} \cdot d$), pH (6.3 to 7.8), and hydraulic retention time (HRT) (4 to 12h). The pilot-scale reactor had a volume of 48.8 L. The RSM-CCD was used for the determination of the number of runs and the optimization of operational parameters. According to the derived model, the reactor exhibited optimal results under the following conditions: Temperature (23.0°C), OLR (2.3 kg $COD/m^3 \cdot d$), pH (7.5), and HRT (11.4 h). Removals of 84.1 %, 99.9 %, and 100 % for chemical oxygen demand (COD), total suspended solids (TSS). and volatile suspended solids (VSS), respectively, were achieved using the optimized parameters. In comparison with the inlet concentrations, the outlet concentrations of volatile fatty acids (VFAs) and alkalinity decreased. Whereas, the outlet concentrations of sulfate ions increased, since the sulfur-reducing bacteria effect was hindered due to the anaerobic condition of VFA and alkalinity. Even though the removal efficiency of the locally utilized wastewater treatment plant employing a UASB reactor was set at 55 % and 70 % for COD and TSS, respectively, the experimental results showed that it was possible to achieve higher removal efficiency at psychrophilic temperatures for unregulated sewage by optimizing controllable operational parameters.

Keywords: Operational parameters Optimization; Performance enhancement; Surface response method; UASB reactor.

1. INTRODUCTION

UASB reactors are considered a common anaerobic treatment method for wastewater. In developing countries, the UASB reactor is viewed as an effective strategy for treating domestic and industrial wastewater [1, 2].

The primary goal of their use is to achieve high efficiency in removing organic and inorganic contaminants from wastewater [3]. For evaluating UASB performance, some common indicators include the removal efficiencies of COD, Biological Oxygen Demand (BOD), and TSS [4-6]. The locally existing wastewater treatment plant employing UASB reactor was designed for the removal efficiency of 55 % for COD, BOD₅ and 70 % for TSS at 20°C but the OLR and HRT were not fixed. Since the set removal efficiencies of the UASB reactor for COD, BOD, and TSS were below global practices, enhancing the removal

efficiencies of the UASB reactor presents a challenge. Several studies have examined the optimization of UASB reactors. Some studies identified temperature as the sole factor affecting the efficiency of the UASB reactor [7-9], while others considered only HRT as the parameter affecting UASB performance [10-12]. Other studies investigated the effects of two operational parameters, such as temperature and OLR, on the efficiency of UASB reactors [13-15]. However, due to the nature of anaerobic biological systems, the performance of UASB reactors is not a simple function of two or three operational parameters; rather, it is influenced by a combination of controllable and uncontrollable parameters.

The seasonal variations also result in the concentration differences and the HRT needed for removal of the pollutants [16]. The research took seasonal differences into account to improve the performance of the UASB reactor. Data were collected for rainy seasons for 30 different operational parameters and surface response method central composite design was used for optimization of operational parameters and data analysis.

The main aim of this research was optimization of operational parameters such as temperature, HRT, OLR, and pH, and their interactions on the efficiency of the UASB reactor in removing COD, VSS, and TSS. The combinational effects were also observed in the removing of alkalinity, VFA, VFA-to-alkalinity ratio, and sulfate ion concentrations.

1.1. Effects of operational parameters

Microorganisms used in wastewater treatment operate at psychrophilic (0 to 30° C), mesophilic (30 to 45° C), and thermophilic (45 to 70° C) temperatures. Anaerobic treatment is possible in these three temperature ranges, and generally, the removal efficiency of anaerobic reactors

increases as the temperature shifts from psychrophilic to mesophilic conditions [17, 18].

In a UASB reactor, an appropriate OLR should be maintained to enhance the COD removal efficiency, biogas production, and process stability. A decrease in OLR would result in a gradual rise in pH. Conversely, an increase in OLR increases the probability of contact between biomass and substrate, potentially leading to poor degradation of incoming COD [19]. HRT is the average retention time of wastewater inside the UASB reactor. It is one of the major parameters affecting the performance of anaerobic reactors treating municipal Prolonging HRT wastewater [20-22]. beyond a certain limit can produce granular sludge or might cause re-suspension of granules. However, a large HRT could be beneficial in shortening the start-up time of the reactor. On the other hand, biomass washouts can occur with short HRT [23, 24].

The pH in UASB reactors should be maintained between 6.3 and 7.8 to enhance methanogenesis due to the buffering capacity of the acid-base system [25, 26]. When the OLR is low in the UASB reactor, the pH will increase. To reduce the pH, the temperature should be changed from mesophilic to hemophilic conditions, demonstrating that pH affects the removal of COD, TSS, VFA concentration, and biogas production in UASB systems [27].

