Exposure of the static magnetic fields on the microbial growth rate and sludge properties in the complete-mix activated sludge process (a Lab-scale study)

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Abstract

In this study, the effect of static magnetic fields (SMFs) on improving the performance of the activated sludge to enhance the microbial growth rate and improve sludge settling characteristics in the real operation conditions has been investigated. The effect of SMFs (15 mT), hydraulic retention time, the sludge age, the aeration time on mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), α-factor, and pH in the complete-mix activated sludge (CMAS) process during 30 days of the operation were evaluated. There were not any differences between the mean concentration of MLSS in the case and control samples, however, the mean concentration of MLVSS in the case (1463.4±419.2 mg/L) was more than the control samples (1244.1±295.5 mg/L). Changes of the concentration of MLVSS over time, follow the first and second-order reaction with and without exposure of SMFs, respectively. However, the slope of the line in the case samples was 6.255 higher than the control samples. The mean of α-factor in the case samples increased by -0.001 higher than the control samples. Changes in pH in both groups of the reactors were not observed. 15 mT intensity of SMFs can promoted oxygen transfer to the biomass and, increase the concentration of MLVSS in the aeration reactor of CMAS. SMFs have a potential to be consider as an alternative method to stimulate the microbial growth rate in the aeration reactor and produce bioflocs with higher density in CMAS.

Key words: Activated sludge; Magnetic fields; Aeration reactors; α-factor; Mixed liquor volatile suspended solids; Bioflocs
1. Introduction

Activated sludge (AS) process is the most well-known and worldwide technic for the wastewater treatment. This process has been commonly applied to remove pollutants by a microbial community of active biomass (Fang et al., 2018, Xia et al., 2018). This type of biological wastewater treatment is used for the treatment of industrial and domestic wastewater. The main goal of this process is to remove the organic matter from wastewater (Yuan et al., 2015). The efficiency of AS is 85-95% for the removal of chemical oxygen demand (COD), depending on the characteristic of the wastewater, design criteria, environmental factors, and the operation conditions (Metcalf et al., 2014).

There are various modifications of AS process for the wastewater treatment, such as the complete-mix activated sludge (CMAS) process. In CMAS, the suspended growth of the variety of the microorganisms has the most critical component in the aeration reactors and the operation of a system (Metcalf et al., 2014).

The aerobic methods which are used for any type of wastewater have disadvantages. One of them is bulking of the sludge in the second clarifier, due to the overgrowth of the filamentous bacteria (4). Rising costs of sludge disposal and, the risk of the presence of toxic materials in unsanitary disposal of solids in the environment, are the other disadvantages of this procedure (Pang et al., 2020).

Nowadays, the improvement in the AS process in terms of the higher sludge settling by a combination of other technics is the target of many studies (Hreiz et al., 2015). An important point in the operation of AS process is the higher gravity settling of the bioflocs in the secondary clarifiers. Higher removal of the flocs in the second clarifier basins has a basic effect on the raise of the sludge in the quantity of the effluent and, in the other processes in the continuous wastewater treatment steps (Daigger et al., 2018).

The interaction of magnetic fields (MFs) and the growth rate of microorganisms is an interesting discussion. Studies, focused on the positive effect of the MFs on stimulating the growth rate of microorganisms in biological wastewater treatment (Tomska and Wolny, 2008). A classification of the MFs is according to the frequency of the electric current. If the frequency of the electric current is 0 Hz or no changes observed in intensity overtime, the generated MFs are called, static magnetic fields (SMFs) and, when the electric current has a frequency more than 0 Hz, the generated MFs are called dynamic MFs (Zhang et al., 2017).

MFs are an alternative method for the treatment of wastewater (Zieliński et al., 2017a). Some of the changes happened by the exposure of SMFs on the liquid such as changes in the structure of molecules, electric potential and, the polarization of the particles (Krzemieniewski et al., 2004). Some of the fundamental properties of the liquids are viscosity, density and, tension of the surface. These components are affected when exposed to MFs are present in the surrounding (Rusanowska et al., 2017). Łebkowska et al. summarized the effect of SMFs on the living things in 9 categories. Changes in the properties of water, pH, higher rate of coagulation and, settling of solids are certain of them (Łebkowska et al., 2018).

