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Pilot-scale study of hydraulic retention time and energy consumption in biological treatment of raw municipal wastewater by air micro-nanobubble aeration in different seasons

Soheil Farajzadehha[a], Seyed Fazlolah Saghravani[a,*], Jalal Shayegan[b]

[a]*Department of Civil Engineering, Shahrood University of Technology, Shahrood, Iran,*
emails: saghravani@shahrootut.ac.ir (S.F. Saghravani), soheil.farajzadehha@shahroodut.ac.ir (S. Farajzadehha)
[b]*Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran, email: shayegan.jalal@gmail.com*

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ABSTRACT

The improvement of wastewater treatment plants is an environmental and economic priority. The highest energy consumption in wastewater treatment plants relates to aeration units with the current technologies. Microorganisms can use air micro-nanobubbles (AMNBs) for biological activity due to AMNB's high oxygen transfer rate, high stability in the aeration reactor, and increased contact surface. This study investigated the effect of aeration with AMNB on hydraulic retention time (HRT), energy consumption, chemical oxygen demand (COD), and nitrogen removal efficiency in the biological treatment of raw municipal wastewater without pretreatment for an extended time during cold and hot seasons. Suspended growth treatment and attached growth mode were studied by installing an active bio-curtain (ABC) as an improvement solution. In the presence of AMNB and ABC, effective micro-organisms significantly increased ten times as conventional activated sludges (CAS). Micro-nanobubble aeration helped the oxygen transfer and accelerated the aerobic layer formation, which took advantage of partial nitrification and denitrification for total nitrogen removal and resulted in total nitrogen removal by 99%. In comparison, 78% and 97% of COD removal in suspended and attached growth modes were achieved in the warm seasons at the optimum HRT of 9 h. In the cold seasons, with a decrease of about 5%, the COD removal yields 73% and 90% in the identical HRT (9 h), respectively. The aeration energy consumption in this study to eliminate the definite ratio of organic loads shows a reduction of about 40% compared to CAS. Excess sludge reduction has also been achieved by up to 70%. In conclusion, this research shows the potential and possibility of practical exploitation of the AMNB aeration and ABC to treat municipal wastewater.

Keywords: Air micro-nanobubbles; Raw municipal wastewater; Biological treatment; Aeration efficiency; Oxidation

1. Introduction

Due to the lack of available water resources and the importance of environmental protection, municipal wastewater treatment plants (WWTP) are being constructed. Reducing hydraulic retention time (HRT) and consequently reducing unit volumes will significantly help to save construction costs. On the other hand, optimizing energy consumption and reducing operating costs are priorities for governments. Conventional activated sludge (CAS) is intensified reactors featuring high bacterial loads and relatively short HRT. Treatment efficiency and decomposition rate are depended on an instant supply of dissolved oxygen (DO) constantly for the bacteria as they decompose

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* Corresponding author.

the organics [1]. Aeration is critical in these reactors and could significantly affect the efficiency of treatment plants and treatment costs [2]. Oxygen is poorly soluble in water, and typical saturation levels are between 8 (at 27°C) and 10 (at 15°C) [1]. Treatment processes based on air-water mixtures include many purposes, such as advanced oxidation and biological decomposition of organic matter. Although the CAS treatment is a practical chemical oxygen demand (COD) and biochemical oxygen demand (BOD) reduction method, it has been ineffective in phosphorous and nitrogen removal and is not cost-effective [3]. The addition of air with new technologies such as micro-nanobubble (MNB) aeration plays a critical role in the metabolism of aerobic organisms and biochemical reactions [4]. It helps to downsize the treatment facilities, reduce the operation time and expenses, and at the same time, increase the treatment and energy efficiencies [1,5]. Nanobubbles (NB's, bubble diameter < 200 nm) and microbubbles (MB's, bubble diameter < 50 μm) will remain in the liquid due to their buoyancy force that is lower than the water surface tension resistance. The combined term MNB will be used to avoid confusion. Macrobubbles tend to decrease in size gradually and subsequently collapse due to the dissolution of interior gases into the surrounding water, whereas MNBs remain as such for months, enhancing their durability by 16 times longer than macrobubbles [6] and don't burst out at once [7]. MNBs can stay in water for days or even months and remain stable for an extended period [8,9]. Previous researcher reported that MNBs suspension was stable over periods of several months [10]. The stability of MNB's results from a lower interfacial curvature than expected due to a high contact angle [11] and low rising velocity [12]. Based on the properties of the MNBs, they will remain available for microorganisms in wastewater until they are entirely consumed and accelerate the removal of pollutants [13]. Therefore, it can be said that MNBs will help increase the aeration efficiency and maximum oxygen consumption injected into the wastewater. It has been demonstrated that MNBs improve gas solubility through the bubble interface and large specific surface area [14]. The surface of MNBs in water is charged and can be measured in terms of zeta potential, the potential of the "plane of shear" [15]. The charge at the gas–liquid interface plays a very crucial role in the stability of the MNBs against coalescence with neighbor bubbles in the dispersion [16,17] and in the generation of free radicals [18] such as OH, which is immensely important and being considered for application in advanced oxidation processes to treat persistent organic pollutants in wastewater treatment that are difficult to biodegrade [4,19–23].

