Evaluation of an Anaerobic/Aerobic System for Reactive Black 5 Removal: Kinetic Study

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ABSTRACT: *Colored effluents are one of the most important environmental pollutants in the world . This study focused on the removal of dye Reactive Black5 (RB5) using a combination of an anaerobic digester and an Integrated Fixed-film Activated Sludge (IFAS) reactor. The effects of Hydraulic Retention Times (HRT), temperature and filling ratio in the anaerobic digester, the effect of hydraulic retention time and filling ratio in IFAS, and also the effect of initial dye concentration on color removal and Chemical Oxygen Demand (COD) reduction efficiencies were investigated. The Maximum efficiency of color removal and COD reduction in the anaerobic digester at HRT of 2.5 days, 35 °C and 50% of filling ratio was 81% and 99%, respectively, and in IFAS at HRT of 6 h and 70% of filling ratio was 25% and 100%, respectively. According to the Scanning Electron Microscope (SEM) photographs of the microbial community, there were morphological differences in the microorganisms of the two reactors. The Stover–Kincannon and Monod models were used to describe kinetic data. The results showed that Stover–Kincannon model follows the experimental data well. Maximum utilization rate constant and Saturation value constant of Stover–Kincannon model for dye and COD were determined as Umax = 0.15 g / (l day), KB = 0.165 g / (l day) and Umax = 11.31 g / (l day), KB = 11.31g (l day) respectively.*

KEYWORDS*:* *Decolorization; Reactive Black 5 dye; anaerobic digester; aerobic integrated fixed-film; activated sludge; kinetic model.*

INTRODUCTION

Synthetic dyes are widely used in various industries, including in the textile, food, cosmetics, leather and tanning, plastics, pharmaceutical, printing, and dye manufacturing centers. The entrance of these materials into the water resources causes dangerous effects on human beings and the environment [1]. There are various types

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of water and wastewater treatment processes that can be used for the removal of the colored compounds such as adsorption [2], ion exchange [3], membrane [4], chemical oxidation [5], electrochemical process [6], and biological treatment processes [7] Physical and chemical techniques have limitations such as excess sludge production, high cost, low efficiency, and toxic residual materials due to incomplete mineralization. In contrast, biological treatment is an economical, efficient, and environmentally friendly method [8]. Many colored substances are reduced easily in anaerobic conditions. The Azo reduction mechanism is proposed by Gingell and Walker [9] which consists of two steps (Eqs. (1) and (2)).

$$2e^- + 2H^+ + \left(R - N = N - R^1\right) \rightarrow \left(R - NR - NR - R^1\right) \quad (1)$$

$$2e^- + 2H^+ + \left(R - NR - NR - R^1\right) \rightarrow \left(R - NH_2\right) + \left(R^1 - NH_2\right) \quad (2)$$

The compounds produced in reaction 1 are colorless and unstable substances, and the azo bond is modified again by oxidation. The main problem of the reduction of the dye is the formation of aromatic amines that do not degrade under anaerobic conditions and are resistant. On the other hand, aerobic processes can degrade organic materials such as aromatic amines [10].

RB5 (Fig. 1) dye has azo chromophores that are chemically stable and this dye is toxic because of the presence of toxic amines in the effluent, so its Biodegradation is of great importance. Azo dyes are no longer applied only for textiles, paper, plastics, paints, inks, and food processing, but rather serve as key components in the high-tech plan such as optical data storage, display devices, dye-sensitized solar cells, light-emitting diodes [11,12].

Hence, the biological anaerobic/aerobic treatment is the most effective and economic approach for the removal of azo dyes. These materials degrade in two steps. In the first step, the azo bond is broken, and the aromatic amines are produced under the anaerobic process that these intermediates are more toxic than the primary dye. In the second stage, aromatic amines are degraded under aerobic conditions [13].

In a study performed by Sponza and Isik, an anaerobic-aerobic system has been used to remove RB5 with the concentration of 100 mg/L. Using an Up-Flow Anaerobic Sludge Blanket (UASB)/ Completely Stirred Tank Reactors (CSTR) sequential reactor system, 98% of the color and 96% of COD were removed. Glucose was used as a carbon source in this study [14]. *You* and *Teng* were investigated the color removal and COD reduction efficiency using a sequential anaerobic and aerobic membrane bioreactor. Approximately 92.3% and 5.2% of COD and 74.6% and 9.1% of true color were removed in this system, respectively [15]. *Karatas et al.* removed RB5 under batch anaerobic conditions by mixed microbial culture. The color removal efficiency was obtained 93.4% for RB5 with an initial concentration of 2400 mg/L [16]. *Silva et al.* used the anaerobic-aerobic system to treat 200 mg/L of RB5 and 1000 mg/L of COD, that ethanol was a carbon source. This system consisted of UASB and aerobic SBR (sequencing batch reactor). In this study, the removal efficiency of RB5, 75%, and COD reduction efficiency was 85% [13].