The present work examined the effects of temperature, HRT, OLR, and pH, and their interactions on the efficiency of the UASB reactor in removing COD, VSS, and TSS. The combined effects were also observed in the removal of alkalinity, VFA, VFA-to-alkalinity ratio, and sulfate ion concentrations.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

The influent and effluent COD concentrations were determined according to the Standard Methods for the Examination of Water and Wastewater, using medium (0-1500 mg/L) and high range (0-15,000 mg/L) COD kits provided by Hach. NaOH and H₂SO₄ were used to adjust the pH of the raw



wastewater during the experiments. A Hach DR890 Colorimetric instrument and relevant medium kits (0-700 mg/L) were used to measure the influent and effluent concentrations of sulfate ions. Alkalinity was measured using Alkaphot tablets and a Palintest 7100 photometer. The pH was measured using a pH meter purchased from Hach.

- 1. Infulent
- 2. Stirrer
- 3. Perlistatic Pump
- 4. Open and Close Valve
- 5. Water Barrel
- 6. 18cm×18cm×150cm Plexiglass Reactor
- 7. Thermometer
- 8. Heating Element
- 9. Blogas Outlet
- 10. Effulent Outlet
- 11. Effluent Collection Tank
- 12. Radiator
- 13. Supply Fan
- 14. Capillary Tube
- 15. Compressor
- 16. Gas outlet
- 17. 1,1,2,2-TetraFlouroethane gas inlet
- 18. Evaporator
- 19. Sampling Port
- 20. Heater and cooler controller(PID control)
- 21. Power Supply
- 22. Power to heater and coller
- 23. Power to cooling system

Figure 1 Process integrated derivative control board with pilot scale UASB reactor, fan, radiator , compressor and barrel filled of water

2.2. Experimental reactor set up

The configuration of the pilot-scale reactor used is shown in Figure 1. The UASB reactor consisted 100 L water-filled barrel and evaporator installed atthe sides of the barrel. The evaporators were attached with compressors, radiators, and a supply fan mounted outside the setup to lower the temperature to the appropriate value. A 2500 W heater was fitted inside the waterfilled barrel and used to elevate the temperature when needed. The control board panel(PID) included thermostat, temperature sensors and timer. The heating,cooling system, sensors, and power sources were all linked to the Process Integrated Derivatives control board. The control board was the central control unit of the pilot-scale UASB reactor, managing temperature, HRT, heating. cooling and systems. The determination of initial COD concentration of the sample wastewater was used in the HRT determination, which was later became input into the timer. The thermostat was used to set the desired temperature, estimated from the CCD-RSM, and the sensor measured the temperature of the wastewater in the barrel. Inside the barrel, a 0.0486 m³ square reactor was submerged and filled with wastewater. This reactor volume was chosen because it was not possible to obtain a larger capacity compressor for cooling, and heating the wastewater in the barrel. The barrel was wrapped with aluminum foil to maintain a stable wastewater temperature inside the reactor and the water temperature in the barrel. One benefit of the employed system was that it made it possible to integrate the PID board for data collecting through temperature control using HRT and heating and cooling systems. The reactor setupintegrated to the PID board could operate independently, but the influent for the reactor setup was sourced from the WWTP using UASB reactor.

2.3. Inoculation of the pilot-scale UASB reactor

Starting up a UASB reactor typically takes nearly four months [28]. However, in this experimental study, 10 L of sludge from the sludge blanket of an operating UASB reactor was extracted and inoculated into the UASB reactor.

2.4. Sampling Methods

The grab sampling method was used to the determine influent and effluent For all runs, concentrations. influent samples were collected daily in the early morning from the inlet of a WWTP employing a UASB reactor. The effluent concentration for every run was determined from the UASB after the wastewater remained for the specified OLR. temperature, pH, and HRT. For each experimental run, samples were taken twice. The first sample was taken at half of the determined HRT, and the second at the end of the experiment. The influent and effluent concentrations of VFA, sulfate ions, and VFA to alkalinity ratios were measured to control the UASB reactor's functionality.

2.5. HRT Determination

After determining the initial COD, the wastewater should remain in the reactor setup for the predetermined duration before taking the effluent sample. The HRT can be calculated using the following equation [29]:

$$HRT = \frac{So}{OLR} * 24 * 10^{-3}$$
(1)

where:

OLR is in kg $COD/m^3.d$,

 S_o is initial COD concentrations in mg/L, HRT is in hours.