MNBs have a wide range of applications in water treatment, such as degradation of organic pollutants, water disinfection, cleaning, and de-fouling solid surfaces [24]. Also, MNBs have been applied to treat various wastewaters [5,25–27]. The wide use of oxygen and air MNBs has been anticipated due to their high bioactivity, mass transfer efficiency, and ability to generate free radicals without toxic chemicals and increase DO [4]. MNBs can be used in environmentally friendly techniques to oxidize organic compounds [24]. In order to treat organic wastewater with good biodegradability, such as municipal wastewater, biological methods such as conventional activated sludge, consumes less energy and is environmentally friendly using the metabolism of microorganisms by providing oxygen for bacteria [4,28]. MNBs have several physical–chemical properties, making them suitable for biological wastewater treatment. It has been respected that the concentrated ions at the bubble boundary prevent the escape of the internal gas and stabilize MNBs [7,29], which reveals that internal gas will be available for chemicals and biochemical consumption.

One major challenge of MNB technology for wastewater treatment is reducing power consumption [24]. Due to the aeration, wastewater treatment primarily consumes 1%–2% of the United States' annual energy demand [6]. The secondary treatment with aeration and sludge pumping is the biggest energy consumer [1]. Energy consumption in aeration often constitutes 50%–90% of the total required energy and more than 30% of the total operating costs [30,31]. It is pretended that the cost of construction and operation of excess sludge removal facilities is very high. For this reason, it is preferable to produce less sludge instead of using methods to stabilize and purify excess sludge. In terms of cost-efficiency, it is ideal for reducing sludge production in the biological process of WWT rather than sludge treatment [32,33]. The dissolved oxygen accelerates the autolysis process in bacteria and reduces sludge production [34]. Therefore, using MNBs in synthetic wastewater treatment specifies that energy consumption decreases up to 80% more than conventional fine bubbles [35]. In previous studies, synthetic wastewater treated with aerated activated sludge using MNBs and COD's removal efficiency (RE) were higher than conventional bubbles [9,36,37].

Yet, despite the consensus regarding the positive effect of MNBs on gas transfer efficiency and potential additional beneficial reactions, the long-time impact of the suggested approach should only be answered using long pilot tests with actual wastewater of different qualities [1]. There is rare research particularly highlighting the improvement of organic biodegradation efficiency via MNB and aerobic technologies [1,4,5]. Various substances in raw wastewater and the influence of operating conditions on treatment need to be investigated [4].

Therefore, this study investigated air micro-nanobubbles (AMNBs) aeration in the biological treatment of raw municipal wastewater as further research. Long-time operation in a different season with various operating temperatures has been considered. In addition, the system's performance at various HRTs was investigated for COD removal and energy consumption. Due to the hydrodynamic function of MNB generators during long operation, the stability of active microorganisms in aerobic biological treatment in the presence of MNBs was investigated. In addition, installing an active bio-curtain (ABC) to reduce the negative impact of generators on the bacterial population during the optimal retention time was investigated. In previous studies, treatment efficiency is improved by effective supplementary methods such as MBBR. Moving beds helped to remove COD up to 62.7% in HRT of 48 h in 35°C [38].

This study explored optimum HRT and estimated the energy consumption to treat raw municipal wastewater in Iran by applying AMNB aeration. Another parameter affecting the performance of the biological treatment system is the ambient temperature. Since the characteristics of raw sewage in most cities of Iran are similar, a municipal sewage treatment plant in the moderate climate of Iran was selected to check the treatment system's performance in the cold and hot seasons of the year. Furthermore, this system operated in the reactor's pilot scale dimension and was fed by the steady-state inflow of raw wastewater without pretreatment. AMNB generator operated continuously without clogging and was fed by a mixture of sewage and biological mass. The negative effect of the high speed and pressure of fluid flow passing through the generator was also investigated. Using the attached growth media as an improvement solution was explored.

