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# Performance evaluation and substrate removal kinetics in a thermophilic anaerobic moving bed biofilm reactor for starch degradation

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## ABSTRACT

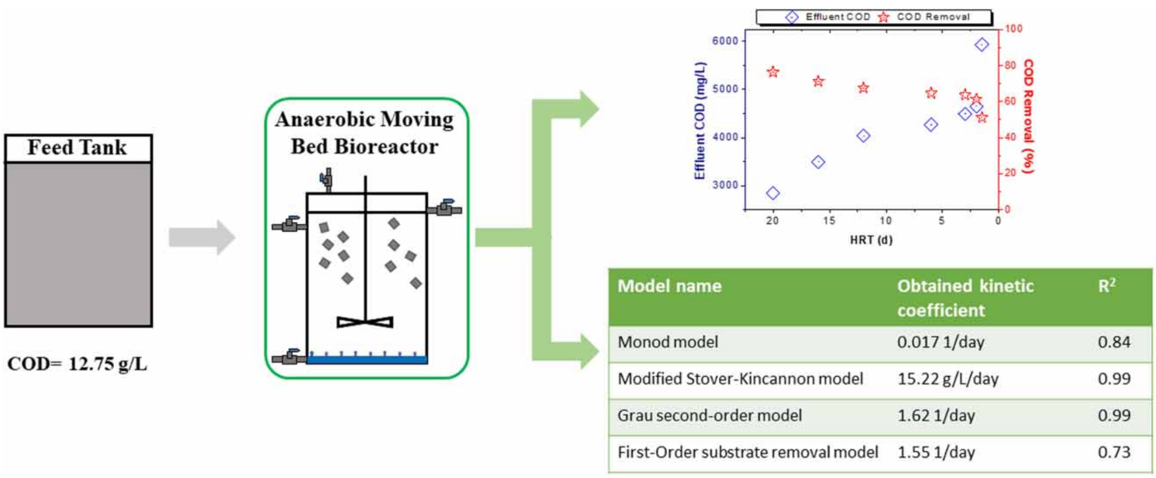
A laboratory-scale anaerobic moving bed biofilm reactor (AnMBBR) was installed and operated at various hydraulic retention times (HRTs) of 20 to 1.5 d with surface area loading rate (SALR) of 0.86 to 11.43 gCOD/m$^2$/d. Synthetic starch containing desizing wastewater with chemical oxygen demand (COD) of 12.75 g/L was prepared and fed into the reactor. Monod, modified Stover-Kincannon, Grau second-order and First-order substrate removal models were used to evaluate the results of AnMBBR. COD removal efficiency of bioreactor was dwindled by increasing the SALR or reducing the HRT. Decay coefficient ($K_d$) and yield coefficient (Y) for the Monod model were 0.027 1/d and 1.01 mgVSS/mgCOD, respectively. Maximum substrate utilization rate ($U_{max}$) and kinetic constant ($K_b$) for the Modified Stover-Kincannon model were estimated as 12.57 and 15.22 g/L/d, respectively. The constants (a and b) for the Grau second-order model were found to be 1.09 and 1.31 whilst kinetic coefficient for the Second-order model and First-order substrate removal model were 1.62 and 1.55 1/d, respectively. Modified Stover-Kincannon model and Grau second-order model were found to be the best fit for experimental data with $R^2$ value of 0.99. The findings suggest that these models can be applied to predict the behaviour of AnMBBRs on various scales.

**Key words:** anaerobic digestion, kinetic modeling, starch removal, wastewater treatment

## HIGHLIGHTS

- Starch containing desizing wastewater was treated using an anaerobic MBBR.
- COD, TKN and $PO_4^{3-}$-P removal of 61, 43 and 10% was achieved, respectively.
- Maximum biogas production of 11.6 L/day was achieved at optimum HRT of 2 days.
- Modified Stover-Kincannon and Grau second order were found to be best fit models.

