New containerized wastewater treatment technology: system description and evaluation of treatment capacity of a highly efficient MBBR system

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Abstract

In a recent R&D project, an innovative biofilm micro-carrier was invented by Inno-Water Inc., that has a much smaller size compared to the traditional carriers. In this article, we show the capacities and structure of a new wastewater treatment system, that contains this new type of biofilm carrier. The "MICROBI" technology has an average of 99,4% ammonium, 94,3% COD, and 75,0% total nitrogen removal rates in municipal wastewater. During the period of bacterial colonization, we measured chemical parameters (COD, NO3-N, NO2-N, PO4-P, NH4-N) of the inflow and effluent twice a week for seven weeks. After that, a 24-hour measuring campaign was carried out to determine the full capacity of the system. Simultaneously we measured dissolved oxygen, pH, temperature, and conductivity in the reactor, and monitored the development of biofilm on the carrier with the light microscope and TTC colouring method. The results show a significant nitrification ability and high COD and ammonium removal at an inflow of 27-28 m³/day. Future improvement plans include the optimization of denitrification and increasing the daily wastewater treatment capacity.

Keywords

Biotechnology, MBBR, wastewater treatment, biofilm monitoring, technology introduction.

Új, konténer alapú szennyvíztisztítási technológia: a technológia ismertetése és a tisztítási kapacitást vizsgáló kísérletek bemutatása egy nagy hatékonyságú mozgóágyas biofilmes rendszerben

Kivonat

A közelmúltban zajlott kutatási és fejlesztési projektben az Inno-Water Zrt. innovatív biofilm mikrohordozót fejlesztett ki, amely jelentősen kisebb méretű, mint a hagyományos hordozók. Ebben a közleményben egy új szennyvíztisztító rendszer kapacitását és felépítését mutatjuk be, amely rendszer biológiai tisztítási lépcsője erre az új típusú biofilm hordozóra épül. A "MICROBI" technológia átlagos szennyezőanyag eltávolítási hatékonysága kommunális szennyvízben 99,4% az ammónium, 94,3% a KOI és 75,0% az összes nitrogén komponensre . A bedolgozási időszak alatt hetente kétszer mértük a szennyvíz kémiai paramétereit (KOI, NO3-N, NO2-N, PO4-P, NH4-N). Ezt követően egy 24 órás mérési kampányt végeztünk a rendszer teljes kapacitásának meghatározása érdekében. Vizsgáltuk a reaktorok oxigénszintjét, pH értékét, hőmérsékletét és vezetőképességét, valamint fénymikroszkóppal és TTC színezési módszerrel nyomon követtük a hordozón lévő biofilm fejlődését. Az eredmények kiváló nitrifikációs képességet és magas KOI és ammónium eltávolítást mutatnak 27-28 m³/nap hidraulikai terhelés esetén. A jövőbeni fejlesztési tervek között szerepel a denitrifikáció optimalizálása és a szennyvíztisztító kapacitás növelése.

Kulcsszavak

Biotechnológia, mozgóágyas biofilmes rendszerek, szennyvíztisztítás, biofilm vizsgálatok, technológia bemutatás.

INTRODUCTION

In the modern world, there is a trend that more and more people move out of large cities, for example, smaller suburban towns around big centres. Another tendency since COVID-19 is the opportunity of working from home (home office). These trends led to expanding towns around large cities, which has created an urgent need for the increasing capacity of infrastructure, like roads, wastewater management, etc. It is difficult to solve the problem of the ever-growing need for more wastewater treatment capacity because building new wastewater treatment plants is expensive and needs a large space as well as redesigning the sewage system of a city. Not to mention that these new trends can change quickly, and not many large wastewater treatment plants can follow. To solve these problems, mobile, flexible, and effective systems need to be developed. MICROBI technology is the size of a standard container, yet it can clean wastewater up to $25{\text -}30$ m³ per day. Its core element is the freshly developed biofilm micro-carrier, which is significantly smaller than traditional carriers. To study the carrier's capacity and measure the amount and activity of the biofilm on this carrier, new methods are required, as described in this article.