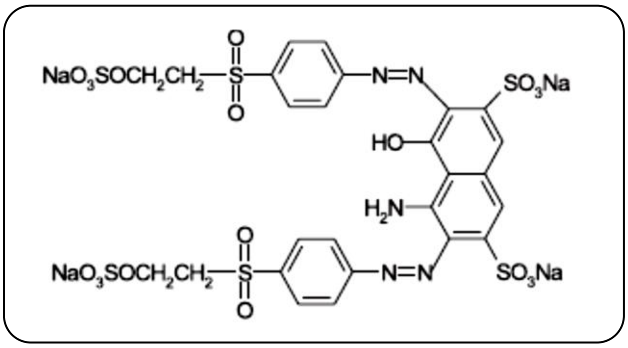

Fig. 1: Chemical structure of RB5.

The use of media in wastewater treatment technologies increases biomass production and high biofilm growth on them, and these systems have the advantages of attached and suspended growth methods [17]. Media provide better conditions for the transfer of the substrate to biofilm [18].

In this study, a continuous system with aerobic and anaerobic reactors using media as attached growth was used to remove RB5 and COD in the wastewater. The effect of operating conditions (hydraulic residence time, temperature, and filling ratio) on color removal and COD reduction efficiency were investigated in these two systems.

EXPERIMENTAL SECTION

Experimental system

The laboratory-scale wastewater treatment system consisted anaerobic digester followed by the Moving Bed

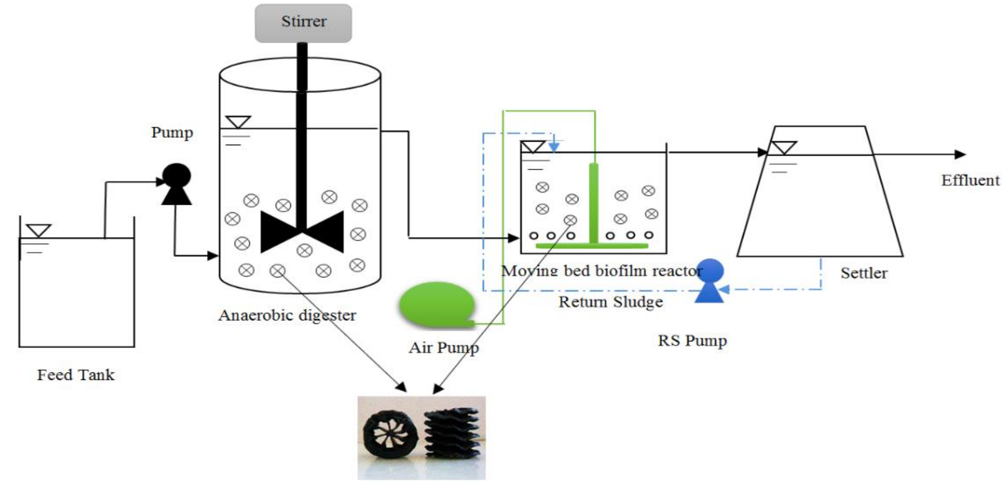

Fig. 2: Schematic diagram of the anaerobic/ aerobic systems.

Biofilm Reactor (MBBR) and settling tank with activated sludge return line as Integrated Fixed-Film Activated Sludge (IFAS), which was fed continuously. The effective volume, height, and internal diameter of the anaerobic digester are 10 L, 27 cm, and 6 mm, respectively. The Oxidation-Reduction Potential (ORP) of the anaerobic digester was -200mv. The effective volume of MBBR and settling tank are 2.5 L (20cm×20cm×11cm) and 2.6 L (23cm×12.5cm×12.5cm). A schematic of the anaerobic digester and IFAS in this study is shown in Fig. 2. Both reactors were packed by media (2H-BCN018 kk3) with high-density polyethylene (specific surface area of 339 m^2/m^3).