## GRAPHICAL ABSTRACT




| Model name | Obtained kinetic coefficient | $R^2$ |
|---|---|---|
| Monod model | 0.017 1/day | 0.84 |
| Modified Stover-Kincannon model | 15.22 g/L/day | 0.99 |
| Grau second-order model | 1.62 1/day | 0.99 |
| First-Order substrate removal model | 1.55 1/day | 0.73 |

## INTRODUCTION

Textile industries have been reported to be major consumers of water, which generates a surplus amount of wastewater. Generally, wastewater generated from textile industry contains wide variety of pollutants including organic matter, dyes and other chemicals (Yaseen & Scholz 2019). Among others, the wastewater generated from desizing process having COD above 10 g/L is the prime cause of chemical oxygen demand (COD) in wastewater of the textile industry (Shahzad *et al.* 2020). The specific COD of starch is 0.9 g of $O_2$ per g of starch (Baumann *et al.* 2018). So, the high COD is caused by high starch content applied to yarn during sizing prior to the weaving process. The operational cost of plant required to treat the combined textile wastewater is high owing to high energy, nutrient and chemical requirements, besides producing more sludge. The economics of treatment process can be improved through anaerobic pretreatment of starch containing desizing wastewater. Anaerobic moving bed biofilm reactor (AnMBBR) was chosen for this study as it can retain more biomass within the system for better COD degradation and reduce the acidification due to superior hydrodynamic conditions (Wang *et al.* 2009).

Kinetic modeling is an effective technique for explaining and forecasting the performance of anaerobic reactors. It helps to obtain a deeper knowledge of anaerobic reactors and serves as a useful tool to eliminate the complicated and laborious experimental work through mathematical equations (Turkdogan-Aydinol *et al.* 2011). Different mathematical equations have been devised and successfully utilized to envisage and describe the performance of anaerobic reactors (Turkdogan-Aydinol *et al.* 2011; Faekah *et al.* 2020). However, to the best of the authors' knowledge, kinetic evaluation of starch degradation by AnMBBR has not been reported until now.

In this study, the performance of an AnMBBR was assessed based on the COD removal, total Kjeldahl nitrogen (TKN) removal, orthophosphates phosphorus removal, alkalinity, pH, volatile fatty acids (VFA) and biogas production for HRT of 20, 16, 12, 6, 3, 2 and 1.5 d on high strength synthetic starch containing desizing wastewater at thermophilic temperature of 55 °C. Furthermore, the kinetic evaluation was carried out by using conventional Monod, Modified Stover-Kincannon, Grau second-order and First-order substrate removal models.

## MATERIALS AND METHODS

### Experimental setup

In the present study, a lab scale AnMBBR having a total liquid volume of 12 L was used. Figure 1 shows the schematic diagram of the AnMBBR reactor. Plastic carrier having specific surface area of 700 $m^2/m^3$ was used as media for biofilm growth in the AnMBBR. Synthetic starch containing desizing wastewater with COD of 12.75 g/L was fed to the AnMBBR with a peristaltic pump from the bottom of the reactor. A temperature controller, connected with a heater and thermocouple, was used in the AnMBBR to control the temperature of