2. Material and method

2.1. Pilot design and operation

The main experiments took place in a larger tank of 2 m^3 (2 m length × 1 m width × 1 m depth), as shown in Fig. 1. At the starting point of the stream, the pump was installed to control the flow rate, which helped control the HRT. In the first section of the pilot, the anaerobic area is created behind the baffle with the dimension of (0.2 m^3 × 0.9 m^3 × 0.9 m^3) in the next section, as shown by Part 12 in Fig. 1, returned mixed liquor from Part 13 that aerated with MNB generator (Model Gen7-180 by Minab Toos New Technologies, Mashhad, Iran). Part 12 and 13 create an aeration zone. At this stage, the water was enriched with air MNB (air flow rate = 2.5 LPM) at a water rate of 70 L/min. ABC, Part 15, acts as the attached growth media that provide a bed for the microorganism for biological treatment. The next part, Part 14, is the pre-anoxic zone [39]. In this section, DO is less than 1 mg/L because microorganisms used the oxygen in Parts 12 and 13 and ABCs. In the final area (Part 7), the post-denitrification zone was designed using innovation and results presented by Urbini et al. [39]. An upward flow with less sludge overflowed from the orifice, numbered 8 in Fig. 1. A secondary settling tank (Part 9) was used to reduce effluent total suspended solids (TSS). A schematic of the pilot is shown completely in Fig. 1.

The attached growth media was installed in two sections inside the aeration reactor. In this case, it was operated in different HRTs in cold and warm seasons. The measurements were made when the conditions were considered stable.

2.2. Wastewater

The wastewater used in this research was from the daily influent of Mahmudabad City Wastewater Treatment Plant (WWTP). The municipal raw wastewater was taken after the grit chamber. Properties of raw sewage are given in Table 1.

The influent flow rate was adjusted so that the HRTs of 6, 9, 12, and 15 h were achieved through electronic controllers. The inlet pipe (1″ of nominal diameter) was attached to a filtered feed pump to prevent the entrance of particles larger than 10 mm. The wastewater contained solid particles of up to 10 mm diameter, fabrics, hairs, leaves, and other common solids in raw wastewater. This is a crucial feature

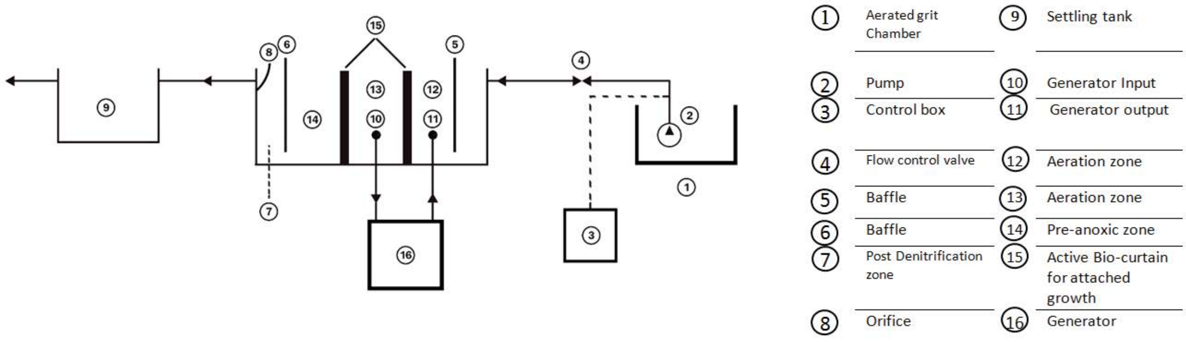

Fig. 1. Pilot design.

Table 1
Properties of raw wastewater

Parameter	Value	Test method
pH	7.72	Standard Methods 2012-4500-H+-B/catalog HACH
COD, mg/L	580	Standard Methods 2012-5220D/HACH Method 8000
BOD, mg/L	305	Standard Methods 2012-5210D/Catalog WTW
TDS, mg/L	762	Standard Methods 2012-2540-C/Catalog HACH
TSS, mg/L	160	Standard Methods 2012-2540-D
Sulfate, mg/L	180	Standard Methods 2012-4500-SO42-E/HACH Method 8051
Total alkalinity, mg/L as $CaCO_3$	471.38	Standard Methods 2012-2320-B
Total phosphate, mg/L	6.4	Standard Methods 2012-4500-P-C/ HACH Method 10127
Total nitrogen, mg/L	30.6	Standard Methods 2012-4500-N-CMERCK to DIN EN ISO 11905-1

that distinguishes this research from those performed by synthetic wastewater.

2.3. MNBs generator

The MNBs generator (Model Gen7-180 by Minab Toos New Technologies, Mashhad, Iran) was connected directly to the aeration tank to inject AMNBs into the feed solution by rapid hydrodynamic mixing of the air and solution. The air was selected as a suitable gas for MNBs production since it is free and available everywhere with less energy consumption and a practical effect on the biological process [40]. Several methods have been applied to generate MNBs [41–43]. This study used the mentioned MNB generator optimized for working with raw sewage without clogging during continuous operation. The machine worked non-stop for consecutive days. Table 2 shows the properties of the MNBs generator.