Synthetic dyeing wastewater

The studied color is RB5 (55% purity, Sigma-Aldrich, USA) . Glucose was chosen as the electron donor and carbon source. The basal medium consisted of (mg/L): NH_4Cl (280), K_2HPO_4 (250), $MgSO_4.7H_2O$ (100), and $CaCl_2$. $2H_2O$ (10) and 1 mL/L of trace elements containing (mg/L): H_3BO_3 (50), $FeCl_2.4H_2O$ (2000), $ZnCl_2$ (50), $MnCl_2.4H_2O$(500), $CuCl_2.2H_2O$ (38), $(NH_4)_6$ $Mo_7O_{24}.4H_2O$ (50), $AlCl_3.6H_2O$ (90), $CoCl2.6H_2O$ (2000), $NiCl_2.6H_2O$ (92), Na_2 $SeO_3{\cdot}5H_2O$ (162), EDTA (1000) and HCl 36% [19]. To keep the pH around 7.0, the wastewater was buffered with sodium bicarbonate ($NaHCO_3$). The synthetic wastewater was prepared daily.

Seed inoculum

The seed culture obtained from the return sludge of municipal wastewater treatment plant (Ghaemshahr, Iran) with initially Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS) concentrations of 5854.14 and 4904.76 mg/L respectively. The facultative microorganisms, which can grow under anaerobic and aerobic conditions were applied in this work.

Operating conditions

The system was fed with synthetic wastewater without dye for biofilm formation on media for 90 days. The Anaerobic digester was started up high HRT (2.5 days) for permitting biofilm acclimation to the synthetic wastewater. COD gradually increased from 500 to 3000 mg/L during this period. The biofilm on the media surface is shown in Fig.3. After the formation of suitable biofilm on the media, the dye was added to the system at a concentration of 50-200 mg/L.

Analytical methods

Samples taken in each experiment from the anaerobic digester, the MBBR, and the settling tank were centrifuged for 20 minutes at 3000 rpm to obtain a clear supernatant. The absorbance of RB5 was read at the wavelength (λ) whose absorbance is maximum (598 nm). RB5 concentrations calculated from the calibration curves of absorbance (ABS598 nm = 0.0195 RB5 dye + 0.018; R2 = 0.9949) versus dye concentration. RB5 concentration has been determined using UV–Vis spectrophotometer (PG Instruments, England). The concentration of COD was measured using HACH testing kits (DR5000, Germany).

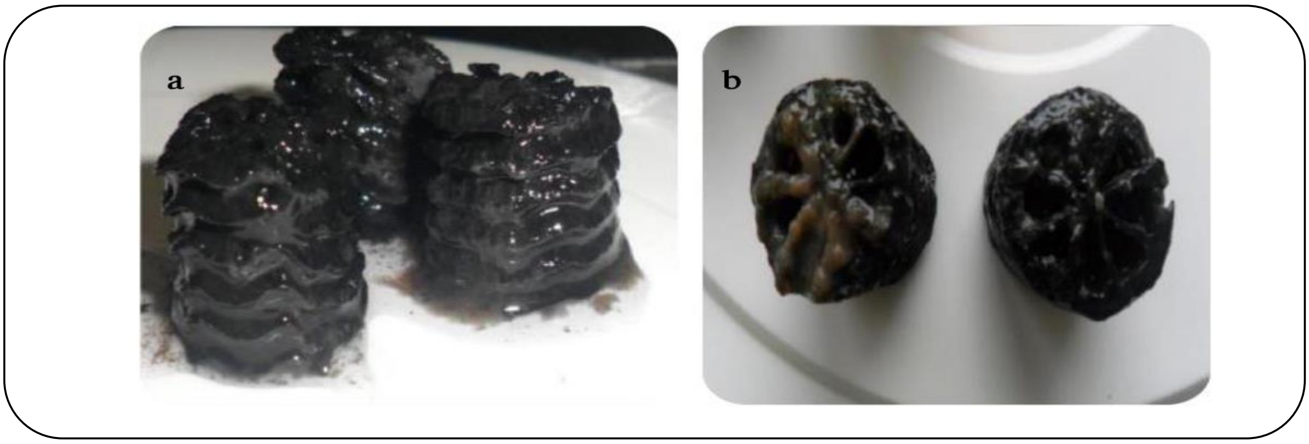

Fig. 3: Biofilm formed in the aerobic and anaerobic systems (a, b).

MLSS and MLVSS were carried according to the Standard Methods [20].

Biofilm sample preparation for SEM

Biofilm samples were washed with NaCl (0.9%) three times. The samples were fixed with glutaraldehyde (2.5%) for 2-4 h and then washed in 0.1 M phosphate buffer three times. The specimens were dehydrated using 30, 50, 70, 90, and 100% graded ethanol. For SEM analyses, dried specimens were coated with gold.