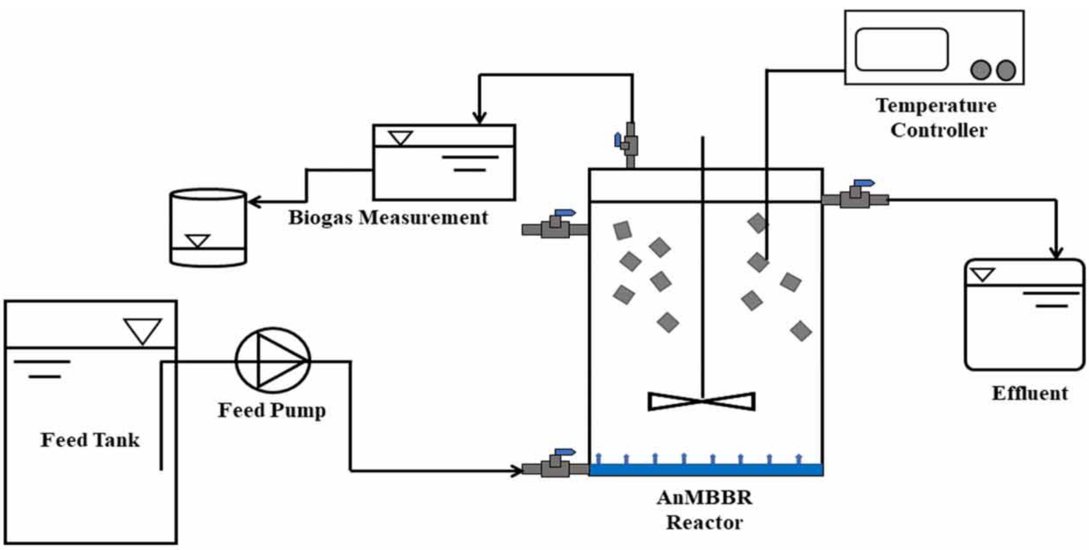



**Figure 1** | Schematic illustration of bench-scale anaerobic moving bed biofilm reactor.

55 °C while a mixer was provided for mixing of wastewater and media. The water displacement method was used to measure the volume of produced biogas from the AnMBBR.

## Operational protocol

Sludge used in the study was procured from the bottom of old wetland constructed at National University of Sciences and Technology, Islamabad Pakistan and acclimatized to thermophilic (55 °C) anaerobic conditions prior to this study. AnMBBR performance was evaluated at various HRTs of 20, 16, 12, 6, 3, 2 and 1.5 d with feed concentration of 12.75 g/L. Surface area loading rates (SALR) against these HRTs were 0.86, 1.07, 1.43, 2.86, 5.71, 8.57 and 11.43 gCOD/$m^2$/d, respectively. The AnMBBR was initially started from a higher HRT, i.e. 20 d and operated until a steady state condition was achieved (determined by a stable COD removal). Afterward, HRT to the AnMBBR was reduced to 16, 12, 6, 3, 2 and 1.5 d to study the effect of varying HRTs on AnMBBR performance.

## Synthetic wastewater composition

For this study, synthetic wastewater was prepared having COD of 12.75 g/L using sizing chemicals (starch, sizing fat) and desizing chemicals (hydrogen peroxide, hydrogen persulfate, caustic soda and surfactant). pH of the influent wastewater was adjusted to 7.50–8.0 before supplying to the AnMBBR. Macronutrients (ammonium chloride and potassium dihydrogen phosphate) and micronutrients (cobalt, zinc and nickel chloride) have also been added to the feed. The characteristics of feed wastewater are portrayed in Table 1.

**Table 1** | Feed wastewater characteristics

| Parameters | Value (g/L) |
|---|---|
| pH | 7.75 |
| Chemical oxygen demand | 12.75 |
| Sulfate | 0.98 |
| Total suspended solids | 0.99 |
| Total Kjeldahl nitrogen | 0.103 |
| Orthophosphates phosphorus | 0.021 |

## Analytical methods

The performance efficiency of the anaerobic MBBR was assessed using various key parameters including alkalinity, pH, VFA, TKN, orthophosphates phosphorus, COD and biogas production. The closed reflux titrimetric method was used to determine the COD. Whereas, the titration method was applied for VFA and alkalinity measurement. A handheld digital meter (Hanna Instruments Ltd, HI 83141, UK) was used to assess pH. The analytical methods aforementioned parameters were acquired from guidelines in standard methods described by American Public Health Association (APHA 2017).

## Kinetic modeling for substrate removal

Kinetic modeling is an effective approach to predict the performance of anaerobic reactors and results of the study might be used to determine the performance of full-scale reactors at similar operating conditions. Monod, modified Stover-Kincannon, Grau second-order and First-order substrate removal models are widely used mathematical expressions for kinetic coefficient determination. The efficacy of the model was assessed based on the COD removal efficiency obtained from AnMBBR operation at steady state condition for all applied HRTs.

### Monod model

The rate of increase in biomass concentration in a biological treatment system is directly related to the initial concentration of biomass in the reactor and the rate of proportion is known as the specific growth rate constant (U). The equation of specific growth rate is given below:

$$U = \frac{(S_i - S_e)}{HRT.X} = \frac{KS_e}{K_s + S_e} \quad (1)$$

where, K and $K_s$ are maximum substrate utilization rate (1/d), and half velocity constant (g/L), respectively, $S_i$ and $S_e$ are influent and effluent COD (g/L) and X is the biomass concentration within the reactor. The value of both (K and $K_s$) can be determined by graph plotting between $1/S_e$ versus HRT.X/($S_i$-$S_e$). To calculate the total amount of biomass produced, the yield coefficient (Y) is employed. It's calculated as the number of new cells produced per unit of substrate removed. The equation is defined as:

$$\frac{1}{HRT} = \frac{(S_i - S_e)}{HRT.X} Y - K_d \tag{2}$$

where, $K_d$ is death rate constant (1/d). The value of Y and $K_d$ can be estimated from a straight line of a plot between 1/HRT against ($S_i$-$S_e$)/(HRT.X). Maximum specific substrate utilization rate (K) is related to the bacteria's maximum specific growth ($\mu_{max}$). The Michaelis-Menten equation, shown below, links the specific growth rate of bacteria with the substrate removal.

$$\mu_{max} = K.Y \tag{3}$$

**Modified Stover-Kincannon model**

This model in particular has been used successfully for attached growth anaerobic systems for treatment of different types of wastewater (Wang *et al.* 2015; Oktem 2020). Based on this model, COD removal efficiency from AnMBBR can be determined using the COD removal rate as a function of the COD concentration. Equations of this model can be described as:

$$\frac{ds}{dt} = \frac{Q(S_i - S_e)}{V} \tag{4}$$

$$\frac{ds}{dt} = \frac{U_{max}\left(\frac{QS_i}{V}\right)}{K_b + \frac{QS_i}{V}} \tag{5}$$

where, ds/dt is the COD removal rate (g/L/d), $S_i$ and $S_e$ are influent and effluent COD (g/L), Q is the feed flow rate (L/d), V is the working volume of AnMBBR, $U_{max}$ and $K_b$ are maximum COD removal rate and the kinetic coefficient (g/L/d) for the Modified Stover-Kincannon model. When written in terms of HRT, the above Equation (5) is linearized as:

$$\left(\frac{ds}{dt}\right)^{-1} = \frac{HRT}{(S_i - S_e)} = \frac{K_b}{U_{max}}\frac{HRT}{Si} + \frac{1}{U_{max}} \tag{6}$$

By plotting the HRT/($S_i$-$S_e$) versus the HRT/$S_i$, $K_b/U_{max}$ and $1/U_{max}$ is the slope and intercept of the straight line.

**Grau second-order model**

The derived equation of this model is given as follows:

$$-\frac{ds}{dt} = \left(\frac{S_e}{S_i}\right)^2 K_2 X \tag{7}$$

where, $K_2$ is the coefficient for the second order COD removal rate (1/d) and X is the concentration of biomass (g VSS/L). The above equation is simplified and linearized as:

$$\frac{S_i \times HRT}{S_i - S_e} = HRT + \frac{S_i}{K_2 X} \tag{8}$$

$(S_i - S_e)/S_i$ represents COD removal efficiency and $S_i/K_2X$ is a constant, so Equation (8) can be written as:

$$\frac{HRT}{COD\ Removal} = b(HRT) + a \tag{9}$$

Here, a and b are a constant and can be obtained from a straight line of a plot between HRT/COD removal and HRT.

**First-order substrate removal model**

This model was used to explain the hydrolysis of organic contaminants. In the anaerobic system, for COD removal the mass balance equation can be defined as:

$$-\frac{ds}{dt} = \frac{(S_i - S_e)}{HRT} - K_1 S_e \tag{10}$$

where, $K_1$ is the coefficient of first order for COD removal rate. Under stabilized conditions the above Equation (10) can be written as:

$$\frac{(S_i - S_e)}{HRT} = K_1 S_e \tag{11}$$

$K_1$ can be found by graph plotting between $(S_i - S_e)$/HRT versus $S_e$.