STATE-OF-THE-ART

Traditional biological wastewater treatment technologies are based on activated sludge reactors and were invented over a century ago, which has a well-known literature and performance. The most widespread used wastewater treatment technology is the activated sludge (AS) system, where the treatment process includes "active" microorganisms, such as bacteria, fungi, protozoa, and even animals like rotifers, insect larvae, and worms. These microorganisms live (among non-living particles, organic and inorganic matter) in flocs, which are the core of the process. These flocs are continuously circulated with oxygen and the influent wastewater to reduce their organic substances to as low as possible, as soon as possible (*Wang et al.*

2009). The AS system performance is based on the settleability of flocs and usually cannot handle more than 5 g/l biomass constantly. Also, there is a massive sludge production, that needs additional treatment methods to work with less biological waste (*Barwal and Chaudhary 2014*).

In MBBR (moving bed biofilm reactor) systems, the flocs are mainly replaced with different-sized and shaped carriers, that host a high specific surface area for the microorganisms to attach (*Kawan et al. 2016*). *Schmidt and Schaechter (2011)* studied that over 90% of biomass is on the surface of the carriers in MBBR systems. These carriers are usually made of HDPE, PE, or PP with a density of 0,95 g/cm³ (*Barwal and Chaudhary 2014*). Their typical size range is 2,2-50 mm in length and 9-64 mm in width (*Barwal and Chaudhary 2014*).

In MBBR systems, *Ødegaard (2006)* says the suggested volume of carriers is 67% of the reactor, but not above 70%. This means around 500 m²/m³ surface area for the biofilm resulting in a growth of around $350 \text{ m}^2/\text{m}^3$ (70% colonization rate). *Ashkanani et al. (2019)* used different carrier sizes 500-800 m^2/m^3 and 1 200 m^2/m^3 surface area and had a 30% colonization rate. *Kermani et al. (2008)* applied a scale laboratory-scale MBBR reactor with a specific surface area of $260 \text{ m}^2/\text{m}^3$.

Madan et al. (2022) found that the concentration of fixed biomass can be up to 10 000-12 000 mg/l, however, *Benakova et al. (2018)* had a biofilm mass of 12 000- 16 000 mg/l. The biomass thickness is also important in the effectiveness of an MBBR system. *Dezotti et al. (2011)* suggest that to maximize the substrate diffusion, a maximum biofilm thickness is 100 µm. *Torresi et al. (2016)* found that a biofilm thickness over 200 µm does not significantly increase the functionality of nitrification activity.

Chu and Wang (2011) showed that PCL (polymer polycaprolactone) carrier has TOC and ammonium removal rates of 72% and 52% with a 14 h HRT (hydraulic retention time). *Ashkanani et al. (2019)* reached 87.3%, 71.8%, and 47.2% ammonium removal rates at 20 °C with different surface areas. An HRT of 6 h revealed that 86.67% and 91.65% ammonia removal was achieved from aquaculture wastewater *(Shitu et al. 2020).* With MBBR systems *Goswami and Mazumdar (2016)* reached a 90% COD removal rate for tannery effluent, and in the textile industry, *Erkan et al. (2020)* reached 98,5% COD removal. *Martin-Pascual et al. (2016)* carried out a pilot-scale study with an MBBR system with urban wastewater. With 24-hour HRT, they reported that 86% COD removal rate. *Kermani et al. (2008)* used 4 reactors in the laboratory with a clarifier in an MBBR system and reached 84,6% total nitrogen removal rate at 24 h HRT. *Phanwilai et al. (2020)* reviewed different types of MBBR systems and concluded that a total nitrogen removal rate was between 64% and 77%, with an HRT of 5-11 hours.

MBBR systems have several benefits over traditional AS systems, like reduced sludge production, smaller footprint, and the ability to operate with higher biomass concentration which might have a higher specific activity than AS. Another great advantage of these systems is that they are more resistant to overloading and toxic compounds (*Kawan et al. 2016*). However, there might be drawbacks compared to AS like the settling characteristic is poorer in some cases (*Lee et al. 2006*), the carriers might cause mechanical failures (*Kawan et al. 2016*), and it is more difficult to separate the carriers from the sludge (*Lee et al. 2006*).