RESULTS AND DISCUSSION

Effect of HRT on COD reduction and color removal

The effect HRT on the removal of color and reduction of COD in the anaerobic/aerobic system is shown in Fig. 4. By increasing HRT from 4.6 hours to 69 hours, the reduction efficiency of COD and the removal efficiency of color increased from 70% to 97%, from 53% to 82.6%, respectively. According to the results, there is a direct correlation between HRT and color removal efficiency. By increasing HRT, the activity of azo reductase that is responsible for cleavage the azo bond, increases [8]. But in high HRT, the color removal efficiency has not changed much due to the complete cleavage of the azo bond of RB5 in very short HRT [14]. The effect of HRT on the removal of color and the reduction COD in the anaerobic and aerobic systems is shown in Fig. 5 and 6, respectively. As can be seen in Fig. 5, most of the color is removed in the anaerobic unit. In contrast, the aerobic unit's contribution to color removal is negligible. The results of this study are similar to the research by *Mohanty et al.* [21] and *You et al.* [22]. In this study, the color removal efficiency in HRT 2.5 days in anaerobic and aerobic units was 79.5% and 11%, respectively. As can be observed in Fig. 6, increasing HRT causes COD reduction efficiency enhancement. The reason for this occurrence is related to biofilm growth and decreasing of F/M ratio.

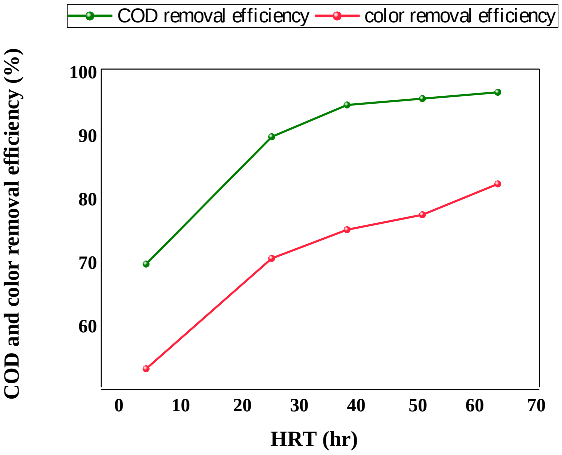

***Fig. 4:** The effect of HRT on color and COD reduction efficiency in the anaerobic/aerobic system (50% filling ratio; initial RB5 concentration 50 mg/L; Temperature 23°C)*

The absorption spectrum of the influent and effluent of the synthetic wastewaters containing RB5 in the influent has been seen in the wavelength of 598 nm. The disappearance of the absorption peak in the 598 nm region in the anaerobic and aerobic effluents shows that the decolorization process is effective.

As previously explained, Fig. 7 shows that more color reduction has occurred in the anaerobic unit and the aerobic unit performance is negligible. It is observed that at the effluent of the anaerobic reactor a peak has been formed

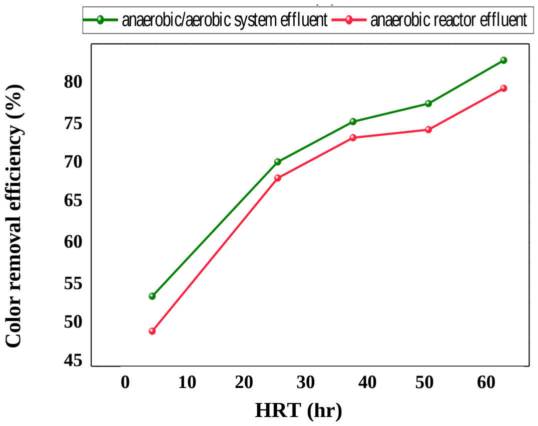

Fig. 5: The effect of HRT on color removal efficiency in the anaerobic/aerobic system and anaerobic unit (50% filling ratio; initial RB5 concentration 50 mg/L; Temperature 23ºC).

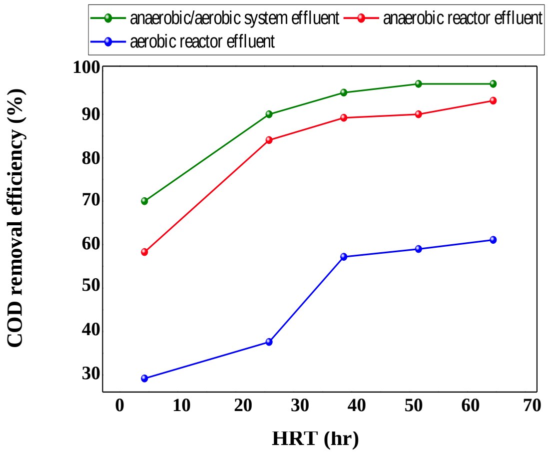

Fig. 6: The effect of HRT on COD removal efficiency in the systems (50% filling ratio; initial RB5 concentration 50 mg/L; Temperature 23ºC)).