Wastewater could be treated by MFs in terms of removal of the organic materials, suspended solids, and turbidity (Łebkowska et al., 2011). Improvement of the biomass and settling of
sludge has been studied by many researchers when the application of the MFs in AS is considered (Zaidi et al., 2016, Nur Syamimi et al., 2014). In the process of the wastewater treatment, more growth of microorganisms is a critical point to degradation of the organic compounds. In other words, the higher growth rate of microorganisms is equal to the higher consumption of the organic materials (Zieliński et al., 2017a). With the application of external MFs or magnetic powder in the biological treatment process of the wastewater, the efficiency of the generation of sludge with higher density, have happened (Zaidi et al., 2014). 550 mT intensity of MFs reduces 60-80% of the biological oxygen demand (BOD) of the landfill leachate, for example (Othman et al., 2009).

Based on available articles, there is a few scientific reports on the discussion of the biological and chemical components changes in the real CMAS process with the application of SMFs. In this case-control study, the impact of SMFs on temperature, pH, α-factor (rate of dissolved oxygen transfer rate into the biomass), the concentration of mixed liquor suspended solids (MLSS) and, mixed liquor volatile suspended solids (MLVSS) of CMAS in the aeration reactors during the 30 days operation of the reactors in Lab-scale in order to estimated the role of SMFs on the optimization of oxygen transfer in the aeration reactor have been considered. Moreover, stimulate the microbial biomass growth rate and, generate the sludge flocs with higher density and, increasing the rate of the efficiency for removing the sludge flocs in the clarifier reactor were the other main target of this research.

2. Materials and methods

2.1. Source of the wastewater sampling

All samples of this study were taken from the effluent of the primary settling basin of CMAS process in Sanandaj's WWTPs (a city located in the west of Iran) during the summer of 2022 and sent to a small feeding container (40 cm×100 cm×100 cm = 40 L) for distribution among the reactors.

2.2. Experimental setup

Two series reactors consisted in course of experimental setup devices (feeding and distribution containers, the aeration and settling devices, air and peristaltic pumps, and returing sludge pipes). These devices and other accessories such as DC power, valves, and a solenoid are illustrated in Fig. 1.
It must be mentioned that, the design parameters of the Lab-scale reactors, were according to the real wastewater treatment plants and effluent guideline criteria (Metcalf et al., 2014). Therefore, the rate of flow (mL/min) into the aeration reactors when microbial retention time was 5 days can be calculate using the following equation:

\[ \frac{S_0 - S}{1 + kd\theta_c} \]

\[ XV = YQ\theta_c A = \pi r^2 (1) \]

\[ S_0 \] is MLVSS in the aeration tank (mg/L), \( V \) is the volume of reactors (L), \( Y \) is a degradable portion of the organic materials (kg VSS/kg COD), \( Q \) is the flow of wastewater (mL/min), \( S_0 \) is primary BOD5 (mg/L), \( S \) is the effluent BOD5 (mg/L), \( K_d \) is a kinetic coefficient which is equal to 0.06 d\(^{-1}\) and, \( \theta_c \) is the microbial retention time (d) (Metcalf et al., 2014).

Hydraulic retention time (HRT) estimated by using the following equation:

\[ \text{HRT(min)} = \theta_c = VT / Q \]

2.3. Type of the aeration system

Aeration and agitation of MLSS in the aeration reactors are carried out by an electric ambient air pump. The diffuser aeration has two main benefits in this study. Provided oxygen between 2 and 3 mg/L that was regulated by airflow meter (Yokogawa RAGL 41 Laboratory Rotameter) during the operation periods (30 days) and, mix the contents of the aeration reactors with uniform distribution of air by stone air diffusers (0.6±0.1 L/min) to obtain complete-mix conditions. DO concentration in the aeration reactors was measured by DO meter (Hach HQ30D), daily.