2.4. Attached growth media

In this system, two ABCs were installed in the reactor section and perpendicular to the direction of the flow. The dimensions of these curtains were chosen so that the whole area was covered, but the wastewater could flow through the openings. The specific cross-section of each curtain used for attached growth is 50 m^2/1 m^2 of the curtain (50 m^2/m^2) made with polyethylene threads considered waste from lathing machinery.

2.5. Reactor setup and operation condition

To set up the reactor, approximately 100 L of activated sludge from the sludge return line of Mahmudabad WWTP was discharged to the pilot aeration reactor. The startup was run on 1st June when the ambient temperature varied from 25°C to 35°C. Raw wastewater was added to the test reactor with a hydraulic retention time of 6 h. For two weeks, MNBs aeration was performed at 70 L of MNB enriched wastewater per minute, and the output parameters were measured daily to assess the system stability. DO can reach a peak value of 10.4 to 13.5 mg/L for air MNB [44,45]. Aeration and steady-state operation were performed for one month to ensure the microorganism adaptation to the system by controlling the effluent COD and DO. In this study, DO in an aeration tank was maintained in the range of 2–2.5 mg/L to supply oxygen required by microorganisms and optimize energy consumption.

Table 2
Properties of MNBs generator

Item	Value
Model	Gen7-180
Voltage (V)	220
Frequency (Hz)	50
Input power (W)	180
Head (m)	7
Flow rate (LPM)	70

The main tests were started on 1st July and took place in the open air in summer, autumn and winter. Ambient temperature during this period varied between 5°C and 35°C. The incoming raw sewage was collected at a temperature between 17°C and 24°C. With the help of a control box designed to adjust the inlet flow by the system feed pump, different HRTs in the reactor operation were investigated. The system performance was examined at 6, 9, and 12 h hydraulic retention times in warm and cold seasons. The duration of each stage was two weeks. This program was repeated in every test and all seasons.

2.6. Sampling

For experiments, samples were taken from the reactor inlet, filtered outlet, and taken from various parts inside the reactor. Sampling was performed at specific periods for each item. This process was based on standard methods for water and wastewater examination [46].

2.7. Data analysis

The samples were prepared from the mixed liquor inside the reactor, the effluent, and the influent. The samples were filtered through Whatman Grade 4 Filter Paper. The concentration of dissolved oxygen (DO) and pH were measured using SD 335 Multi (Set 2) - pH/DO by Lovibond. In addition, a particle size analyzer (Litesizer 500, Anton Paar, Austria) was employed to determine the size distribution of MNB. COD, total nitrogen, nitrate, and total phosphorous were measured using spectrophotometry techniques (HACH-DR6000). Other analyses were performed based on standard methods (Ahmadi et al. [46]).

3. Results and discussion

3.1. Dynamic light scattering tests

The generator enriched pure water with AMNB at an altitude of approximately 1,250 m above sea level. The fluid flow through the generator was 70 L/min, and the inlet air rate to the generator was kept at 2.5 L/min, similar to the condition intended for the main tests. Various techniques can estimate bubble size distribution [47,48]. The samples underwent the dynamic light scattering and mobility (zeta potential) tests presented in Figs. 2 and 5. AMNBs properties (particle size distribution and zeta potential) in the feed solution were determined to better understand their effects on the scaling process. One day after AMNBs production, the measurements were made using a particle size analyzer (Litesizer 500, Anton Paar, Austria) with a particle size detection range of 55–105 nm zeta-potential measurement range of –30 mV in the pH of 7, respectively. The zeta potential in distilled water is negative [49]. The values reported for MNBs in pH of 5.8 by [50] and [51] are –35 and –57.9 mV, respectively. In another study, the negative zeta potential was observed at –45 mV for air bubbles of 100 nm in water at pH 7, respectively [14,52]. Zeta potential will be decreased (manifested) as pH increases [50]. The charge on the gas–water interface is developed due to OH^- and H^+ [49]. In addition, the zeta potential of MNBs

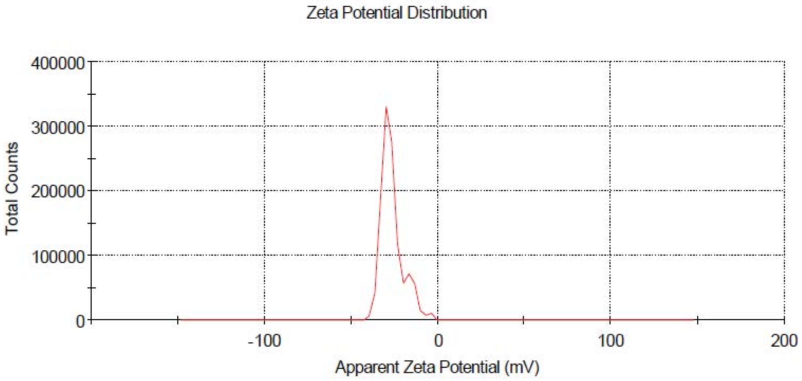

Fig. 2. Zeta potential distribution.

significantly depends on the presence of surface-active compounds (e.g., Surfactants and alcohols) in water [49].