DESCRIPTION OF THE TECHNOLOGY

The concept of using special micro-carriers for wastewater biofilm was tested in earlier research on a lab scale (*Fleit et al. 2008, Sándor et al. 2009, 2012, 2017*). Early experiments used PVA-PAA (polyvinyl alcohol/polyacrylic acid) hydrogel micro-carriers that could be produced only in small amounts using materials with high environmental risks.

Based on the early results, researchers of Inno-Water Inc. have invented new types of micro-carriers, first on a laboratory scale, then a production technology has been developed for the automatized synthesis of micro-carriers.

During the recent R+D project, we implemented the production of micro-carriers with special structures, defined durability and sedimentation properties for bacteria responsible for pollutant transformation processes in wastewater treatment, as well as laboratory, semi-industrial, and operational scale testing, which can be used to optimize the performance of wastewater treatment by regulating the properties of carriers bacterial culture properties and thus processes that degrade/remove contaminants.

Our goal was to create different micro-carriers having a compact structure but surface unevenness, helping to form surface biomass. To achieve this, a special additive has been incorporated into hydrogels. To ensure suitable mechanical properties, such as pumpability and applicability under conditions of municipal wastewater treatment, the degree of crosslinking of hydrogels has also been optimised.

"MICROBI" technology is based on freshly developed, innovative hydrogel biofilm micro-carriers. In our pilot system described in this article, we utilize micro-size copolymer hydrogel micro-carriers synthesized with iron oxide. The special composition and structure of the biofilm carrier allow high biomass concentration with high specific activity and a magnetic separation that can be much more efficient than single sedimentation.

"MICROBI" is a unique system offering a technical solution to the problems of spontaneously formed flocks, thereby providing an engineering solution in the field of biological wastewater treatment with controlled parameters (sedimentation/separation rate, size distribution range, and other functional characteristics).

The mobile "MICROBI" technology pilot system is placed in a 6.0 x 2.4 x 2.7 m standard container. The walls are made of EPS sandwich panels and the bottom is marine plywood. The interior of the container is full of reactors, which are reachable from the top. The pilot unit examined contains:

- 1. Primer clarifier, net. volume 2.2 m³,
- 2. 2 aerated tanks with a separation wall in the middle, net. volume 6.9 m³ each,
- 3. 2 micro-carrier separator with magnets,
- 4. after clarifier, net. volume 2.16 m^3 ,
- 5. blower for aeration, the compressor for pneumatics, pumps, etc.

1. ábra. A reaktorokkal felszerelt konténer sematikus ábrája

In *Figure 1,* the overview of the containerized MI-CROBI technology is shown, where the red arrows mean the order of the wastewater flow. Raw wastewater arrives in the primary clarifier tank. Primary sludge is removed by a pump from the bottom of the tank. After that, the wastewater flows to the first reactor. The first and second reactor separated with a polypropylene wall, that has holes in the bottom and at the top. After the second section wastewater flows to the second aerated part, which is like the first one, except that it has a moving wall after the fourth reactor, around 40 cm from the outlet. Its purpose is to settle the biomass from the water phase. After this, two simultaneous reactors, the carrier separators receive the wastewater. They have electromagnets inside them to separate the carrier from the sludge. The last step of the technology is the secondary clarifier tank.

The containerized pilot technology is proven to handle 28 m³/day of communal wastewater. The path of the wastewater and the HRT times of the units are shown in *Figure 1*.

The overall retention time in biological reactors is 11.8 hours, which is lower than *Chu and Wang (2011)*'s and *Martin-Pascual et al. (2016)*'s experiments (14 h and 24 h HRT), but almost double that of *Shitu et al. (2020)*, they had 6 h of HRT.

MATERIALS AND METHODS

Pilot experiments lasted from 12.10.2023. to 08.12.2023. In the first period (until 01.12.2023.), point samples were taken from the effluent and the influent for chemical analysis. In this period, the following parameters were measured twice a week for seven weeks:

- 1. Temperature.
- 2. Dissolved oxygen (DO) concentration.
- 3. Chemical oxygen demand (COD).
- 4. Phosphate-phosphorus.
- 5. Nitrite-nitrogen.
- 6. Ammonium-nitrogen.
- 7. Nitrate-nitrogen.