The rate of DO concentration in the aeration basin of AS must be 1.5–4 mg/L (Metcalf et al., 2014).

2.4. SMFs generation
DC power (DAZHENG PS-305D) was used for SMFs generation on the Lab-scale. The number of turns of the coil was 750 rounds (0.5 mm thickness) in three rows and they were wrapped around a sheet of galvanized iron in order to the ever-increasing intensity of MFs. Based on the measurement by Tesla meter (GM-511 Polytronic), the intensity of generated MFs was 15 mT.

The field intensity of 15 millitesla was chosen because it is a intensity that can be easily produced in the laboratory using a DC power device, and it has been determined in past studies that this field intensity can stimulate the growth rate of the microorganisms.

2.5. Seeding the aeration reactors

At the beginning of the processes, seeding of the aeration reactors as initial inoculum by MLSS (1750±100 mg/L) from the effluent of the primary settling tank of the WWTPs was done. For this reason, a half volume of the aeration reactors (1250 mL) was filled with MLSS. We have to seed the reactors because, even after 30 days of the starting the reactors without seeding, the mean concentration of MLSS in the case and control samples were only, 176.8±118.9 and 120.3±73.7 (mg/L), respectively.

It must be mentioned that, the mean concentrations of MLSS was recommended for the CMAS processes are from 2500 to 6000 (mg/L) (Metcalf et al., 2014).

2.6. Design of experiment

A basic parameter that has the main effect on the CMAS process is the number of microorganisms in the bioreactor or the aeration reactor. Therefore, in terms of evaluating the efficiency of the processes, the concentration of MLVSS in the aeration reactors is the key factor. The time required to reaches this low level concentration (at least 2500 mg/L) is called start-up time and, this takes 7 to 28 days (Metcalf et al., 2014). The flow rate of the returning sludge from a secondary clarifier or settling reactor has the main effect on MLVSS concentration and performance the aeration reactors and, is calculated as the following equation:

$$100 \frac{Q_r}{Q} = 100[(100/p_w \times SVI) − 1] \quad (3)$$

100 to 150 percent of average flow is suggested to be returned to the aeration reactors in AS processes (Metcalf et al., 2014).

In Table 1. Design parameters of the CMAS reactors ($\Theta_c$, HRT, $Q$, aeration flow rate, and 100 $Q_r/Q$) are illustrated.

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Theta_c$</td>
<td>3</td>
<td>day</td>
</tr>
<tr>
<td>HRT</td>
<td>297</td>
<td>min</td>
</tr>
<tr>
<td>Q</td>
<td>6.7</td>
<td>mL/min</td>
</tr>
<tr>
<td>Aeration flow rate</td>
<td>0.6 ± 0.1</td>
<td>L/min</td>
</tr>
<tr>
<td>100 $Q_r/Q$</td>
<td>%100</td>
<td>-</td>
</tr>
</tbody>
</table>
In this study, 100% of sludge was returned to the aeration reactors by peristaltic pumps. In Table 2. The mean of the fundamental parameters in the feeding container and, the case and control samples are shown.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of samples</th>
<th>Feeding container</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>93</td>
<td>22.86 ± 0.80</td>
<td>29.75 ± 2.02</td>
<td>23.74 ± 0.70</td>
</tr>
<tr>
<td>pH</td>
<td>93</td>
<td>7.69 ± 0.25</td>
<td>7.80 ± 0.23</td>
<td>7.73 ± 0.24</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>93</td>
<td>0.38 ± 0.15</td>
<td>2.6 ± 0.32</td>
<td>2.6 ± 0.31</td>
</tr>
<tr>
<td>MLSS (mg/L) + seeding</td>
<td>62</td>
<td>-</td>
<td>2260.1 ± 296.0</td>
<td>2148.8 ± 235.6</td>
</tr>
<tr>
<td>MLVSS (mg/L) + seeding</td>
<td>62</td>
<td>-</td>
<td>1463.4 ± 419.2</td>
<td>1244.1 ± 295.5</td>
</tr>
<tr>
<td>MLSS (mg/L) + no seeding</td>
<td>62</td>
<td>-</td>
<td>176.8 ± 118.9</td>
<td>120.3 ± 73.7</td>
</tr>
<tr>
<td>MLVSS (mg/L) + no seeding</td>
<td>62</td>
<td>-</td>
<td>105.7 ± 70.8</td>
<td>77.6 ± 55.1</td>
</tr>
</tbody>
</table>