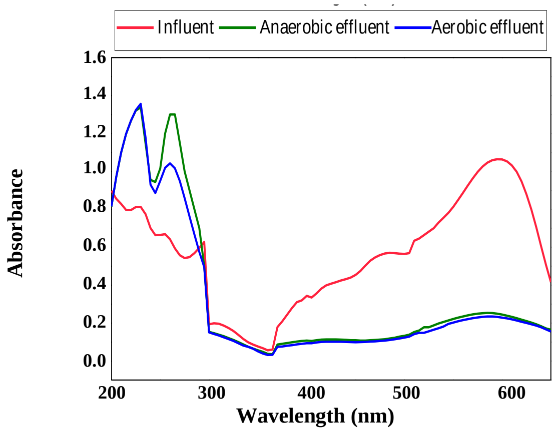

Fig. 7: Absorbance fluctuation of influent and effluent containing RB5 from the anaerobic and aerobic processes (initial RB5 concentration 50 mg/L).

in the range of 260 nm, which is associated with the decomposing products of the RB5 in an anaerobic unit. In the anaerobic process, the azo bond is broken down, and aromatic amines are produced which the aromatic amines are more toxic than the dye. Reducing absorption in the aerobic unit may indicate the mineralization of aromatic amines.

Effect of temperature in anaerobic digester on COD reduction and color removal

The temperature affects the growth rate, biomass yield, and reaction mechanism [23]. The degradation of the azo dye is a type of biological process based on the optimal temperature, that's why temperature is an effective factor in decolorization [24]. It has been reported that the decolorization efficiency increases to the optimal temperature [23]. In the study, the effect of temperature of 23, 28, and 35oC on the efficiency of COD reduction and color removal is shown in Fig. 8. With the increasing temperature of 23°C to 35°C, the COD reduction efficiency varies from 97% to 100%, and the color removal efficiency changes from 82.9% to 87.7%. The reason for the results is the increase in respiration, substrate metabolism, and microorganism activity at high temperatures [24]. Such changes have also been observed in the study of others [24, 25].

Effect of filling ratio in anaerobic digester and IFAS on COD reduction and color removal

The effect of the filling ratio in anaerobic digester on the color removal efficiency is shown in Fig. 9. As can be seen from the figure, by increasing the filling ratio from15% to 50%, the color removal efficiency of the anaerobic digester increased from 61.5% to 79%. As shown in Fig. 9, the effect of different filling ratios on the COD reduction efficiency is negligible. By increasing the filling ratio from 15% to 50%, the COD reduction efficiency in anaerobic digester has changed from 92.2% to 95.5%. In this work, indeed the effect of filling ratio in IFAS on the efficiency of COD reduction was investigated. By changing the filling ratio from 30% to 70%, the reduction efficiency of COD from IFAS has increased from 40% to 61.1%. The reason for phenomena is related to increasing of the specific surface between the biomass and the media.

Effect of initial dye concentration on COD reduction and color removal

As explained in Fig. 10, with an increase in the initial dye concentration from 50 to 200 mg/L, color removal efficiency is reduced from 88.5% to 75.4%. High

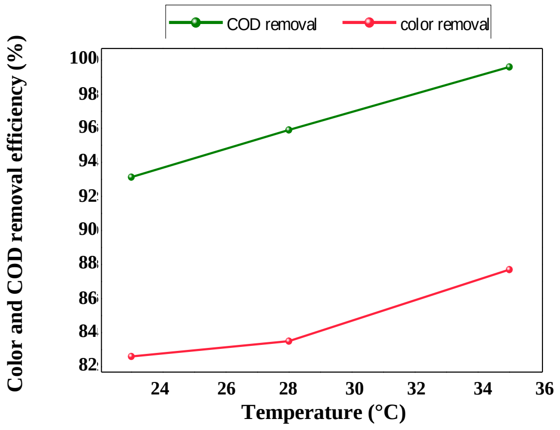

Fig. 8: The influence of temperature on COD reduction and color removal efficiency in systems (50% filling ratio; HRT 2.5d; initial RB5 concentration 50 mg/L).