## RESULTS AND DISCUSSIONS

### Performance of AnMBBR

AnMBBR was operated continuously for about 150 d at seven different HRTs of 20, 16, 12, 6, 3, 2 and 1.5 d. HRT was reduced from 20 to 1.5 d by increasing the feed flow rate to the reactor. Figure 2 shows the effluent COD concentration and COD removal efficiency from AnMBBR under steady state conditions. Results indicated that COD removal efficiency was reduced from 76.3 to 51.1% with reducing the HRT from 20 to 1.5 d. At the HRT of 20 d, variation in COD removal efficiency was in the range of 43–78%. At HRT of 20 d under steady state conditions, the average COD removal efficiency was 76% and effluent COD concentration was 2.8 g/L. At HRT of 16 d, the COD removal efficiency varied between 48 to 71%, with average COD removal of 71% and effluent COD of 3.5 g/L under steady state conditions. Likewise, at HRTs of 12, 6, 3, 2 and 1.5 d COD removal efficiency varied in the range of 51–69, 54–67, 53–65, 50–61 and 32–54%, respectively. Under steady state conditions at the above-mentioned HRTs, average COD removal was 68, 65, 64, 61 and 51%, respectively, and effluent COD concentration was 4.0, 4.3, 4.5, 4.7, 6.0, and 6.6 g/L, respectively. Performance of the AnMBBR was decreased in terms of COD removal, VFA accumulation and biogas production by increase in HRT due to the shock received to the microorganisms with increasing the feed flow rate (Musa *et al.* 2018; Shahzad *et al.*

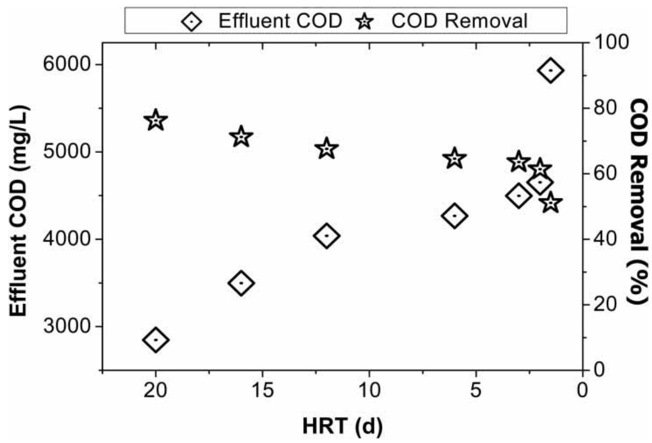



**Figure 2** | Effluent COD and COD removal from AnMBBR.

2021). Overall, the AnMBBR showed that there is a significant difference in COD removal efficiency ($P < 0.05$) with change in HRTs. In this study, maximum removal efficiency of COD was observed at maximum HRT and vice versa. With decrease in HRT, the reduction in COD removal efficiency is due to less time being available to degrade the available organic matter in wastewater (Musa & Idrus 2020).

Table 2 shows the VFA, alkalinity, pH and biogas production from the AnMBBR under steady state conditions. At each change of HRT, initially the VFA production increased abruptly and biogas production decreased, which indicated that the AnMBBR was destabilized; however, the reactor was stabilized to the new conditions thereafter. With decreasing the HRT from 20 to 2 d, VFA and alkalinity concentration were in the range of 0.5 to 1.3 g/L and 3.0 to 4.1 g/L, respectively. However, with further decrease in HRT to 1.5 d VFA, the concentration sharply increased to 1.9 g/L and the high concentration of VFA at minimum HRT affected the buffering potential and decreased the alkalinity of the reactor to 3.5 g/L. This also indicates that the anaerobic reactor was well buffered up to the HRT to 2 d and performance of reactor was disturbed at minimum HRT. The effect of decreasing HRTs to a minimum level with the accumulation of VFA was also expressed by variability in the rate of daily biogas production. As shown in Table 2, the rate of biogas production reduced to 8.3 L/d at HRT value of 1.5 d. At each shift of HRT, the pH of the AnMBBR slightly fluctuated and self-regulation of pH was observed under steady state condition. As seen in Table 2, when HRT declined from 20 to 1.5 d, the pH for methanogenic bacteria remained above the lower threshold of the ideal range (pH 6.8–7.2). Generally, the above-mentioned parameters showed that there was not a complete failure of anaerobic reactor even at minimum HRT (1.5 d). This is mainly associated with the fact that the attached growth media was able to retain more biomass within the AnMBBR.