2.6. Operational parameters

The effects of various operating parameters like temperature (0°C to 30°C), HRT (4 hours to 12 hours), OLR (1 kg COD/m³·d to 3 kg COD/m³·d), and pH (6.3 to 7.8) were investigated to increase the performance of UASB for psychrophilic temperatures for the treatment of unregulated sewage during the rainy seasons [30, 31]. The range of the parameters examined (see Table 1) was chosen according to the literature and the instrumentation used [32].

 Table 1
 Five levels of RSM-CCD and coded parameters [41]:

| Parameters | Cod | Levels of CCD-RSM | | | | |
|----------------------------------|-----|-------------------|-----|------|-----|-----------|
| | C | -α | -1 | 0 | +1 | $+\alpha$ |
| Temperature , °C | А, | -15 | 0 | 15 | 30 | 45 |
| OLR, kg COD/m ³ .d | В | 0 | 1 | 2 | 3 | 4 |
| рН | С | 5.55 | 6.3 | 7.05 | 7.8 | 8.55 |
| HRT, hrs | D | 0 | 4 | 8 | 12 | 16 |

2.7. Response Surface Methodology and process efficiency

Surface Methodology Response using Central Composite Design (RSM-CCD) was employed to estimate the effects of four operational parameters (temperature, OLR, HRT, and pH) and their interactions on the removal efficiency and to further optimize the system. RSM-CCD was constructed and analyzed using Stat-Ease, Inc. software, version 13.0.1 (Minneapolis, USA). RSM-CCD was used because it allows the estimation of the main effects and their interactions using a minimal number of experiments compared to the one-factor-ata-time analysis (30 experiments in our case) [33,34] reducing cost of chemicals and time needed for experiments. COD, TSS, pH, VSS, VFA, sulfate ion concentration, and alkalinity were measured as response factors. The following formula was used to determine the efficiency of the pilot-scale UASB reactors: [35].

% Performance efficiency of UASB
Reactor=
$$\left[\frac{C_{influent} - C_{effluent}}{C_{influent}}\right]$$
*100 (2)

where:

 $\begin{array}{ll} C_{influent} & -is \mbox{ the concentration of raw} \\ influent (mg/L) \\ C_{effluent} & -Concentration & of & effluent \\ (mg/L) \end{array}$

2.8. Statistical analysis

According to the RSM-CCD used for the design of the experiments, data analysis, and optimizations [36, 37], there are five levels for each operational parameter. The coding is from $-\alpha$ to $+\alpha$ ($-\alpha$, -1, 0, +1, $+\alpha$). Each parameter was coded as follows:

Temperature as (A), OLR as (B), pH as (C), and HRT as (D) [38]. The following formula was used to determine the total number of pilot-scale experimental runs:[39,40].

$$N = K^2 + 2k + C_0 = 4^2 + 2^* 4 + 6 = 30 \tag{3}$$

where:

N- represents the total runs, K- is the operational parameters considered; C_0 - is center point.

Table 2 HRT values obtained using Eq. (1), inlet COD and %COD removal for 30 experimental runs

| Experimental number | Temperature (°C) | рН | OLR (kg COD/m^3.d) | HRT (hrs.) | Inlet COD (mg/L) | %COD removal |
|---------------------|---------------------|------|-----------------------|------------|---------------------|-----------------|
| 1 | -5 | 7.05 | 2 | 9.60 | 800 | 10.00 |
| | | | | 4.8 | | 5.01 |
| 2 | 0 | 6.30 | 3 | 12.00 | 980 | 15.52 |
| | | | | 6.00 | | 6.23 |
| 3 | 0 | 6.30 | 3 | 3.22 | 403 | 0.00 |
| | | | | 1.61 | | 0.00 |
| 4 | 0 | 6.30 | 1 | 12 | 500 | 72.40 |
| | | | | 6.00 | | 25.52 |
| 5 | 0 | 6.30 | 1 | 16.00 | 1250 | 70.85 |
| | | | | 8.00 | | 44.85 |
| 6 | 0 | 7.80 | 3 | 4.56 | 570 | 34.10 |
| | | | | 2.28 | | 24.23 |
| 7 | 0 | 7.80 | 3 | 6.24 | 780 | 30.24 |
| | | | | 3.12 | | 24.36 |
| 8 | 0 | 7.80 | 1 | 13.20 | 550 | 74.11 |
| | | | | 6.6 | | 52.32 |
| 9 | 0 | 7.80 | 1 | 16.00 | 1570 | 62.20 |
| | | | | 8.00 | | 6.96 |