### 3. Results and discussion

#### 3.1. Change of the temperature in the reactors

It has been proven that the optimum temperature is a stimulating factor on the microbial growth rate, generally (Sigee, 2005). Some of the researchers reported that the effect of temperature (in the low range of changes, especially) on the category of the kind and community of the bacteria is not noticeable. The community of microorganisms can be affected by other main factors, such as pH, total phosphorus concentration, and BOD loading rate (Zhang et al., 2019, Liu et al., 2018b).

The differences between the temperature (°C) of the wastewater in the feeding container, the case 1 (after exposure of the SMFs for one hour), case 2 (without exposure of the SMFs) and, the control samples during the time of the operation is demonstrated in Fig. 2.

---

Fig. 2 Relationship between temperature (°C) and time of operation (d) in the feeding container and the case and control samples

There was a statistical difference \( p < 0.05 \) between the mean temperature of MLSS in the feeding container (22.86±0.80 °C) and, the case 1 (29.75±2.02 °C) samples, between the case 1 and, control (23.74±0.70 °C) samples and, case 2 (24.37±0.60 °C). However, there weren't
any differences between the mean of the feeding container temperature with case 2 and control samples.

In the operation of the biological wastewater treatments processes, the maintenance of the temperature in the recommended ranges is the basic challenge (Obaid et al., 2015). Furthermore, the microbial growth rate and degradation of the organic matters caused generation of heat (Gostomski et al., 1997). However, as the pattern of flow in our study was continuous, the mean temperature raised in control samples was only 0.88 °C more than the feeding container.

Due to the passage of electricity through the solenoid and the generation of internal resistance, heat generation is inevitable. This mechanism is known as Joule heating (Agarwal et al., 2014). Since the only difference between three groups of samples was the exposure of the SMFs, it can be concluded that the application of SMFs causes the higher temperature in the case 1 samples (6.89 °C), (6.04 °C) and, (6.01 °C) more than case 2 and, control samples, respectively. It seems that this increase in the temperature due to the effect of SMFs in case 1 (one hour of 24 hours) in the aeration reactors is a weak stimulus for the growth rate of microorganisms. However, increasing the reactor temperature can affect the dissolution of dissolved oxygen in bioreactors, too.

Finally, it seems that the interaction between the SMFs in increasing the liquid temperature of the aeration reactors in the short period of time and, the continuous flow pattern and keeping the concentration of the dissolved oxygen between 2-3 mg/L during the operation of the systems in the aeration reactor, has no significant effect on the changes of MLVSS concentration.

3.2. DO concentration in the reactors

Microbial processes can be divided into aerobic and anaerobic processes in wastewater treatment processes. However, aerobic processes are more widely applied than anaerobic. Oxygen is the most important element for the metabolic and growth rate of aerobic microorganisms (Liu et al., 2006). The concentration of oxygen in water is called dissolved oxygen (DO) and, in the biological process, this amount of oxygen due to low solubility has the main role to control of bioprocess (Karimi et al., 2013). It has been proven that DO concentration is a factor that can limit the community and distribution of the microorganisms (Tang et al., 2017). Operation of the aeration reactor higher than 2 mg/L of DO concentration has a positive effect on the flocs formation. Nevertheless, providing this amount of oxygen in the aeration reactors is costly (Wilén and Balmér, 1999, Wilén, 2010).