3.2. COD removal

As mentioned in the material and methods section, after adding 100 L of activated sludge, the system took only one month to set up and adapt. AMNBs optimized microbial metabolic pathways by speeding their structural development of microbial aggregates, which resulted in shortening adaptation in CAS systems [53]. The COD and SVI fluctuations were monitored during this time to control the system adaptation process. The main experiments began in the warm season on 1st July with an HRT of 6, 9, 12, and 15 h (1st July–30th Oct) and continued throughout the cold season (1st Nov–28th Feb) for eight months at the same HRTs. In the warm season, the temperature range varies between 20°C and 35°C. Samples were taken from the aeration reactor and the secondary settling outlet at 11 a.m., and the experiments were performed on the same day according to standard methods. The temperature varies between 5°C and 20°C in the cold season. In warm and cold seasons of the year, different HRTs have been studied in Mahmudabad City in Qazvin Province, Iran. The results of average COD removal in different HRTs and temperatures are shown in Figs. 3 and 4.

In the suspended growth mode and mentioned HRTs, the average RE was 71%, 81%, 86%, and 98%. In CAS treatment plants, typical HRT is 5–14 h in conventional units, and for extended aeration activated sludge plants, the range is between 15 and 35 h [54]. This study demonstrated that HRT for degradation of organics in AMNB aeration is less than half of that used in traditional systems [44]. Another study explored the effect of MNBs on the aerobic biodegradation of pollutants. The results showed that the oxygen utilization rate and volumetric mass transfer coefficient of the MNB aerated synthetic wastewater was almost twice conventional aeration [55]. Therefore, these research results are about halving the HRT in 9 h to achieve a treatment efficiency of about 80% rather than extended aeration in the suspended growth mode.

As shown in Fig. 3, increasing the residence time from 6 to 9 h increases COD removal efficiency with a steeper slope. Then with increasing the residence time, the COD removal efficiency finds a smoother gradient. And this indicates that energy efficiency will be at risk with more aeration and energy consumption.

In previous studies, synthetic municipal and domestic wastewater was treated in lab-scale studies by MNB aeration and RE of COD ware in 54%–86% in different HRTs at 22°–25° [35–37].

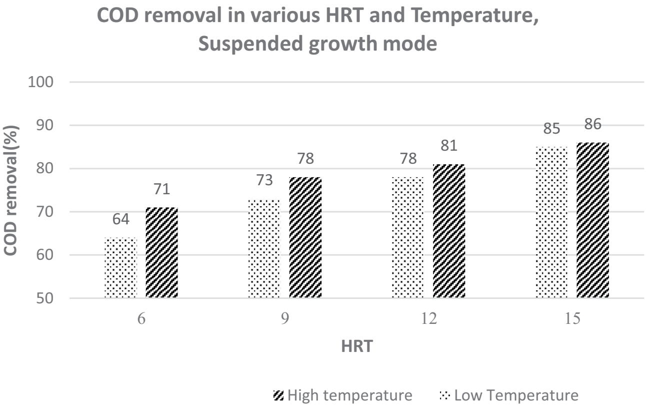

Fig. 3. Average COD removal in various HRT (h) and temperature (low: 5°C–20°C and high: 20°C–35°C), suspended growth mode.

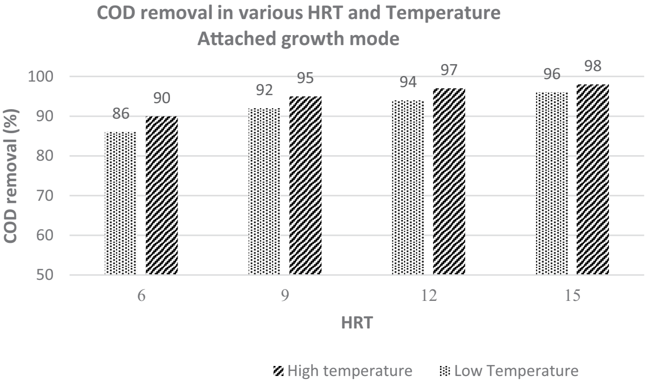

Fig. 4. Average COD removal in various HRT (h) and temperature (low: 5°C–20°C and high: 20°C–35°C), attached growth mode.