| Experimental | Temperature | pН | OLR | HRT (hrs.) | Inlet COD | %COD |
|--------------|-------------|------|----------------|------------|-------------|---------|
| number | (°C) | 1 | (kg COD/m^3.d) | × , | (mg/L) | removal |
| 10 | 15 | 7.05 | 4 | 8.00 | 1333 | 78.20 |
| | | | | 4.00 | | 29.65 |
| 11 | 15 | 7.05 | 0 | 0.00 | 0 | 0.00 |
| | | | | 0.00 | | 0.00 |
| 12 | 15 | 8.55 | 2 | 11.52 | 960 | 96.08 |
| | | | | 5.76 | | 45.96 |
| 13 | 15 | 7.05 | 2 | 0.00 | 1000 | 0.00 |
| | | | | 0.00 | | 0.00 |
| 14 | 15 | 7.05 | 2 | 10.20 | 850 | 73.64 |
| | | | | 5.10 | | 45.21 |
| 15 | 15 | 7.05 | 2 | 14.40 | 1200 | 70.52 |
| | | | | 7.20 | | 28.32 |
| 16 | 15 | 7.05 | 2 | 11.52 | 960 | 11.52 |
| | | | | 5.76 | | 9.65 |
| 17 | 15 | 7.05 | 2 | 16.00 | 1333 | 62.65 |
| | | | | 8.00 | | 35.51 |
| 18 | 15 | 7.05 | 2 | 15.12 | 1260 | 70.00 |
| | | | | 7.56 | | 42.21 |
| 19 | 15 | 7.05 | 2 | 8.00 | 667 | 72.25 |
| | | | | 4.00 | | 25.91 |
| 20 | 15 | 7.05 | 2 | 8.00 | 667 | 72.45 |
| | | | | 4.00 | | 32.10 |
| 21 | 15 | 5.55 | 2 | 13.44 | 1120 | 99.25 |
| | | | | 6.72 | | 52.54 |
| 22 | 30 | 6.30 | 3 | 12.00 | 1500 | 93.86 |
| | | | | 6.00 | | 52.96 |
| 23 | 30 | 7.80 | 3 | 6.72 | 840 | 87.14 |
| | | | | 3.36 | | 45.78 |
| 24 | 30 | 7.80 | 3 | 5.84 | 730 | 82.55 |
| | | | | 2.92 | | 58.54 |
| 25 | 30 | 6.30 | 3 | 7.68 | 960 | 81.00 |
| | | | | 3.84 | | 23.52 |
| 26 | 30 | 7.80 | 1 | 15.60 | 650 | 100 |
| | | | | 7.80 | | 55.23 |
| 27 | 30 | 7.80 | 1 | 10.08 | 420 | 95.32 |
| 20 | | | | 5.04 | 210 | 45.96 |
| 28 | 30 | 6.30 | 1 | 7.44 | 310 | 62.01 |
| 20 | | 6.00 | 1 | 3.72 | ~ 00 | 36.65 |
| 29 | 30 | 6.30 | 1 | 12.00 | 500 | 97.41 |
| 20 | 15 | | | 6.00 | 1005 | 54.78 |
| 30 | 45 | 7.05 | 2 | 14.70 | 1225 | 50.00 |
| | | | | 7.35 | | 29.52 |

2.9. Modeling COD, TSS and VSS

In general, RSM-CCD methodology developed the following mathematical model for % COD, %TSS and % VSS removal [42, 43].

$$Y = \beta_0 + \sum_{i=1}^{k} (\beta_i x_i) + \sum_{i=1}^{k} (\beta_{ii} x_i^2) + \sum_{i=1}^{k} \beta_{ij} x_i x_j + \epsilon$$
(4)

Where Y-is the variable for the experimental response, β_0 -is the intercept, β_i , β_{ii} and β_{ij} are the regression coefficients for the linear effect, double interaction, and quadratic effects respectively. xi and xj are the independent variables (experimental variables), and ε represents random error.

The appropriateness of the model equations estimated from CCD-RSM for predicting COD, TSS, and VSS was assessed using analysis of variance (ANOVA) [44]. Specifically, the Coefficient of Variation (CV), Coefficient of Determination (R²), Adjusted Coefficient of Determination (adj-R²), and Prediction Coefficient of Determination (pred-R²) were used to examine the quality of the model developed.