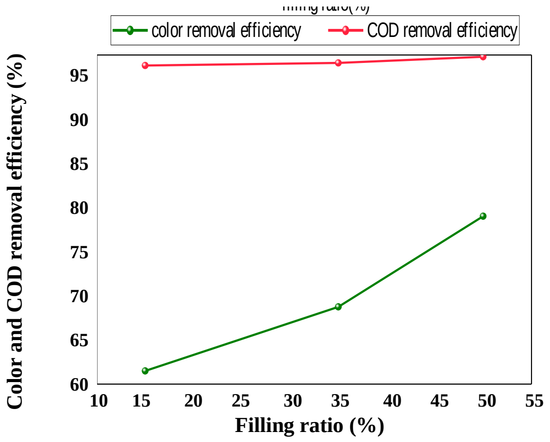

Fig. 9: The effect of filling ratio in anaerobic digester on COD reduction and color removal efficiency in systems (HRT 2.5d; initial RB5 concentration 50 mg/L; Temperature 23ºC).

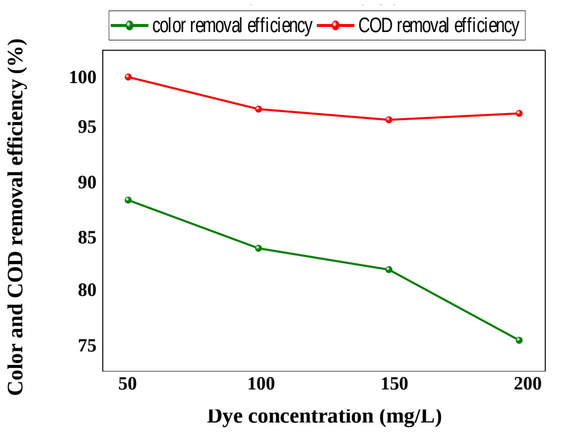

Fig. 10: The effect of initial dye concentration on COD reduction and color removal efficiency in the system (50% filling ratio; Temperature 35ºC; 2.5d HRT).

concentrations of dye may influence microbial growth and decrease the rate of decolorization and microbial dye degradation. The reason for this may be the blockage of active sites of azo reductase by dye molecules and their toxic effects. These results are similar to research by *Bonkadarpour et al.* [26] and Johnstrup et al. [27]. By increasing the color concentration from 50 to 200 mg/L, COD reduction efficiency is increased from 100% to 97%. The negligible decrease of COD reduction due to COD is caused by glucose and increasing color concentration.

Bio-faces of anaerobic and aerobic systems

As seen in Fig.11, the microbial population and the microbial diversity of attached biomass is more than the suspended biomass. Because connection the microbes into the bed causes the biofilm to form and can't be easily deleted from the system and attached biofilm creates micro-colonies.

With this advantage, they can feed on the food in the surroundings and as a result, they will grow better. But in suspended biomass, due to lack of access to the support, they are easily removed from the system and have less chance of consuming food and as a result, their numbers are less. This could be one of the reasons for the increased efficiency of the attached growth system in the anaerobic reactor [11]. SEM images show a discrepancy between the types of microorganisms present in aerobic and anaerobic reactors in terms of shape, which may be another reason for the difference in the efficiency of the two systems in removing RB5 [25]. There is a lot of food in the anaerobic reactor (glucose), so we have more microbial mass but with the activity of germs in the anaerobic reactor, food is also reduced and with the reduction of the color may produce toxic metabolites. So, after the transfer of wastewater to the aerobic reactor, the microbial population is not sufficiently formed. Due to lack of food and the presence of toxic compounds, it may inhibit the growth and activity of aerobic microbes, while it is expected to be in good condition and sufficient food, the microbial population in aerobic conditions is greater than in anaerobic conditions [11].

Decolorization kinetics study

In this research, the Stover- Kincannon kinetic model was used to experimental results from the sequential anaerobic digester, and IFAS for degrading of RB5 and kinetic constant for color removal and COD reduction was obtained.