The mean DO concentration in the case samples was 2.6±0.32 (mg/L) and, in the control, samples was 2.6±0.31(mg/L), during the operation of the reactors, intentionally. This parameter in the feeding container was 0.38±0.15 (mg/L). Based on statistical analysis, there were differences between the DO concentration in the feeding container compared to the case and control samples (p<0.05). However, this difference between the case and control samples was not observed (p=1.000). The concentration of DO (mg/L) in the feeding container and the aeration reactors is demonstrated in Fig. 3.
The main aim of this study was to increase the transfer of DO from the wastewater into the biomass by applying SMFs to make optimal use of oxygen in the aeration reactor at the proposed concentration of DO (2 - 4 mg/L), which is discussed in the following section.

### 3.3. Oxygen mass transfer (OMT)

In the discussion of the operation and design of an aerobic bioreactor, one of the limiting factors is the growth rate of microorganisms, also known as the mass transfer of oxygen (Moucha et al., 2003). Some parameters can affect the rate of oxygen mass transfer (OMT) such as biomass concentration, type and rate of the aeration, hydrodynamic qualification, solids retention time (SRT) and, biofilms conditions (Tang et al., 2015, Garrido-Baserba et al., 2017). OMT and volumetric OMT coefficient ($K_{La}$) are the two most important parameters to evaluate biofilms quality in the wastewater treatment processes (Guimerà et al., 2016, Liu et al., 2018a). In this way, $K_{La}$ and $\alpha$-factor (0.25-0.65 l/h) are used to estimate the OMT quality in the wastewater processes (Pino-Herrera et al., 2018).

In AS process, MLSS (mg/L) is the main propellant agent for controlling $K_{La}$ and $\alpha$-factor. MLSS must be between 10-15 g/L to have a basic efficiency in the transfer of oxygen (Germain et al., 2007, Liu et al., 2018a). The range of $\alpha$-factor on air diffusers is 0.3-0.85 (Baquero-Rodríguez et al., 2019).

$\alpha$-factor can be estimated from the following equation:

$$\alpha = \frac{K_{La \ (process \ water)}}{K_{La \ (clean \ water)}}$$  \hspace{1cm} (4)

$K_{La}$ is a volumetric oxygen transfer coefficient (1/h)

One equation to estimate the $\alpha$-factor is based on the concentration of MLVSS. This is a contrary correlation between the $\alpha$-factor and MLVSS. When MLVSS is 1-12 g/L, equation five is proposed to calculate $\alpha$-factor (Henkel et al., 2011):

$$\alpha\text{-factor} = -0.062 \text{ MLVSS} + 0.972 \pm 0.070$$  \hspace{1cm} (5)
There is a linear relationship between the $\alpha$-factor, and SRT (1-25 days). The relationship between three main parameters (MLSS, $\alpha$-factor and SRT) in the case samples of the aeration reactor is shown in Fig. 4.

Fig. 4 Relationship between MLSS, $\alpha$-factor and SRT in the case samples

As consumption of substrate by the microorganisms increasing SRT during the operation of system, so the rate of $\alpha$-factor increases with increasing sludge age. Equation six is suggested to measure this relationship (Henkel et al., 2011):

$$\alpha\text{-factor} = 0.019 \text{ SRT} + 0.533 \pm 0.093 \quad (6)$$

The correlation between MLSS (mg/L) and $\alpha$-factor is negative linear equation ($y = -0.003x + 0.9269$). Moreover, this correlation with SRT (d) is positive linear equation ($y = x$).

In Fig. 5 the relationship between MLSS, $\alpha$-factor, and SRT in the control samples is demonstrated.

Fig. 5 The relationship between MLSS, $\alpha$-factor and SRT in control samples
The relationship between the MLSS, $\alpha$-factor, and operation time of the system in the control samples was the same with the case samples, too.