At the end of the experiment, a continuous sampler was set to each end of the container, that took samples every 15 minutes and made an average sample each 2 hours. Besides the same parameters measured in the first period, total nitrogen was also monitored.

MSZ ISO 6060 (COD), *MSZ 1484-13:2009* (nitrate-nitrogen, nitrite-nitrogen), *MSZ ISO 7150-1:1992* (Ammonium-nitrogen), *MSZ 448-18:1977*, *LCK238* (total nitrogen) standards were used during the laboratory measurements. pH, dissolved oxygen, and temperature were measured on-site using a Hach HQ40 portable multimeter.

The pollutant removal efficiency was determined after the first stage of biofilm development, considering the 12 hours of HRT of the biological reactors.

The surface and biofilm formation of carriers were monitored by microscope measurements. The average sample from the four sections was taken. 0.5% triphenyl tetrazolium chloride solution was used in the laboratory to colour the living (active) biomass on the surface of the carriers. Zeiss AXIO Lab.A1 light microscope, and Zen 3.1 (Blue Edition) software was used for taking the microscopic photos. 35-50 pictures of each sample were taken and analysed with Image Pro software to measure the overall diameter (mean, min, max) and area of the carriers, and to measure the area of the active biofilm attached to the surface of the carriers.

RESULTS

The rate of biological processes is strongly determined by temperature and oxygen levels of water, therefore DO level was measured in each section of the biological tanks and temperature was monitored in the influent and effluent (*Figure 2*).

Figure 2. Temperature and DO levels at each reactor space and influent, effluent. 2. ábra. A térrészenként és a be- és elfolyóban mért hőmérsékleti és oldott oxigén értékek

Temperature values are decreasing, due to the colder weather. Aeration was different in the fourth reactor due to the more efficient settling process. In a non-aerated reactor, both flocs and carriers are easier to be settled. In the first reactor Dissolved Oxygen (DO) levels were significantly lower than second and third reactors, that's because of the COD removal processes. At the second and third reactor sections the DO levels stay almost always above 3 mg/l, which is required for the nitrification process. DO concentration of the third reactor exceeded 6-8 mg/l, however, lower values would also be sufficient. In the first part of the experiment in Reactor 1 a higher DO level was aimed to maintain COD removal and nitrification. Later,

the aeration was cut to achieve an anoxic environment necessary for denitrification.

In the first phase of the experiment, it was aimed to monitor the removal rates of the main wastewater pollutants during the growth of the biofilm on the carrier's surface. We observed COD removal indicating that heterotroph organisms begin to grow in aerobic conditions quickly (*Figure 3).* The simultaneous decrease of ammonium-nitrogen concentration shows the build-up of nitrifying biofilm and the efficient process of nitrification despite a significant variation of the pollutant concentrations in the inflow.

Figure 3. COD and NH4-N concentration of inflow and effluent during the biofilm development process 3. ábra. A KOI és NH4-N koncentráció változása a be- és elfolyóban a betelepedés során

The average concentration of chemical oxygen demand of the influent was 580 mg/l; however, the maximum was 1 893 mg/l showing great variability and relatively concentrated wastewater characteristics. Low COD values were reached in the effluent in the first ten days.

The ammonium-nitrogen concentration of the influent shows somewhat less, but still high variability having an average of 36.1 mg/l and a maximum concentration of 99.2 mg/l. The decrease of ammonium-nitrogen concentration to a low level took 4 weeks due to the decreasing wastewater temperatures, and the lower growing rate of autotrophic nitrifying bacteria.

During the biofilm colonisation period, nitrite-nitrogen appeared as an intermediate product of nitrification. The final product is nitrate-nitrogen. The second step of denitrification requires nitrate-nitrogen, anoxic conditions, and easily accessible organic matter for the facultative

heterotrophic microorganisms. *Figure 4* shows the nitrite-N and nitrate-N values over the experiment.

Nitrite-nitrogen appeared in the effluent at the beginning of the colonization process at a high (1-9 mg/l) in the first ten days, after that, its value remained zero, or slightly more than that. Nitrate-nitrogen started to increase after the initial period; it reached its maximum value on 13.11.2023. After that, the settings of the technology were changed significantly: aeration of the first reactor was cut, and the recycling rate was raised. These changes led to decreasing nitrate-nitrogen concentrations in the effluent, however a significant amount (26-36 mg/l) appeared in the effluent through the measurement period. Results suggest that the recycling capacity of the system needs to be further upgraded in the future.