3. RESULTS AND DISCUSSION

3.1. Experimental Validation

To evaluate the validity of the obtained model. triplicate experiments (supplementary material) were conducted at the optimal point according to the RSM-CCD design with the following conditions: temperature of 23.0°C, OLR of 2.23 kg $COD/m^3 \cdot d$, pH of 7.50, and HRT 11.4 hours. The experimental values were 84.1% for COD, 99.9% for TSS and 100 % for VSS, which were very close to those predicted by the RSM-CCD model (83.0 % for COD, 99.2 % for TSS, and 100 % for VSS). One common method for estimating a model's capacity to predict overall removal efficiency involves computing the coefficient of determination R^2 . Models exhibiting excellent predictive accuracy, as indicated by minimal differences between experimental and modeled values, typically have R²values close to one [45].

The quadratic model proposed by the RSM-CCD methodology, as compared to the linear model derived from the linear combination of input variables, demonstrates superior accuracy. This is evident from the values of R^2 (the ratio of

explained sum of squares (ESS) to the total sum of squares (TSS), $R^2 = ESS/TSS$), Adjusted $R^2 = (1 - [(1 - R^2)^*(n - 1) (n - k - 1)])$ where 'n' is the experimental observations and k is the predictor variables and Predicted $R^2 = (1 - (PRESS/TSS))$ where PRESS is predicted sum of squares are in Table 3. Furthermore, presented according to the ANOVA, the second-order polynomial model, which includes the coded variables A, B, C, and D was statistically significant.

| Table 3 Quadratic and | l linear models R ² | comparison |
|-----------------------|--------------------------------|------------|
|-----------------------|--------------------------------|------------|

| Quadra | tic | Linear | | | |
|-----------------------------|--------|--------------------------|--------|--|--|
| \mathbb{R}^2 | 0.9977 | R ² | 0.5259 | | |
| Adjusted R ² | 0.9956 | Adjusted R ² | 0.4719 | | |
| Predicted R ² | 0.9863 | Predicted R ² | 0.2704 | | |

3.1.1. COD Model output by RSM –CCD

The % COD removal model is shown as:

% COD= 70.5749 + 18.0757A - 7.9972B + 6.9780C + 15.2942D + 12.5759AB + 6.9919AD - 3.1719BC -11.1343BD-7.8575CD - 19.5888A² + 6.5013B² + 4.8995C² - 9.4604D² (5)

The model, with coded parameters A, B, C, D, AB, AD, BC, BD, CD, A^2 , B^2 , C^2 , and D^2 , was statistically significant, with a pvalue less than 0.05 at the 95% confidence level. A single parameter or the interplay of operational parameters, which might have a positive or negative impact on the model, affected the % removal of COD. The 'F'-test by Fisher was utilized for the ANOVA analysis. The significance of the model was confirmed as its F-value was found to be 465.21. There was only 0.01 % chance that such a large F-value could occur due to noise. In the model, the probability statistics obtained were less than 0.0001. The lack of fit F-value of 232.69 suggested that it was

not significant relative to the pure error. There was a 5.13% chance that this large could occur due to noise. To evaluate the fit between experimental and modeled data, the adjusted R^2 determination coefficient value was estimated 0.9956. An adjusted R^2 close to one indicates that the developed model could approximate 99.56 % of the total variability in the percentage COD removal

data. The signal-to-noise ratio was calculated using the fit statistics with sufficient precision. A ratio greater than four was preferred. The adequate precision ratio of 68.89 in this model indicated an adequate signal, suggesting that the proposed model can adequately describe the COD reduction within the range used.



Figure 2 Studentized residuals against collected experimentally collected data

To further illustrate the appropriateness of the model, a plot was created to compare the predicted percentage removals with the studentized residuals. The values ranged between ± 3.90 , as shown in Figure 2. The plot demonstrates that the model excellently fits the experimental data.

3.1.2. TSS Model out-put by RSM-CCD

Similar to COD, Eq. (6) shows the TSS removal model elaborated from the RSM-CCD methodology.

% TSS = 94.9013 - 5.5261A - 8.8758C +18.3182D + 3.7210AB+ 5.6324AC + 7.2205AD - 3.9458BC - 3.3562BD + 6.3232CD - 6.4446A² - 12.7649D² (6) Equation (6) clearly shows that the TSS percentage removal is influenced either by linear or double interactions of parameters. These parameters can impact the model either negatively or positively, as indicated by their coefficients in the equation.

3.1.3. VSS Model out puts by RSM-CCD

Finally, Eq. (7) shows the VSS removal model equation obtained from RSM-CCD methodology.

% VSS =
$$88.1682 - 7.0277A + 4.4879B + 22.4402D + 7.3832AD - 3.2492A^2 - 9.7178D^2$$
 (7)

Single and double interactions of parameters had a negative or positive effect on the percentage removal of VSS, as indicated by the coefficients of the input parameters.