**Table 2** | Stable performance results obtained at different HRTs

| HRT (d) | SALR ($gCOD/m^2/d$) | VFA (g/L) | Alkalinity (g/L) | pH | TKN Removal (%) | Orthophosphate phosphorus Removal (%) | Biogas Production (L/d) |
|---|---|---|---|---|---|---|---|
| 20 | 0.86 | 0.5 | 3.0 | 7.1 | 54.2 | 17.8 | 1.5 |
| 16 | 1.07 | 0.6 | 3.1 | 7.1 | 50.7 | 13.9 | 2.0 |
| 12 | 1.43 | 0.6 | 3.3 | 7.2 | 49.2 | 11.7 | 2.7 |
| 6 | 2.86 | 0.8 | 3.6 | 7.1 | 44.6 | 8.4 | 3.8 |
| 3 | 5.71 | 1.0 | 3.8 | 7.3 | 36.2 | 7.7 | 9.0 |
| 2 | 8.57 | 1.3 | 4.1 | 7.3 | 33.5 | 4.7 | 11.6 |
| 1.5 | 11.43 | 1.9 | 3.5 | 6.9 | 29.6 | 3.4 | 8.3 |

A summary of TKN and orthophosphate phosphorus removal during the operation of the AnMBBR are reported in Table 2. Removal of TKN and orthophosphate phosphorus from the AnMBBR was statistically significant ($P < 0.05$). Average TKN and orthophosphate phosphorus removal from the AnMBBR was 42.57 and 9.6%, respectively. TKN removal was highest at highest HRT, with maximum removal efficiency of 54.2%, due to the biomass assimilation and the removal efficiency decreased as the biomass acclimatized. Ammonia is also produced in anaerobic process as a by-product due to the mineralization of nitrogen during deamination of protein and results in the increase of the TKN concentration in the effluent (Musa & Idrus 2020). The AnMBBR showed low orthophosphate phosphorus removal efficiency due to its limited capacity to support biomass growth. Due to minute biomass growth with infinite SRT, nutrients demand for microbes was negligible. A little accumulation of phosphates was observed at higher HRT with no significant accumulation at lower HRT. Almost the same total concentration of phosphates at the influent and effluent of the AnMBBR was observed. The slight removal of orthophosphate phosphorus at higher HRT was due to the assimilation of biomass (Haider *et al.* 2018). However, it is also stated that at low HRT, accumulation of ammonia and nitrate in anaerobic biomass also interferes with the phosphate removal (Yilmaz *et al.* 2008). The removal of both TKN and orthophosphate phosphorus by anaerobic digestion was low due to limited utilization of nutrients for growth of biomass, which requires a demand for further treatment by aerobic process. In the textile industry, they can be removed later in a combined centralized wastewater treatment unit.

## Kinetic models for COD removal

### Monod model

Figure 3(a) shows the straight line between HRT.X/($S_i$-$S_e$) versus 1/$S_e$ to obtain the value of $K_s$ and K. Figure 3(b) demonstrates a graph plot between ($S_i$-$S_e$)/(HRT.X) versus 1/HRT for the value of $K_d$ and Y. Maximum substrate utilization rate (K), saturation constant ($K_s$), decay coefficient ($K_d$), yield coefficient (Y) and maximum specific growth rate of bacteria ($\mu_{max}$) can be determined as 0.071 1/d, 6.04 g/L, 0.027 1/d, 1.01 mgVSS/mgCOD and 0.072 1/day, respectively. Coefficient of regression ($R^2$) of this model is in the range of 0.84 to 0.98. The value of $K_s$ obtained in this study is far higher than the value of K. As reported earlier (Ahn & Forster 2000; Faekah *et al.* 2020), this condition is favourable for an anaerobic reactor as the performance efficiency of the system will not be reduced with increase in organic loading rate (OLR). The higher $K_s$ value will result in better biodegradability of substrate (starch) while the higher K value indicates that it is difficult to degrade the organic matter within the bioreactor (Enitan & Adeyemo 2014; Ahmadi *et al.* 2015).