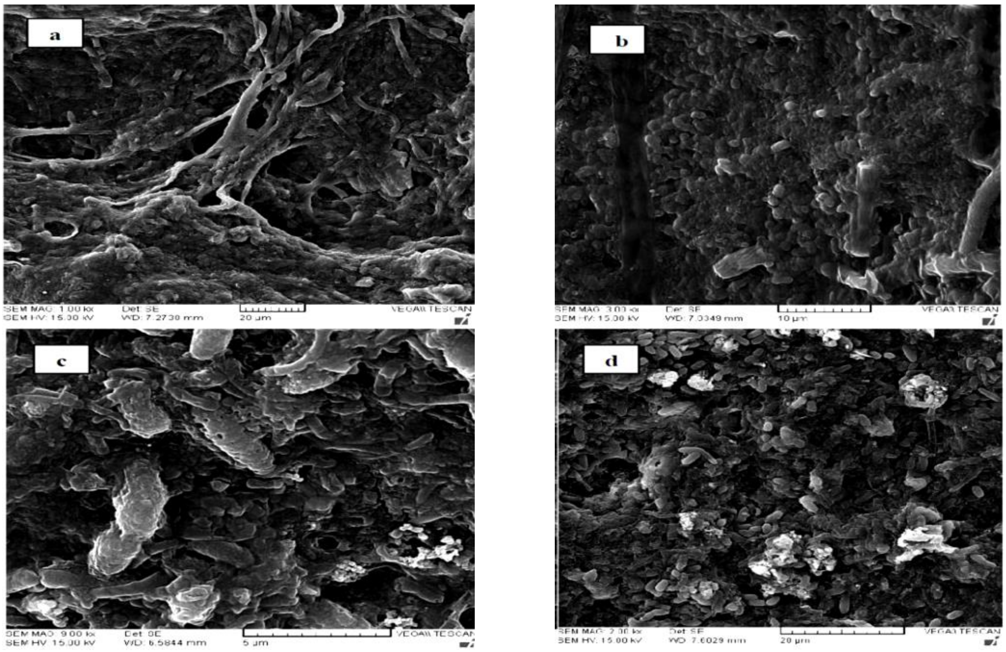

Fig. 11: SEM photographs of (a) suspended and (b) attached biomass in the aerobic unit and (c) suspended and (d) attached biomass in anaerobic unit

Eq. (3) shows the Stover- Kincannon kinetic model of COD reduction and color removal.

$$\frac{dS}{dt}=\frac{U_{mas}\left(QSo/V\right)}{K_B+\left(QSo/V\right)} \tag{3}$$

Where dS/dt is the substrate removal rate (g/L.d), S is the substrate concentration in the reactor (g/L.d), U_{max} is the maximum substrate removal rate constant (g/L.d) and K_B is a saturation value constant (g/L.d). A mass balance of substrate into and out of the volume can be made as follow:

$$\frac{dS}{dt}=\frac{Q}{V}\left(So-Se\right) \tag{4}$$

Using Equation 3 and 4, the following relationship is obtained:

$$\frac{Q}{V}\left(So-Se\right)=\frac{U_{mas}\left(QSo/V\right)}{K_B+\left(QSo/V\right)} \tag{5}$$

Linearization of Eq. (5) gives the relationship [28]:

$$\left(\frac{dS}{dt}\right)^{-1}=\frac{V}{Q\left(So-Se\right)}=\frac{K_B}{U_{max}}\times\frac{V}{QSo}+\frac{1}{U_{max}} \tag{6}$$

Fig.12 and Fig.13 indicate the plot of COD and color loading $V/(QS_0)$ versus $V/[Q(S_0 \_ S_e)]$ COD reduction and color removal rate, respectively. From the slope and intercept of a best-fit line (R^2 = 0.9973), kinetic constants for COD reduction were determined as Umax = 11.31 g/ (l day) and K_B = 11.31 g/ (l day), respectively. Similarly, when the model is applied to color removal, the coefficients are obtained as Umax = 0.15 g / (l day), K_B =0.165 g / (l day) with high regression coefficient (R^2 = 0.9998). According to the obtained data, RB5 decolorization system followed the Stover – Kincannon model, and effluent color/COD concentration can be predicted by using Eq. (7).

$$Se=So-\frac{U_{mas}So}{K_B+\left(\frac{QSo}{V}\right)} \tag{7}$$

Monod model

The Monod model is described as equation (8):

$$\frac{dS}{dt}=\frac{Q}{V}\left(So-Se\right)=\frac{KXSe}{Kx+Se} \tag{8}$$

X is the concentration of Volatile Suspended Solid (VSS) in the reactor. Linearization of Eq. (8) provides Eq, (9):

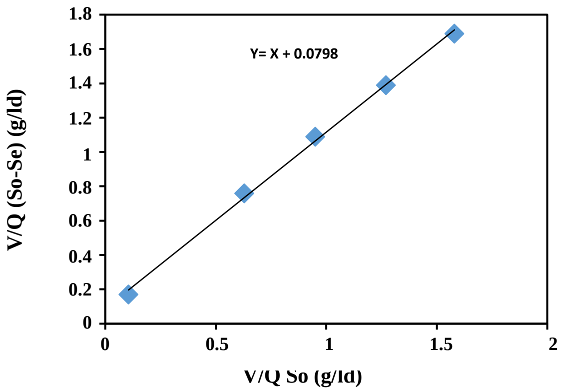

Fig. 12: Stover–Kincannon model plot for COD reduction.