ABCs were then installed in the warm season to test for increased COD removal efficiency. The results in this stage showed that in the HRT of 6, 9, 12, and 15 h, the average COD RE of 90%, 95%, 97%, and 98% were obtained (Fig. 4). The performance and efficiency of the system were significantly improved by the installation of ABCs, 20% on average. The results demonstrated that the system is less sensitive to temperature changes in the attached growth mode, as shown in Fig. 4. The increase in the number and activity of microorganisms on ABCs. Because of the hydrodynamic performance of the microbubble generator at high speeds and pressures, several beneficial microorganisms in the aerobic biological process are likely to be killed during passage through the generator, and the installation of ABCs will increase the growth rate and the number of these microorganisms on the attached growth media. MNBs offer a superior oxygen supply capacity and 1.5 times higher oxygen transfer efficiency than large bubbles, promoting the biofilm's growth and achieving better RE of COD and ammonia [56]. By MNB, aeration of extracellular protein and polysaccharides increased by 3.4 and 1.7 times in biofilm, and floc size increased [53].

In addition, the count of aerobic bacteria was performed in two modes of attached and suspended growth, the results of which are given in Table 3, and the general information of this table shows an increase of about ten times in the number of beneficial aerobic microorganisms in suspended growth. Measurements of other parameters are shown in Tables 4 and 5.

These same steps were repeated throughout the year from November to the end of February, as cold second, for four months, respectively, for suspended and attached growth at different retention times. The test schedule can be seen in Fig. 3. As a result, the RE of COD was 64%, 73%, 78% and 85% in the HRT of 6, 9, 12, and 15 h in the suspended growth mode. The following ABCs were installed in the cold season to test for increased COD removal efficiency. The results in this stage showed that in the remaining 6, 9, 12, and 15 h, the average COD removal efficiencies of 86%, 92%, 94%, and 96% were obtained. It can be concluded that the system is not sensitive to temperature fluctuation in the attached mode (Fig. 4).

In a previous study, synthetic municipal wastewater was treated by an aerated activated sludge system using MBs at 15°C. The RE of COD was higher than those of conventional bubbles (1.73 min^{-1}) with 3.26 min^{-1} at initial COD of 600 mg/L in 90 min. [36]. This research results demonstrated that AMNB aeration in HRT of 9 h in the presence of ABCs could treat the raw municipal wastewater by COD in the normal range of 550–650 mg/L with higher RE than 90% so that the effluent is within the standard range of discharge to surface water in all seasons with various operational temperatures. RE of other compounds and organic pollutants is shown in Tables 4 and 5 and demonstrate that all of them are in the permitted range for discharging to surface water due to standards [57].

Table 4
Average RE in suspended growth mode in various operating temperatures (HRT = 9 h)

Parameter	Influent	Effluent	RE
pH	7.72	7.4	–
COD, mg/L	530	132	75%
BOD, mg/L	290	64	78%
TDS, mg/L	670	127	81%
TSS, mg/L	148	25	83%
Sulfate, mg/L	173	112	35%
Total alkalinity, mg/L as $CaCO_3$	463	314	32%
Total phosphorus, mg/L	6.1	2.4	60%
Total nitrogen, mg/L	32.1	1.3	96%

Table 5
Average RE in attached growth mode in various operating temperatures (HRT = 9 h)

Parameter	Influent	Effluent	RE
pH	7.72	7.4	–
COD, mg/L	580	23	96%
BOD, mg/L	305	14	95%
TDS, mg/L	762	125	83%
TSS, mg/L	160	22	86%
Sulfate, mg/L	180	112	37%
Total alkalinity, mg/L as $CaCO_3$	471.38	295	37%
Total phosphorus, mg/L	6.4	2.3	64%
Total nitrogen, mg/L	30.6	.01	99%

Table 3
Bacteria count (number/mL)

Attached growth zone	Section 1 – suspended growth	Extended aeration reactor	10–100 λ
$>10^5$	10^4–10^5	$>10^5$	E. spp. (3)
$>10^5$	10^4–10^5	$>10^5$	*E. coli*
$>10^5$	10^4–10^5	$>10^5$	*E. coli* inactive
$>10^5$	10^4–10^5	$>10^5$	*Pseudomonas* spp.
$>10^5$	10^4–10^5	$>10^5$	*Alcaligenes*
$>10^5$	10^4–10^5	$>10^5$	*Paracoccus*
$>10^5$	10^4–10^5	$>10^5$	*Flavobacterium-cytophaga*
10^3–10^4	$<10^3$	10^3–10^4	*Klebsiella pneumonia*

3.3. Investigating the energy consumption and excess sludge

The energy consumption of the aeration unit is directly related to the amount of dissolved oxygen. Therefore, optimal oxygen supply has a direct effect on energy savings. DO should be sufficient to ensure the growth of microorganisms. Although excessive DO concentration will not enhance the efficiency of biological processes, it will increase unnecessary costs of pumping air [58].