After the startup period (when all necessary types of bacteria could be developed on the surface of the carriers)

we set the continuous measurement device in the upper region of the preliminary clarifier. Effluent samples were taken from the carrier separator. In *Figure 5* we show the results of COD and ammonium-nitrogen measurements.

Figure 4. NO2-N and NO3-N values over the experiment 4. ábra. A NO2-N és NO3-N értékek változása a kísérlet során

Figure 5. COD and NH4-N values over the continuous measurements 5. *ábra. A KOI és NH4-N értékek változása az automata mintavevővel történt mérés során*

Influent COD values varied between 729 mg/l and 1 308 mg/l and the average COD removal rate was 94.3%. The organic removal efficiency was slightly greater than the efficiency by *Goswami and Mazumdar (2016)* who reached (90%) in MBBR, but less than reported by *Erkan et al. (2020)* (98.5%). Ammonium-nitrogen of the influent was more stable, with values remaining in a narrower range (28.8-33.6 mg/l). The average ammonium removal rate was 99.4%, which is much higher than *Chu and Wang (2011)*'s 52% having similar HRT (14 h). *Ashkanani et al.*

(2019) reached an 87.3% ammonium-nitrogen removal rate at 20 °C, which is still lower than in our technology. However, *Shitu et al. (2020)* reached 91.65% ammonium removal by using only a 6 h retention time.

Nitrate-nitrogen shows the denitrifying capacity of the system, and total nitrogen is important to calculate the overall nitrogen removal capacity. *Figure 6* shows the continuous measurements of nitrate-nitrogen and total nitrogen results.

Figure 6. NO³ and TN values over the continuous measurement 6. ábra. A NO³ és ÖN értékek változása az automata mintavevővel történt mérés során

Treated wastewater had a nitrate-nitrogen concentration between 21.8 mg/l and 25.5 mg/l. This shows that a recycling rate would be required to optimize the treatment process. Increasing the recirculation rate is expected to allow quick adaptation of the denitrifying heterotrophic bacteria causing higher nitrate removal rates.

The total-nitrogen content of the inflow was relatively high (80.5-125.0 mg/l; average: 102 mg/l). The average TN removal rate was 75.0%, which is behind *Kermani et al. (2008)*'s results, however they had 24 h HRT and 28 °C. *Phanwilai et al. (2020)* showed 64% and 77% TN removal, with an HRT of 5-11 hours, which is similar to our results, however they had shorter retention time.

Sauer et al. (2002) stated that there are five major stages of biofilm development, the initial reversible and irreversible attachment of microbes, the formation of microcolonies, maturation of the biofilm, and dispersion or detachment of biofilm. It is difficult to determine/measure the quantity of the attached biomass. In our study, biofilm formation was followed based on microscopic measurements applying Image Pro software. The method was found suitable to determine the diameter (mean, max, min) and the surface area of the carriers, as well as the colonisation rate $(\%)$. Colonisation rate was defined by the percentage of the carrier surface covered by biofilm over the total surface. *Figure 7* shows that the average colonisation rate follows the change in the mean diameter of the carriers.

Figure 7. Colonisation rate and diameter values of the carriers over the experiment 7. ábra. A mikrohordozók betelepedettségi arányának és az átmérőjének változása a kísérlet alatt

Colonisation rate quickly rose over 50%, then fell back to around 30%, which is because fresh carriers were loaded into the reactors. After 06.11.2023. colonisation rate values slowly began to rise around 50%. *Barwal et al. (2014)* observed a 70% colonisation rate over the whole carrier surface, and *Bjornberg et al. (2009)* discovered 30-50% on the outer and 100% on the inside of the carriers. It is important to consider that our new micro-carriers are about 100 times smaller than those used in other studies, therefore we reach a higher specific surface area overall. The measured diameter of the inoculated carriers also changes due the biofilm development. It varies between 400 µm and $750 \mu m$. The diagram shows that there is a correlation between the two values, increasing the colonisation rate means increasing measured diameter values.

