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## **INVESTIGATION OF BIOFILM FORMATION ON KALDNES K1**

# KALDNES K1 ÜZERİNDE BİYOFİLM OLUŞUMUNUN İNCELENMESİ

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## ABSTRACT

In this study, the effect of different aeration rates (0- 0.001-0.004 m<sup>3</sup>air/m<sup>3</sup>reactor) was investigated for biofilm formation. Two types of reactors, AnMBBR1 and AnMBBR2, were used in the study. Both reactors were operated at constant HRT (6 h). The reactors (AnMBBR1 and AnMBBR2) were filled with 40 % carrier material (Kaldnes K1). AnMBBR1 was operated under anaerobic conditions (0 m3 air/m3reactor) while AnMBBR2 was operated at different aeration rates (0.001-0.004 m<sup>3</sup>air/m<sup>3</sup>reactor). The highest biofilm density was observed in AnMBBR2 with a reactor aeration ratio of 0.004 m<sup>3</sup>air/m<sup>3</sup>reactor, corresponding to 4062 mg/L. These results showed that limited aeration improved biofilm formation on Kaldnes K1.

Keywords: AnMBBR, biofilm, kaldnes k1, textile wastewater

## ÖZET

Bu çalışmada, farklı havalandırma hızlarının (0- 0.001-0.004 m<sup>3</sup>hava/m<sup>3</sup>reaktör) biyofilm oluşumuna etkisi araştırılmıştır. Çalışmada AnMBBR1 ve AnMBBR2 olmak üzere iki tip reaktör kullanılmıştır. Her iki reaktör de sabit HRT'de (6 saat) çalıştırılmıştır. Reaktörler (AnMBBR1 ve AnMBBR2) % 40 taşıyıcı malzeme (Kaldnes K1) ile doldurulmuştur. AnMBBR1 anaerobik koşullar altında (0 m3 hava/m3reaktör) çalıştırılırken, AnMBBR2 farklı havalandırma hızlarında (0,001-0,004 m<sup>3</sup>hava/m<sup>3</sup>reaktör) çalıştırılmıştır. En yüksek biyofilm yoğunluğu, 4062 mg/L'ye karşılık gelen 0.004 m<sup>3</sup>hava/m<sup>3</sup>reaktör reaktör havalandırma oranıyla AnMBBR2'de gözlendi. Bu sonuçlar, sınırlı havalandırmanın Kaldnes K1'de biyofilm oluşumunu iyileştirdiğini gösterdi.

Anahtar Kelimeler: AnHYBR, biyofilm, kaldnes K1, tekstil atıksuyu

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#### **INTRODUCTION**

The biological wastewater treatment reactors designed to use free-floating carrier media for biofilm growth and biomass attachment have been widely and effectively used in recent years (Copithorn, 2010; Ødegaard, 2006; Morper, 1994). These carrier media reactors are known as moving bed biofilm reactors (MBBRs) and are also called carrier media, support elements or substrates. The MBBR was developed in Norway in the late 1980s (Ødegaard et al., 1999; Ødegaard et al., 1994). Moving Bed Biofilm Reactor (MBBR) is a highly effective biological treatment process developed on the basis of the traditional activated sludge process and biofilter process. It is a fully mixed and continuously operating biofilm reactor in which biomass is grown on small carrier elements with a density slightly lighter than water and kept in motion with a stream of water inside the reactor. Movement within a reactor may result from aeration in an aerobic reactor and from a mechanical stirrer in an anaerobic or anoxic reactor (Borkar et al., 2013). MBBRs have several advantages such as high biomass concentration, high COD (chemical oxygen demand) loading, high sludge age, low hydraulic residence times, high volumetric removal rates, relatively small area requirements, and no sludge bulking problem (Chen et al., 2008). But, slow biofilm formation rate during the startup stage and easy detachment of biofilm from the plastic media are a few of the stumbling blocks on the practical applications of MBBRs. Biofilm density, thickness, and surface are have long been known to affected MBBR process performance (Li et al., 2016a; Mahendran et al., 2012). In recent years, MBBR carriers have been the focus of further studies aimed at controlling bacterial attachment and biofilm growth. and optimizing MBBR performance (Morgan-Sagastume, 2018). Factors such as oxygen, pH, nutrient levels, shape and material of carriers, and microbial activity play an important role in microbial community structure and biofilm formation rate (Ansari et al., 2012). In Additionally, Chu et al. (2014) reported that biofilm growth and distribution are affected by the structure and properties of the carrier material.

Until now, different kinds of the carrier have been used in MBBRs for wastewater treatment, e.g. suspended plastic bio-carriers, Kaldnes K1, K2, K3 and K5, Kaldnes biofilm Chip M. Kalndnes K1 is widely used in wastewater treatment. It is made of high-density polyethylene and the total surface area is significantly larger than the effective biofilm surface area. The main objective of this research was to analyze the biofilm formation performance of a moving bed reactor using Kaldnes K1.

#### **MATERIAL AND METHODS**

#### Characteristics of the Textile Wastewater

The wastewater was taken from the wastewater treatment plant of Iskur Dye Textile Industry in Kahramanmaras, Turkey and characteristic of real textile wastewater is shown Table 1.