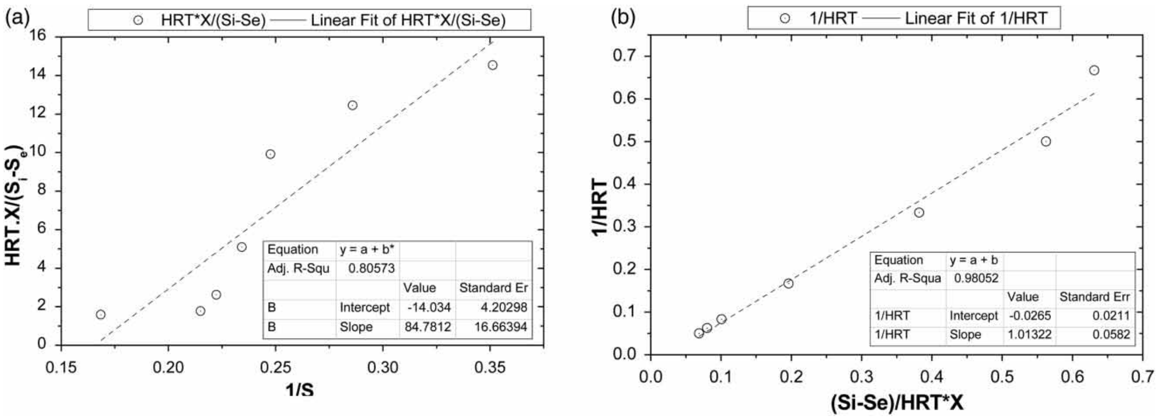



**Figure 3** | Linear plot of Monod model to determine kinetic constants of (a) K and $K_s$ (b) $k_d$ and Y.

### Modified Stover-Kincannon model

Figure 4 demonstrates a graph plot between the HRT/($S_i$-$S_e$) versus the HRT/$S_i$, to obtain the value of $K_b$ and $U_{max}$ for starch degradation by thermophilic anaerobic moving bed biofilm reactor. A straight-line having a value of coefficient of regression of 0.99 strongly supports the validity of this model. The maximum COD removal rate ($U_{max}$) and kinetic coefficient ($K_b$) values were estimated to be 12.57 and 15.22 g/L/d, respectively. $U_{max}$ and $K_b$ obtained in this study are found to be lower than the previous study for treatment of starch based textile wastewater by using an upflow anaerobic fixed bed (UAFB) reactor (Sandhya & Swaminathan 2006). On the other

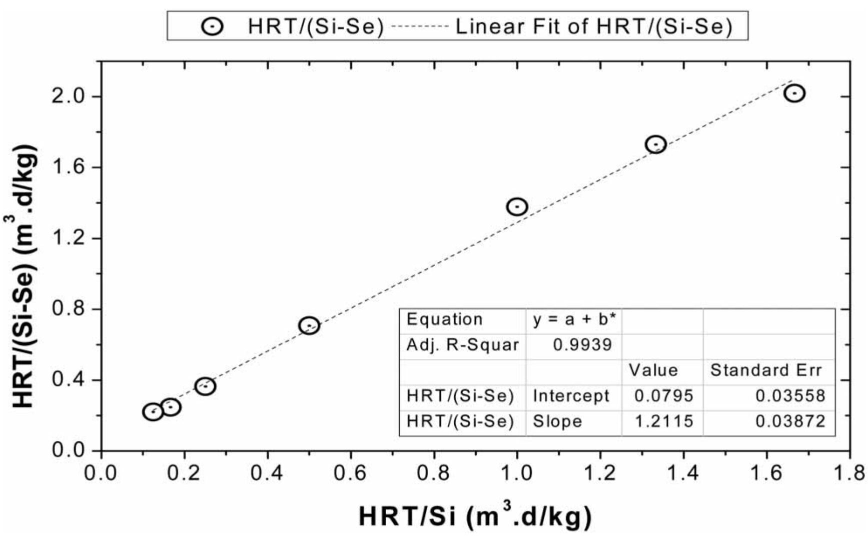



**Figure 4** | Linear plot of modified Stover-Kincannon model.

way, both values ($U_{max}$ and $K_b$) are found to be comparable with values obtained by Işik & Sponza (2005). The variation of the values among different studies might be attributed to the use of different kinds of anaerobic reactors and microorganisms even for the same kind of substrate (Priya *et al.* 2009).

### Grau second-order model

The plot of this model for COD removal from the AnMBBR is shown in Figure 5. Values of a and b can be calculated from the intercept and the slope of the straight line. The values of a and b were obtained as 1.09 and 1.31, respectively, with a strong correlation coefficient ($R^2 = 0.99$). That confirms the model validity for the treatment of starch containing wastewater by AnMBBR. The value of the second order kinetic coefficient ($K_2$) was determined as 1.62 1/d.