3.2. Model generated Results

A percentage removal for COD ranging from 0 to 98.6%, TSS ranging from 0 to 100%, and VSS ranging from 0 to 99.9% was predicted by the obtained model. The goal of the current research was to improve the performance of the UASB reactor in relation to the surrounding temperature. A temperature of 23.0°C, OLR of 2.23 kg COD/m³.d, pH of 7.5, and HRT of 11.4hrs were determined as the optimal operational parameters.

3.3. Combined effects of parameters on percentage COD removal

3.3.1. Combined effect of temperature and OLR

Figure 3 (a) shows the concurrent effects of OLR and temperature on the removal efficiency of COD which is obtained using Eq. 5. The COD removal efficiency increases from 20 to 80 % as the organic load increases from 1 to 3 kg COD/m³.d and the temperature rises to 30°C [46]. At a constant OLR of 1 kg COD/m³·d, increasing the temperature from 0 to 30 decreases the COD removal efficiency from 80 % to 70 %. This reduction is attributed to a decrease in OLR, not temperature. Microorganisms consume substrates more efficiently at higher OLRs, enhancing COD removal efficiency. Maintaining OLR at 3 kg $COD/m^3 \cdot d$ and reducing temperature from 30 to 0°C alters the working mechanism of the WWTP necessitating acclimatization and a startup phase [47, 48]. At a constant OLR of 3 kg COD/m³·d and a temperature of 0° C, COD removal efficiency decreases from 85 % to 20 %, a drop due to the reduced temperature [49].

Figure 3(b) depicts the interaction effect of HRT and temperature. As HRT increases from 4 to 12 hours and temperature from 0 to 30°C, COD removal efficiency improves from 30 % to 85 %. Maintaining HRT at 4 hours and increasing temperature from 0 to 30°C, COD removal efficiency falls from 85 % to 38 %, a decrease due to shortened HRT [50, 51]. Furthermore, keeping HRT at 12 hours and temperature at 0°C, the efficiency of COD removal drops from 85 % to 30 %, this decline in COD removal attributable to decrease in temperature. The interaction between pH and temperature is presented in Figure 3(c). Increasing pH from 6.3 to 7.8 and temperature from 0 to 30°C boosts COD removal efficiency from 45 % to 80 %. However, with a constant pH of 6.3 and increasing temperature from 0 to 30°C, COD removal efficiency decreases from 80 % to 65 %, a decrease due to lower pH levels [52]. If temperature remains constant at 0°C and pH increases from 6.3 to 7.8, COD removal efficiency drops from 85 % to 45 %, a decrease resulting from the lower temperature.

Figure 3(d) shows the interactions between OLR and pH. Increasing OLR from 1 to 3 kg COD/m³·d and pH from 6.3 to 7.8 results in a decrease in COD removal efficiency from 100% to 78. Keeping OLR at 3 kg COD/m³·d and lowering pH from 7.8 to 6.3 decreases COD removal efficiency from 78% to 69 %, a reduction due to the lower pH. Figure 3(e) reveals the combined effects of OLR and HRT for COD removal. Increasing OLR from 1 to 3 kg COD/m³·d and HRT from 4 to 12 hours reduces COD removal efficiency from 100 % to 64 %. Maintaining OLR at 3 kg COD/m³·d and HRT at 4 hours decreases COD removal efficiency from 64% to 58 %, a decrease attributed to the reduced HRT [53]. Figure 3(f) presents the combined effect of HRT and pH for COD removal. Increasing pH from 6.3 to 7.8 and HRT from 4 to 12 hours

improves COD removal efficiency from 80% to 82%. However, keeping HRT at 4 hours and increasing pH from 6.3 to 7.8 decreases COD removal efficiency from 82 % to 65 %, a reduction attributed to the shortened HRT [54]. The fit summary

response, lack of fit tests, ANOVA for the quadratic model response, fit statistics, and 3D surface percentage removal for TSS and VSS are provided in supplementary materials.