Table 1. Characterization of Real Textile Wastewater			
Demonsterne		Unit	Textile
Parameters			Wastewater
pH		-	9.2±0.2
COD		mg/L	$1000 \pm 100$
DOC		mg/L	500±100
Color		Pt-Co	6883±100
	$\lambda_{436}$	$m^{-1}$	3.66±1.0
Color	λ 525	$m^{-1}$	$4.16 \pm 1.0$
	$\lambda_{620}$	m <sup>-1</sup>	$5.05 \pm 1.0$

#### **Experimental Operation**

Two anaerobic moving bed bioreactor (AnMBBR1 and AnMBBR2) was used in this study. The reactors with 5L volume were filled with the Kaldnes K1 carrier material (Table 2) at 40% of the volume of the reactor and fed continuously with real textile wastewater for around 234 days. Different aeration rates were performed to supply oxygen (0.001 and 0.004 m<sup>3</sup>air/m<sup>3</sup>reactor) in AnMBBR2. A magnetic stirrer (Heidolph D-91126 MR Hei-Standart, Schwabach, Germany) was placed under the reactor to mix the reactor at 250 rpm to provide contact between the wastewater and carriers. The pH values of both reactors were kept at 7.2. The reactors were continuously operated at the hydraulic retention time of 6h. The temperature of AnMBBR1 and AnMBBR2 were kept at room temperature.

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Table 2. Technical Characteristics of Carrier Media (Kaldnes K1)		
Type of carrier material	Polyethylene	
Surface area $(m^2/m^3)$	500	
Sizes (mm)	H=7, Ø=10	
Density (g/cm <sup>3</sup> )	0.95	

#### Analysis

The pH values of both reactors were online measured and recorded in situ daily. The oxidation-reduction potential (ORP) in the reactor was measured continuously using an ORP Meter equipped with a redox electrode (M 300, Mettler Toledo, Greifensee, Switzerland). For the mass on the biofilms measurement, 5 carriers were dried at  $105^{\circ}$ C for 2h and weighed. The mixed liquor suspended solids (MLSS) were carried out by the standard methods (Apha, 1998). The mixed sample of 20 mL taken from the system at the end of the reaction was passed through a 0.45 µm pore-sized filter using a vacuum. The filter was dried at a  $105^{\circ}$ C oven for 1 h. Later, it was placed in a desiccator to cool down to room temperature and MLSS measurement was done by weighing it on the analytical balance.

#### **Biofilm Analysis**

First, some carrier was removed from the bioreactor and dried to a constant weight in an oven at  $105^{\circ}$ C for 2 hours. The dried carriers were mixed in 0.1 mol/L of the hydrochloric acid solution for 24h at  $105^{\circ}$ C and then treated in an ultrasonic for 1 h, followed by washing with pure water many times till all the biofilm was removed from the carrier. The sample of clean carriers was again dried and weighed. The weight of the attached biomass was calculated by the equation of X1 - X2 (Chen et al., 2015).

X1: The dry weight including biofilm and carrier.

X2: The dry weight of the net carriers.

## **RESULTS AND DISCUSSION**

#### **Biofilm Formation Performance**





Figure 1. Evolution of Attached Biomass in the AnMBBR1 and AnMBBR2

The process of biofilm formation in lab-scale two moving-bed biofilm reactors (MBBR1 and MBBR2) was investigated. The MLSS concentration was kept stable at 450±50 mg/L in AnMBBRs during the whole operation. As shown in Fig. 1, the biomass concentration in anaerobic was much lower than that in limited aeration conditions. We know anaerobic microorganisms had low microbial growth rates (Annachhatre et al., 1992). At the

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start of MBBRs, a thin layer of biofilms could be observed on the inner wall of the carriers, and then it was found that the biomass concentration was increased with time. Gu et al. (2018) reported that intermittent aeration was of great benefit to biomass accumulation. The biofilm concentration in the carrier of AnMBBR1 was averaged 1846±10 mg/L and increased 3946±10 mg/L where 0.004 m<sup>3</sup>air/m<sup>3</sup>reactor.min of aeration rate was applied. The results meant that electron donor/acceptor and redox potential in biofilms are also known to influence bacterial population growth and microbial diversity, producing stratification in mixed microbial biofilms (Table 3) (Bassin and Dezotti, 2018; Flemming et al., 2016; Mahendran et al., 2012).

Table 3. ORP Profiles of the Operating Conditions		
Reactors	ORP	Aeration Rate (m <sup>3</sup> air/m <sup>3</sup> reactor)
AnMBBR 1	-450±10	-
AnMBBR2	-369±10	0.001
AIIIVIDDK2	-345±10	0.004

The further increase in aeration rate increased biofilm concentration and reached the maximum value of over 4500 mg/L at the end of the study. Lima et al. (2016) reported that the added biomass concentration in MBBR systems is generally between 2000 and 8000 mg/L, which is in line with the average values obtained for MBBR in this study. Also Duyar et al. (2021) showed that the biofilm concentration in AnoxMBBR was reached up to 9 g/L. However, Li et al. (2016) observed higher biofilm formation in MBBR at a low aeration rate. This is because organic carbon is an energy substrate for many microorganisms, and microorganisms can cause the degradation of carbon sources and nutrients when adequate oxygen is provided. As a result, increasing carbon sources cause microorganisms to grow faster and increase biofilm density (Kozak et al., 2021).



Figure 2. Biofilm Formation on Kaldnes K1 in AnMBBR1 and MBBR2

## CONCLUSION

In this study, biofilm formation performance was investigated on Kaldnes K1. The high biofilm formation was 2380 and 4062 mg/L in AnMBBR1 and AnMBBR2, respectively. The biofilm formation mainly occurred in conditions where limited aeration was to 0.004 m<sup>3</sup>air/m<sup>3</sup>reactor in AnMBBR2; however, the anaerobic condition was limited at biofilm formation. The experimental results showed that the limited aeration had considerable impacts on biofilm formation. Further research is needed to ensure biofilm attachment or prevents biofilm separation on the carrier which seems pretty much challenging for long-term industrial-scale processes. Also, the combination and behavior of attached biomass on biocarriers during different operation conditions shall assist in a better understanding of the mechanism and advantage of using biocarriers.

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