The aeration methods in WWTPs is a costly process (about 15 to 49% of total energy consumed by a plan) and, saving energy, especially in the discussion of increasing the efficiency of oxygen to generate higher biomass must be considered (Drewnowski et al., 2019). Nowadays, the operation of the aeration reactors with a low concentration of DO for saving energy suggested (Fan et al., 2017). These methods are not suitable for diffuser aeration, as the agitation of the MLSS, has been supplied by the force of air which entering the depth of the reactors.

By exposure of MFs in the biological processes, the rate of oxygen transfer into the cell of the microorganisms increased. This impact was on the increasing amount of $\alpha$-factor. When the differences between the two figures (4 and 5) were carefully evaluated, it was found that the slope of the regression equation was different, especially, through the application of SMFs in the intensity of 15 mT for one hour, daily. At the beginning of the processes, the amount of $\alpha$-factor in both reactors was -48.63 and over time it decreased. However, the decrease of $\alpha$-factor at the end of one-month operation of the systems in the case reactor was -131.243 (slop of line was -0.003) and, in the control was -107.032 (slop of line was -0.002), therefore $\alpha$-factor at the end of the operation in the case samples -24.211 unit was less than the control samples and, the rate of the slop decreased by -0.001 in the case samples, approximately.

Fan et al. reported that with increasing SRT in the process of AS, the rate of $\alpha$-factor is reduced. In this condition, with increasing sludge age, the size of flocs gets smaller than the start-up of a system (Pendry and Salvatore, 2015).

### 3.4. Changes of pH in the reactors

MFs could increase the pH of water when the intensity of MFs is 0.15 and 0.2 T. This phenomenon is related to the increases the concentration of carbonate in water (Alabdraba et al., 2013). Dissociation of bicarbonate (calcium and magnesium) and, water happened by exposure of MFs. The result of this interaction is hydroxide calcium and magnesium (strong bases) which causes an increase in pH (AbdelHady et al., 2011). As MFs stimulated the rate of the bacterial growth rate in the wastewater, a fall in pH in the solution or the external of bacteria (such as *E.coli*) by decomposition of glucose, happened (Sánchez-Clemente et al., 2018). So it seems that the changes in the wastewater pH are unimpressive when the intensity of SMFs is 15 mT and the flow pattern is continuous.

Based on statistical analysis, no significant changes were observed in the pH of all samples in the aeration reactors ($p>0.05$). Changes in pH value during 30 days of the operation in the feeding container, the case, and, control samples in the aeration reactors are illustrated in Fig. 6.
It must be considered that the intensity of 15 mT of SMFs for one hour in the continuous flow pattern of CMAS, was not in such a way to cause major changes in the pH of the solution between the case and, control samples. According to this, there is no need to adjust the pH of MLSS in the aeration reactors, as the SMFs did not change the pH of the aeration contents more than the recommended range. Moreover, the typical pH for the most biological processes is 6 to 9 (Metcalf et al., 2014).

In 1987, McMeekin et al suggested the gamma hypothesis for the microbial growth rate. Based on this hypothesis which has been later confirmed by other researchers, the effect of environmental conditions on the growth of the microorganisms, has an independent role (Chandler and McMeekin, 1989, Leroi et al., 2012).

3.5. Mixed liquor suspended solids (MLSS)

According to the result of the analysis of the data in terms of evaluation of MLSS in the aeration reactors, the concentration of MLSS (mg/L) in the case and, control samples was not statistically different (p=0.107), as shown in Fig. 7. However, In both reactors, the concentration of MLSS increased, daily.
Removal of BOD in the aeration reactors is based upon first-order kinetic, however, changes of BOD in the reactor with the application of SMFs were similar to second-order kinetic.

At the beginning of the experiments (first 10 days), the changes in MLSS concentration were similar in both reactors. However, MLSS concentration trend changes in the second and third 10 days was growing. This correlation is illustrated in Fig. 8.