Figure 3 Response 3D surfaces for %COD removal (a) OLR vs. Temperature (b) HRT vs. Temperature (c) PH vs. Temperature (d) HRT vs. OLR (e) pH vs. OLR (f) HRT vs. pH

3.4. Sulfate ion (SO₄²⁻) Concentration

In this research, the effluent SO_4^{2-} ion concentration was greater than the influent concentration. This phenomenon was attributed to the high rate of methanogenesis, which inhibits the activity of sulfur-reducing bacteria, and showing that the reactor set-up was well functioning [55, 56].

| No | Temperature | OLR | HRT | pН | Inlet SO ₄ ²⁻ | Outlet SO ₄ ²⁻ |
|----|-------------|----------------------------|--------|------|-------------------------------------|--------------------------------------|
| | (°C) | (kg COD/m ³ .d) | (hrs.) | | (mg/L) | (mg/L) |
| 1 | 30 | 3 | 12 | 6.30 | 6.0 | 80 |
| 2 | 30 | 1 | 10.08 | 7.80 | 23 | 52 |
| 3 | 30 | 3 | 7.68 | 6.30 | 21 | 80 |
| 4 | 30 | 3 | 6.72 | 7.80 | 29 | 70 |
| 5 | 30 | 3 | 5.84 | 7.80 | 70 | 80 |
| 6 | 30 | 1 | 7.44 | 6.30 | 33 | 80 |
| 7 | 30 | 1 | 15.60 | 7.80 | 30 | 20 |
| 8 | 30 | 3 | 12.00 | 6.30 | 43 | 80 |
| 9 | 15 | 2 | 11.52 | 8.55 | 37 | 80 |
| 10 | 15 | 0 | 0.00 | 7.05 | 0.0 | 0.0 |
| 11 | 15 | 4 | 8.00 | 7.05 | 19 | 72 |
| 12 | 15 | 2 | 8.00 | 7.05 | 22 | 0.0 |
| 13 | 15 | 2 | 8.00 | 7.05 | 22 | 76 |
| 14 | 15 | 2 | 16.00 | 7.05 | 28 | 61 |
| 15 | 15 | 2 | 10.20 | 7.05 | 26 | 66 |
| 16 | 15 | 2 | 11.52 | 7.05 | 26 | 67 |
| 17 | 15 | 2 | 15.12 | 7.05 | 20 | 54 |
| 18 | 15 | 2 | 14.40 | 7.05 | 28 | 51 |
| 19 | 15 | 2 | 0.00 | 7.05 | 28 | 56 |
| 20 | 15 | 2 | 13.44 | 5.55 | 26 | 54 |
| 21 | 0 | 3 | 3.22 | 6.30 | 70 | 80 |
| 22 | 0 | 3 | 4.56 | 7.80 | 50 | 80 |
| 23 | 0 | 3 | 12.00 | 6.30 | 21 | 80 |
| 24 | 0 | 3 | 6.24 | 7.80 | 25 | 75 |
| 25 | 0 | 1 | 12.00 | 6.30 | 59 | 80 |
| 26 | 0 | 1 | 13.20 | 7.80 | 53 | 80 |
| 27 | 0 | 1 | 16.00 | 6.30 | 45 | 59 |
| 28 | 0 | 1 | 16.00 | 7.80 | 28 | 73 |
| 29 | -5 | 2 | 9.60 | 7.05 | 45 | 5.0 |
| 30 | 45 | 2 | 14.47 | 7.05 | 27 | 22 |

Table 4 Inlet and outlet sulfate ion concentrations

At a temperature of -15° C, pH of 7.05, OLR of 2 kg COD/m³·d, and HRT of 9.6 hours, it was observed that the outlet SO₄²⁻ ion concentration significantly decreased. This is attributed to the fact that at extremely low temperatures, the activity of anaerobic microorganisms diminishes [57].

3.4.1. VFA to Alkalinity ratio

The VFA to alkalinity ratio was observed to be in the range of 0.055 to 0.15. This indicates that the UASB reactor was operating under normal conditions. In the system, saprophytes break down complex molecules, producing acids such as acetic, propionic, and butyric acids. The alkalinity in the solution neutralizes hydrogen ions released by these acids and methanogenesis subsequently takes over to maintain the pH balance. At a temperature of -15°C, pH of 7.05, OLR of 2 kg COD/m³.d, and HRT of 9.58 hrs, the outlet volatile fatty acid to alkalinity ratio was out of the set range since acid genesis and methanogenesis were inhibited by decreasing temperature.