In the main experiment, DO in the aeration reactor changes over time. This is due to microorganisms' changes in oxygen consumption rate throughout the process [59]. Massive decay of aerobic bacteria occurs at critical levels below 2 mg/L [1]. In this research, operators controlled DO in the aeration reactor at 2–2.5 mg/L. Also, the DO was about 0.1–0.5 mg/L behind the exterior baffle.

In this study, according to the measurements, 0.4 kW has been used to treat 1 m^3 wastewater with 650 mg/L organic loads with the help of AMNBs aeration in both suspended growth and attached growth modes. The conventional system consumes 0.65 kW to treat the same municipal wastewater in different countries [60,61]. Comparing the efficiency of the MNB aeration system in the present study, one could conclude that MNB aeration saves at least 40% of electrical energy.

In the CAS treatment plants, disposal and treatment constitute 60% of the total operation cost [62]. Using AMNBs in sequencing batch reactor (SBR), SVI increased and reached 120 mg/L with 5 h aeration and is lower than aerated with macrobubbles because higher cell mass lysis resulted in less sludge production [46]. MNBs improved the efficiency of sludge reduction [63].

In this study, excess sludge compared with the Mahmoudabad MLE treatment plant (extended aeration) found about a 70% reduction in excess sludge production.

3.4. Nitrate removal in the attached growth state and the final anoxic phase

Nitrogen compounds can be removed through physic-chemical and biological treatments [35,64]. The last ones are cost-effective and based on aerobic and anoxic bacteria [39]. If an anoxic or intermediate unit is placed after the aeration unit to act as a pretreatment before the denitrification unit to reduce the dissolved oxygen to about 0.11–0.31 mg/L, the resulting denitrification efficiency of 73% increases to 91% [39]. Using the result of this research and considering that the dissolved oxygen decreases after passing the aerated sewage through the second ABC. Therefore, Part 7, as shown in Fig. 1, acts as a post-anoxic zone in which dissolved oxygen is reduced to approximately 0.15 mg/L and operated as a denitrification unit. Therefore, this configuration has removed up to 99% of total nitrogen and is the notable result of this research. In other studies, by MNB aeration, extra oxygen supplied for microbial aggregates and nitrogen removal rate increased by 10.58% [53]. Synthetic municipal wastewater treated using a MBs aerated biofilm reactor and confirmed that NH_4^+–N RE was almost 99% [9].

Table 6 shows the calculated bio-volume of fluorescence nuclei acid and the relative volume of EPS, where a different variation of activated sludge (AS) and biofilm was observed. Previous studies of varying aeration intensity suggested that the AS would become tighter under higher aeration intensity [65,66], the result indicated the influence was attributed to the shear stress along with the traditional aeration rather than the DO improvement. For biofilm samples, the participation of MNB significantly decreased the bio-volume of the nuclei acid from 2.13 to 1.74 μm3/μm2. A previous study demonstrated biofilm thickness is less than 60 μm without MNB aeration. The result suggested MNB enhanced the longitudinal growth whereas the biofilm thickness kept

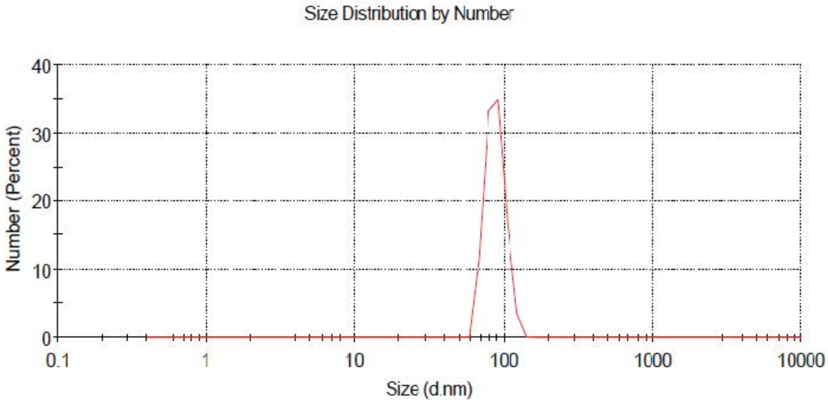

Fig. 5. Size distribution of MNBs.