The red dots of the places indicate the concentration of MLSS. As it is clear, at a temperature about 30 (°C) and pH of around 7.8, the concentration of MLSS was more than in the other area in the case samples (Fig. 9 on the right). However all the red dots (concentration of MLSS) for control samples accumulated at temperatures under 25.34 (°C) and pH around 7.75 (Fig. 9 on the left).

Zieliński et al. in their research reported that SMFs could improve MLSS in the aeration reactors of AS by about 470±20 mg/L in the case samples (3420±710 mg/L) higher than the control samples (2950±670 mg/L) (Zieliński et al., 2017b).
3.6. Mixed liquor volatile suspended solids (MLVSS)

In AS process, the density of the microorganisms in the aeration reactor can be estimated by measurement of the concentration of MLVSS (mg/L), approximately (Gerardi, 2011).

In Fig. 10 effect of SMFs (15 mT) on MLVSS in the case samples compared to the control samples, was illustrated. Although the changes in MLVSS at the beginning of the process were imperceptible, however, the difference between the mean concentration of MLVSS in two groups of samples increased and becomes statistically significant, over time (p<0.05).

![Graph showing relationship between MLSS and time of operation in the case and control samples]

The correlation between the concentration of MLSS in both groups (the case and control) was not statistically significant. However, in terms of MLVSS, this difference is significant. The SMFs, have the properties to affect on the growth rate of living components of MLSS, not the non-living components. The relationship between the improvement of MLVSS over time in both groups is shown in Fig. 10.

![Graph showing relationship between MLSS and time of operation in the case and control samples]

There was a linear correlation between MLVSS and the time of the operation of the systems in the control samples. However, the pattern of the growth of MLVSS in the case samples was look like a second-order reaction. Therefore, it can be said that the rate of growth in the
average concentration of the microorganisms or MLVSS in the case samples was higher than the control samples.

The correlation between MFs and the growth rate of the microorganisms is not linear. This phenomenon is called "biological window effect" (Kříklavová et al., 2014, Zieliński et al., 2018). It must be mentioned that the changes of MLVSS concentration in first 10 days of the operation in the case and control samples were not observed as shown in Fig. 11.

![Fig. 11 Trend of MLVSS (mg/L) concentration changes during the operation of system in the case and control samples](image)

In the second 10 days of the operation of the system, changes the MLVSS (mg/L) were observed. To determine the trend changes and a correlation between temperature, pH value, and concentration of MLVSS, contour line diagrams have been used. The red dots were gathered around a pH of 7.89 and, temperature was gathered between 28.62 to 32.5 (° C) in the case samples, as shown in Fig. 12 on the right. These trends and correlations of pH and temperature are illustrated for the control samples, too (Fig. 12 on the left). As specified, the accumulation of data was between 22.8 and 25.32 (° C) and pH 7.75, approximately.

![Fig. 12 Contour line diagram for the case (right) and control samples (left)](image)
It seems that, despite the positive and incremental trend changes of MLVSS in the case samples, when compared to the control samples, no changes occurred in the pH of both groups. One main reason for this is related to a pattern of flow. As a continuous flow pattern was used in our study, changes in pH in two groups of samples were not significant.

### 3.7. Flocs density and bonds structures

In Fig. 13 the flocs structure (1000x magnification) at the outlets of the aeration reactors in the case (on the right) and the control samples (on the left) on the 30th day of the operation of systems by light microscope is shown.

![Fig. 13 Light microscopic image (1000× magnification) of flocs in the outlet of the aeration reactors in the case (right) and control (left) samples](image)

As it is clear, the number and density of flocs on the case sample was higher than the control sample. In Fig 14. image of Atomic Force Microscopy (AFM) in the case (on the right) and, control samples (on the left) on the 15th day of the operation of the system is illustrated.