Figure 8 shows that with different colonisation rates (above 50 and 75 percent, below 15 and 20 percent) the changes of diameter (max – blue, mean – orange, min – grey) values over the experiment.

The first two diagrams (below 15 and 20 percent colonisation rate) show, that the carriers have low interaction with the microorganisms. It is like a control sample, which shows how the carrier's diameter changes due to pumping, mixing, aeration, etc. The first diagram (below 15%) shows a large variation in the values, which makes it harder to analyse trends. The second diagram shows that the carriers have a slight decrease in diameter, which may be caused by mechanical loads like the pumps, or the mixing. However, the carriers have starch and PVA inside them, that can diffuse from the inside and may cause a decrease in size.

The colonisation rate above 75% has similar diagram trends like the below 15%, which is caused by low sample numbers visible in *Figure 9*. The 50% colonisation rate diagram however shows less variability and more sample numbers. It has an increasing-decreasing tendency, which is due to the microorganism flocs depart and rebuilt on the surface area.

Figure 8. Diameter changes according to the colonisation rate 8. ábra. Átmérő változás a betelepültség függvényében

Figure 9. Number of carriers according to the colonisation rate 9. ábra. Mikrohordozók száma betelepültség függvényében

Figure 9 shows the number of carriers equivalent to a certain colonisation rate (15-20-50-75%). Higher numbers mean more accurate analysis in terms of the amount of biomass. Most data belong to a 50% colonisation rate (minimum $9 -$ on 20.10.2023.), while there are sampling dates when only 1 data belong to 15 and 75% colonisation rate. This suggests that below 20 percent and above 50 percent is preferable for biofilm analysis.

Figure 10. Biofilm thickness through the experiment 10. ábra. A biofilm vastagságának változásai a kísérlet során

Biofilm thickness was calculated by subtraction of the mean diameter values of carriers below 20 percent colonisation rate from the mean diameter of carriers above 50 percent colonisation rate and dividing by 2. *Figure 10* shows the changes and the values of biofilm thickness over the measured period. It shows a maximum biofilm thickness of 200 µm, with an average of 140 µm. According to *Barwal et al. (2014)* for a full substrate penetration, 100 µm biofilm thickness is ideal, and *Torresi et al. (2016)* claims that above 200 µm biofilm thickness there is no significant nitrification increase.

CONCLUSIONS

MBBR systems have been a well-studied area in wastewater treatment, there is a lot of information about the biofilm carriers, their specific surface area, and biomass. Also, a lot of knowledge about different types of experiment setups, and their capabilities in terms of ammonium, total nitrogen, or COD removal rate. In conclusion, MBBR is a promising and well-working technology, that has a wide variety of base knowledge. However, with the new micro-carrier developed in the MICROBI R&D project, we can have a significantly more specific surface area, as this carrier is in the size range of micrometres, instead of the traditional carrier's millimetre size. With this monitoring program, which contains the method to paint the living biomass, take many pictures of it, and analyses it with software we can monitor and measure the primal parameters of new carriers, which is going to be useful in calculations required for modelling. This study shows that:

the biofilm on the carrier's surface is between 60 and 200 micrometres, which is optimal according to literature data,

- the biofilm grows and detaches on the surface of the carriers, but overall shows an increasing tendency,
- for biofilm thickness monitoring, the carriers with over 50 percent colonization rate are most suitable,
- the containerized technology can clean communal wastewater up to $28 \text{ m}^3/\text{day}$ in cold weather (12 °C influent) and
- has above 90% of COD and ammonium-nitrogen removal rate,
- also, above 70% of nitrate-nitrogen removal rate.

Further improvements still need to be made, like upgrading the recirculation rate or controlling the DO levels to a minimum for energetic optimization. The results however show that even in these pilot conditions with cold influent, the technology can reach the efficiency found in the literature. With further optimization, it is possible to reach a stable, highly efficient system, thanks to the new type of micro-carriers.

The statistical analysis of microscopic pictures shows promising results about the carrier's diameter changes over time, both in terms of biomass growth and material properties. According to the literature, the examined biofilm developed in our experiments has an ideal thickness and a slightly low colonisation rate.

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