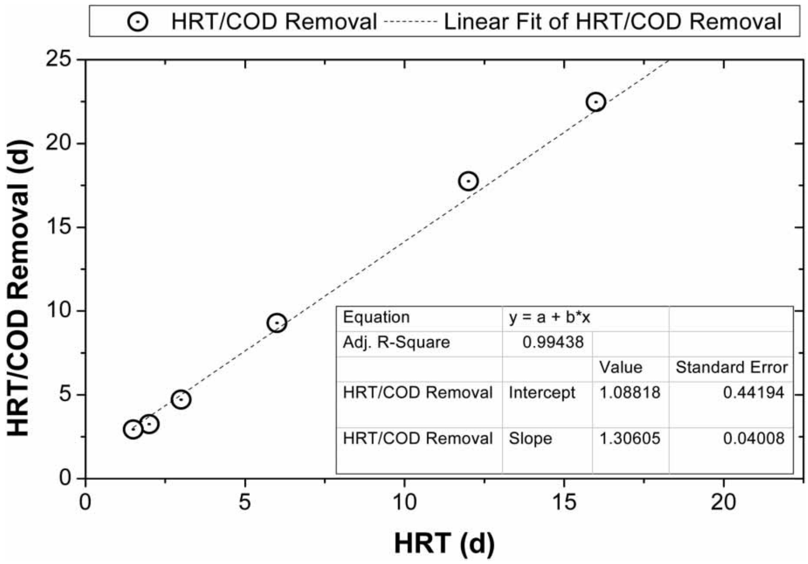



**Figure 5** | Linear plot of Grau second-order model.

### First-order substrate removal model

The plot of the first-order substrate removal model for the AnMBBR is shown in Figure 6. For this model, at steady state conditions, to get a straight line the ($S_i$-$S_e$)/HRT values were plotted against the corresponding $S_e$ values. The line slope reflects the values of $k_1$, which was determined as 1.55 1/d with an $R^2$ value of 0.73. Işik & Sponza (2005) also obtained a similar value of the $K_1$ for the treatment of a similar kind of substrate.

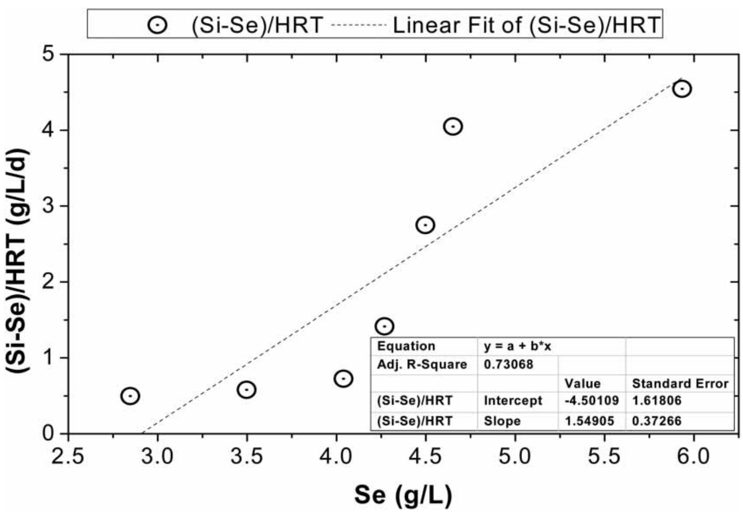



**Figure 6** | Linear plot of first-order substrate removal model.

## CONCLUSIONS

It is demonstrated from the obtained results that in AnMBBR, starch can be degraded effectively at various HRTs (20, 16, 12, 6, 3, 2 and 1.5 d). Also, the COD removal efficiency was in the range of 76 to 51% between the HRT of 20 to 1.5 d and SALR of 0.86 to 11.43 g/COD/$m^2$/day. Modified Stover-Kincannon and Grau second-order models suits well ($R^2 = 0.99$) with the collected experimental data for the degradation of starch under thermophilic conditions by using AnMBBR. Monod model and First-order model with a lower coefficient of regression value as compared to other two models have not been favourable to predict the efficacy of the AnMBBR.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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