The size of flocs (0.22 µm/div) in the case sample was 1.28 µm higher than the control sample (1.5 µm/div). Mikkelsen and Keiding (Mikkelsen and Keiding, 2002) reported that, the typical flocs’ size in the aeration reactors is 129±109 µm. SMFs could improve the density of flocs in the aeration reactors of CMAS when the intensity of SMFs was 15 mT (Asgari et al., 2021). MFs can improve sedimentation of sludge due to an inhibitory effect on the growth rate of filamentous bacteria (Zieliński et al., 2018).

In addition to the generation of higher flocs by SMFs, the size of flocs increased, too. By application of 15 mT SMFs for one hour daily on the aeration reactor density of flocs could be increased. An interesting point in this section of the study was that the surface of flocs when exposed to SMFs was rough. However, the surface of the flocs without the application of SMFs was not so uneven.

In order to determine possible the chemical changes in the sludge properties FTIR (Fourier-transform infrared spectroscopy) spectra was used for flocs in output of settled sludge in the clarifier reactors in two groups of samples that were demonstrated in Fig. 15.
Fig. 15 FTIR spectra of flocs in the output of settled sludge in the clarifier reactors in the case (up) and the control samples (down)

FTIR analysis is a method to determine the functional variation of the chemical groups in flocs of MLSS. Based on analyses of wavenumbers in Fig. 15 the percent of transmittance in the sharp peaks of the case (17 sharp peaks) was higher than the control (10 sharp peaks) sample in all bonds of the chemical substances. These sharp peaks indicated that the density of materials in the case samples was higher. It can be concluded that by application of SMFs (15 mT) the ultimate target of the wastewater treatment in the aeration reactors is obtained by generating a higher density of flocs for higher settling in the clarifier.

Ren et al reported that intensity of 15-25 mT of MFs improves the metabolism of bacteria by the effect of more generation of dehydrogenase and, more consumption of substrate (Ren et al., 2018). In this study, The spectra of amid I’ (1440 cm-1) and II’ (1650 cm-1) areas related to hydrogenase bonds in the case samples were higher than the control samples and, this indicates that the microbial growth rate in the samples in which the SMFs applied was higher due to higher production of hydrogenase enzyme.

4. Conclusion and outlook

The higher growth rate of the microorganisms in the aeration reactors to the transformation of soluble organic matter into the flocs or removal of BOD is a basic point of the operation of
the CMAS process. The supply of oxygen required for the metabolism of the organic matter in the aeration reactors has always been one of the challenges in the operating of the aeration system due to the need for higher energy consumption. Furthermore, as oxygen solubility in water is low, the use of new methods (such as the application of SMFs) which can increase the efficiency of the aeration process, is an alternative method for the process of the wastewater treatment in aerobic conditions. Application of 15 mT intensity of SMFs on the aeration reactor could effect of the $\alpha$-factor as the basic parameter on the transition of oxygen into the MLVSS. Furthermore, higher settling of flocs in the secondary clarifier is related to higher density of them is the main key to improving the effectiveness of the clarifier and generating an effluent with lower turbidity. By the application of 15 mT intensity of SMFs a new view of the wastewater treatment process can be achieved without consuming the chemical materials as a coagulant, too.

Effect of SMFs on components of the reactors limited to development of MLVSS. Although MLVSS is one vital part of MLSS, the impact of SMFs on MLSS was not significant. The reason for this issue may be due to the presence of the microbial mass in MLVSS as a portion of MLSS.

The effect of SMFs on $\alpha$-factor or rate of oxygen transfer is stimulated. Even when the concentration of DO was adjusted by a flow meter during the processes. In other words, the SMFs cause a more efficient use of oxygen in the aeration reactors.

By application of SMFs, sharp peaks of amids related to dehydrogenase bond as the indicator of the microbial growth rate were higher.

**Ethics approval and consent to participate**

This article is a part of Ph.D student research that has Ethics Committee Code IR.UMSHA.REC.1399.1077 from Hamadan University of Medical Sciences and Health Services.

**Consent for publication**

Not applicable.

**Data availability and materials**

On request from the corresponding author.

**Competing interest**

There is not conflict of interest among the authors of this paper.

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