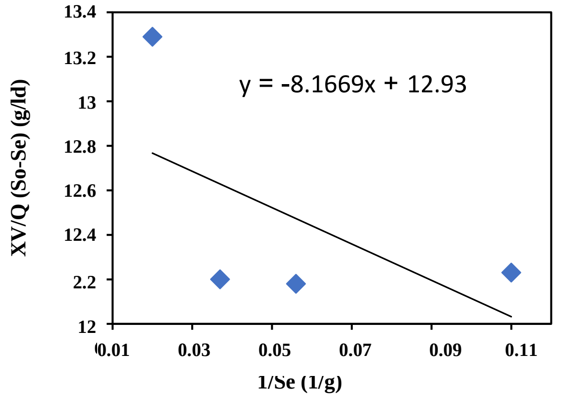

Fig. 14: Monod model plot for color removal.

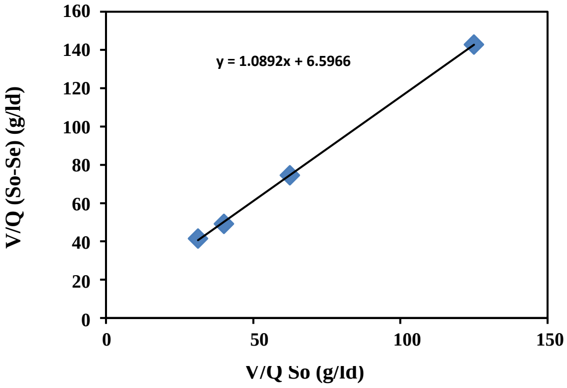

Fig. 13: Stover–Kincannon model plot for color removal.

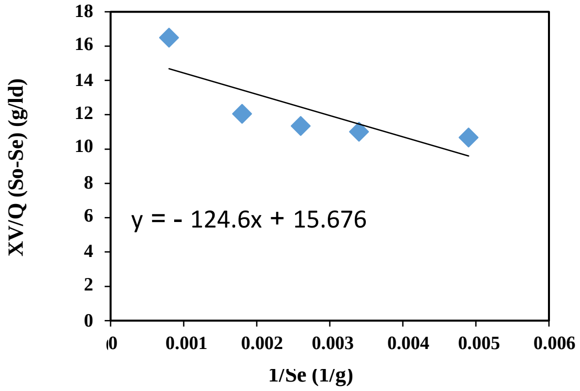

Fig. 15: Monod model plot for COD reduction.

$$\frac{XV}{Q(So-Se)}=\frac{Ks}{K}\frac{1}{Se}+\frac{1}{K} \tag{9}$$

Based on equation (9), by plotting XV/ Q(Si-Se) against 1/Se for color removal and COD reduction, kinetic constants were determined (Fig. 14, Fig. 15). From the slope in Fig. 14. Kinetic constants for color removal were determined as K = 0.077 g/ (l day) and Ks = -0.63 g / (l day), respectively. The regression coefficient (R^2) in the figure predicted 0.342 which is a lower Kincannon-Stover model. Similarly, when the model is applied to COD reduction (Fig. 15) kinetic constants determined as K = .063 g/ (l day) and Ks = -79.1 g/ (l day), with low regression coefficient (R^2 = 0. 658). Based on the regression coefficient the Kincannon-Stover model is very best the Monod model for describing the color removal and COD reduction in an anaerobic digester and IFAS sequential reactor system.

CONCLUSIONS

The results of this study indicate that the anaerobic/ aerobic system is effective for the removal of Reactive Black 5 dye. In this research, the parameters of the temperature (35, 28, 23°C), the hydraulic residence time (4.6, 27.6, 41.45, 55.2, 69h), initial concentration of RB5 (50 to 200 mg/L), and filling ratio (15%, 35%, 50%) were investigated. The maximum color removal and COD reduction efficiency at the initial concentration of 50, the temperature of 35°C, and the filling ratio of 50% in the sequential anaerobic/aerobic system was 87% and 100%, respectively. Most degradation of RB5 and COD has occurred in the anaerobic digester. RB5 decolorization in this system followed Stover- Kincannon kinetic well. Kinetic constants and regression coefficient of Stover–Kincannon model for dye and COD were determined as Umax = 0.15 g/(l day), KB = 0.165 g/(l day), R2 =0. 9998 and Umax = 11.31 g /(l day), KB = 11.31 g /(l day), R2 =0.9973 respectively. One of the limitation of this study was the lack of facilities to identify and quantify the types of microorganisms involved in RB5 removal and COD reduction. Therefore, investigating the types of microorganisms affecting dye removal such as bacteria, actinomycetes and fungi may be necessary for the future.

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