| Expt. n <u>o</u> | Temperature (°C) | OLR (kg COD/m ³ .d) | HRT | pН | in let VFA | out let VFA |
|---------------------|---------------------|-----------------------------------|-------|------|---------------|----------------|
| | | | | | Alkalinity | Alkalinity |
| 1 | 30 | 3 | 12.00 | 6.30 | 0.080 | 0.075 |
| 2 | 30 | 1 | 10.08 | 7.80 | 0.090 | 0.065 |
| 3 | 30 | 3 | 7.68 | 6.30 | 0.065 | 0.060 |
| 4 | 30 | 3 | 6.72 | 7.80 | 0.090 | 0.065 |
| 5 | 30 | 3 | 5.84 | 7.80 | 0.060 | 0.050 |
| 6 | 30 | 1 | 7.44 | 6.30 | 0.090 | 0.140 |
| 7 | 30 | 1 | 15.60 | 7.80 | 0.090 | 0.060 |
| 8 | 30 | 3 | 12.00 | 6.30 | 0.098 | 0.080 |
| 9 | 15 | 2 | 11.52 | 8.55 | 0.100 | 0.150 |
| 10 | 15 | 0 | 0.00 | 7.05 | 0.000 | 0.000 |
| 11 | 15 | 4 | 8.00 | 7.05 | 0.088 | 0.060 |
| 12 | 15 | 2 | 8.00 | 7.05 | 0.080 | 0.060 |
| 13 | 15 | 2 | 8.00 | 7.05 | 0.055 | 0.080 |
| 14 | 15 | 2 | 16.00 | 7.05 | 0.090 | 0.088 |
| 15 | 15 | 2 | 10.20 | 7.05 | 0.100 | 0.080 |
| 16 | 15 | 2 | 11.52 | 7.05 | 0.099 | 0.054 |
| 17 | 15 | 2 | 15.12 | 7.05 | 0.080 | 0.090 |
| 18 | 15 | 2 | 14.40 | 7.05 | 0.060 | 0.090 |
| 19 | 15 | 2 | 0.00 | 7.05 | 0.000 | 0.000 |
| 20 | 15 | 2 | 13.44 | 5.55 | 0.100 | 0.067 |
| 21 | 0 | 3 | 3.22 | 6.30 | 0.090 | 0.060 |
| 22 | 0 | 3 | 4.56 | 7.80 | 0.089 | 0.075 |
| 23 | 0 | 3 | 12.00 | 6.30 | 0.043 | 0.020 |
| 24 | 0 | 3 | 6.24 | 7.80 | 0.080 | 0.053 |
| 25 | 0 | 1 | 12.00 | 6.30 | 0.086 | 0.050 |
| 26 | 0 | 1 | 13.20 | 7.80 | 0.120 | 0.050 |
| 27 | 0 | 1 | 16.00 | 6.30 | 0.055 | 0.053 |
| 28 | 0 | 1 | 16.00 | 7.80 | 0.060 | 0.050 |
| 29 | -5 | 2 | 9.60 | 7.05 | 0.110 | 0.095 |
| 30 | 45 | 2 | 14.47 | 7.05 | 0.106 | 0.103 |

Table 5 Volatile fatty acid to alkalinity ratio

4. CONCLUSIONS

According to the results derived from the RSM-CCD the main factors influencing the performance of the pilot UASB reactors were the following operational parameters: Temperature (0 -30°C), OLR of (1- 3 kg COD/m³.d), pH of (6.3-7.8), and HRT of (4-12hrs). CCD-RSM with five levels was used

to optimize these operational parameters. An optimized operational parameter of temperature (23.0°C), OLR of (2.23 kg COD/m³.d), pH of (7.5), and HRT of (11.4 hrs) were achieved. Using optimized operational parameters, 84.1 % for COD, 99.9 % for TSS and 100 % for VSS were obtained. The CCD-RSM predicted a

removal efficiency of 83.0, 99.2, and 100 %, for COD, TSS, and VSS. Furthermore, the influent and effluent concentration of sulfate ions. total nitrogen, alkalinity, total phosphorous, VFA, pH, and volatile fatty acid to alkalinity ratio, were measured to check the functionality of the pilot scale reactor set up. Relative to the influent concentration, the effluent concentration of volatile fatty acid and alkalinity were decreasing. The reason was as microorganisms break the organic compounds, acids like acetic, propionic and butyric acids were produced and release hydrogen which later accepted by the alkalinity to maintain the pН by methanogenesis. The effluent concentration of sulfate ion (SO₄²⁻) was seen increasing since the activities of sulfur reducing bacteria were hindered due to anaerobic conditions created by acid, and alkalinity. This creates higher production of methane gas than hydrogen sulfide. In general, there is a possibility to increase the removal efficiency of the WWTP employing UASB reactor by optimizing operational parameters such as temperature, HRT, pH and OLR without incurring energy. This can be achieved practically either through dilution of the wastewater at an inlet by the service water or heating the wastewater using the solar panel. The amount of heat energy needed and the source of energy will be the future potential research.

CONFLICT OF INTEREST

No conflict of interests exists between the authors

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