Table 6
Structural characteristics of activated sludge (suspended growth mode) and biofilm on ABC (attached growth)

AS				Biofilm			
Nucleic acid (μm3/μm2)	Protein (μm3/μm2)	Polysaccharide (μm3/μm2)	Size (D90) (μm)	Nucleic acid (μm3/μm2)	Protein (μm3/μm2)	Polysaccharide (μm3/μm2)	Thickness (μm)
2.13	1.14	0.92	209	1.74	1.44	1.51	84

increased by 34% to 80 μm. MNB addition enlarged the size and thickness of AS and biofilm by 34% and 86.67%, respectively, which subsequently enhanced the removal of total nitrogen (TN) by as much as 10.58% [67].

Besides the thickening of biofilm, the mean size of AS floc was also observed at the end of the experiment, in contrast to the size at the early stage. Previous research pointed out that the thickness of biofilm corresponds to the transition of the biofilm layer, which provides a suitable environment for denitrifying bacteria and benefits the removal of TN [41,53]. On this occasion, the participation of MNB helped with the oxygen transfer and accelerated the anaerobic layer formation, which took advantage of partial nitrification and denitrification for TN removal.

3.5. Confirmation of aerobic condition in the reactor

The count of active microorganisms in the aeration reactor was performed to investigate the aerobic condition in the reactor. To ensure that these microorganisms are continuously involved in the presence of AMNBs, different parts of the aeration reactor were sampled in suspended growth and attached growth. The presence and number of several types of index bacteria in the samples of MNBs aeration reactor were compared with their number in the raw wastewater and the extensive aeration reactor of the Mahmoudabad treatment plant.

Each sample was cultured after 100 and 10-micron dilutions in blood agar and MacConkey agar media. After 24 h of incubation and examination of colonies, seven types of gram-negative bacteria were observed in each of the eight plates. Each of which was isolated separately, and for each, a diagnostic gallery for gram-negative bacilli was provided. The results of diagnostic tests and the count of bacteria obtained are given in Table 3. It is noteworthy that this method could not count more than 100,000 count/1 mL of bacteria despite diluting.

The results show that with the passage of the wastewater mixture through the MNB-generator, even though the biological mass has survived and continued the biological removal activity, their number could be decreased, affecting the treatment efficiency. However, the average number of index bacteria has elevated to about ten times with attached growth curtains installed like a large aeration tank. As a result, by installing the fixed bed, or ABCs, the negative effect of the MNBs on reducing the number of active bacteria and microorganisms has been resolved. The results of the count tests are given in Table 3. Further investigation of extracellular polymeric substance and surface of microbial aggregates showed the composition of active substances of microbial aggregates were shifted by applying nanobubble, especially the oxygen-sensitive ones [53].

In addition, as shown in Table 7, In the presence of AMNB, the filamentous bacteria count that has negative impacts on bulking is less than 100 mL in suspended growth mode and less than 10 mL in attached growth mode. Subsequently, there was no bulking in the aeration reactor.

Table 7
Filamentous bacteria count (number/100 mL)

	Type of bacteria	Suspended growth	Attached growth
1	*Nostocoida limicola*	$<10^2$	<10
2	*Haliscomenbacter hydrosis*	$<10^2$	<10
3	*Thiothrix*	$<10^2$	<10
4	*Beggiatoa*	$<10^2$	<10
5	*Microthrix parvicella*	$<10^2$	<10

4. Conclusion

In current research as a long time and pilot-scale investigation on raw wastewater biological treatment, it has been found that with an AMNB aeration, the aerobic condition in wastewater treatment is achievable. In the presence of MNBs, the productivity of bacteria and microorganisms significantly increases in attached growth mode due to the availability and activity of MNBs. In addition, energy consumption is reduced by about 40% compared to CAS systems. With the installation of the fixed bed in ABCs, the number of microorganisms increased at least ten times, the same amount as extended aeration reactors. The negative effect of the speed pressure of fluid flow passing through the generator also improved using the attached growth media as an ABC. The MNBs aeration reactor maintained dissolved oxygen at 2–2.5 mg/L. In the final stage, as a post-anoxic zone with reduced dissolved oxygen, it reaches the level of about 0.1 mg/L, providing a perfect condition for denitrification. In the case of 9 h retention time, the total nitrogen removal was as high as 99% after denitrification. The participation of MNB helped with the oxygen transfer and accelerated the anaerobic layer formation, which took advantage of partial nitrification and denitrification for TN removal. In the identical HRT and warm seasons, 80% and 97% COD removal were achieved in suspended and fixed bed growth modes. With a decrease of about 5% in the cold season, these numbers yield 75% and 90%, respectively. Laboratory studies have shown that aerobic microorganisms survive and continue their activities more efficiently using MNBs. Excess sludge reduction has also been achieved by up to 70%.

Data availability statement

Some or all data, models, or codes that support the findings of this study are available from the corresponding author upon reasonable request.

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