EVALUATION OF NANOFIBER STABILITY AND TOXICITY IN BIOLOGICAL WASTEWATER TREATMENT

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Abstract

Nanotechnology is now used in a range of wastewater treatment applications. As an example, polymer nanofibers are starting to be used as carriers for bacterial populations. The primary advantages of nanofiber technology are its high specific surface area, which allows rapid colonization, and the high stability of the resultant biofilm, thanks to its characteristic surface morphology. A question still remains, however, as to possible leakage of (nano)fibers into the surrounding environment, and what effect these (nano)fibers have. This study assesses a) to what extent nanofibers break away from the supporting-fiber's surface; b) how to prevent or limit any impact of breakaway; and c) how to evaluate possible toxicological impacts of breakaway nanofiber particles on aquatic organisms.

Keywords:

Nanofibers, biomass carrier, breakaway, toxicity, wastewater treatment.

1. INTRODUCTION

The ever-increasing needs of the human race for material goods necessarily leads to an increased capacity for fabrication, which in turn produces more highly-polluted industrial wastewaters and an increase in municipal wastewater volume. This necessitates the use of methods and materials that enable effective and rapid cleaning of such wastewaters. Today, nanoparticles (NPs) are commonly used, e.g. silver NPs [1, 2], zero-valent iron NPs [3], CeO_2, TiO_2 and Fe_3O_4 NPs [4], and magnetic iron oxide NPs [5].

Alongside the growing use of NPs, there are increasing worries about their possible negative effects on microorganisms, higher organisms and on the environment generally. While the actual negative effects of such particles are still not very well known [6, 7], they are most likely to be connected with their release in wastewater treatment plants, where the volume of NPs in use is constantly growing [8, 9]. For example, NPs may accumulate in the bodies of higher life forms following release during the treatment plant cleaning process [7]. There is a clear necessity, therefore, for characterization of treatment, transport routes and activity of such NPs, especially as regards their application in wastewater treatment plants. Universally recognized NP ecotoxicity protocols for organisms are presently unavailable, which could lead to serious problems at wastewater treatment plants [7]. Understanding the treatment and behavior of NPs, as well as their potential toxicity to organisms, now has highest priority.

This paper focuses on gaining an understanding of the treatment and behavior of nanofibers presently used as bacterial biofilm carriers in wastewater treatment plants [10, 11]. The paper also aims to provide a method for nanofiber evaluation, a means to characterize the volume of nanofibers released into the water, and a consequent measure of their potential toxicity to water organisms.

2. MATERIAL AND METHODS

2.1. Characteristics of nanofiber carriers

Testing of fiber breakaway was undertaken using samples of supporting fibers covered with nanofiber PUR-260. Nanofiber carrier is composed of three parts: a basic (supporting) fiber composed of polypropylene Prolenvir (660 dtex) and a coating composed of polyurethane Larithane nanofiber (50 dtex, electrospinning method, diameter approx. 260 nm); both of which are twice-wrapped in a protective polyethylene fiber (167 dtex) for fixation. Three different kinds of nanofiber fixation were selected for testing: a carrier with no fixation wrap, a carrier with thick and sparse fixation fibers and a carrier with thin and dense fixation fibers (Fig. 1).

Fig. 1 PUR nanofiber carriers. (a) with no fixation wrap, (b) with thick and sparse nanofiber fixation, and (c) with thin and dense nanofiber fixation.

2.2. Laboratory-scale model

The experiment took place over four weeks, using 0.2 L volume distilled water bottles containing 15 meters of nanofiber carrier. Tests were undertaken with no bacterial population. For ease of identification on the membrane filter, the nanofiber carrier was dyed with textile color in advance. Carriers were raised in water using an air pump (fine-bubble aeration, intensity 200 L air / hour) and it was the impact of the air bubbles, (specifically, their cutting/shearing forces) that led to fiber breakaway.

2.3. Sampling and evaluation of breakaway (nano)fibers

Each bottle containing a nanofiber carrier was shaken well before sampling, and 50 mL of water suspension taken for analysis. The water suspension sample was then filtered through a 0.22 μm porosity membrane filter. The dried membrane filters were scanned and evaluated for the presence of breakaway fibers and nanofibers (Fig. 2).

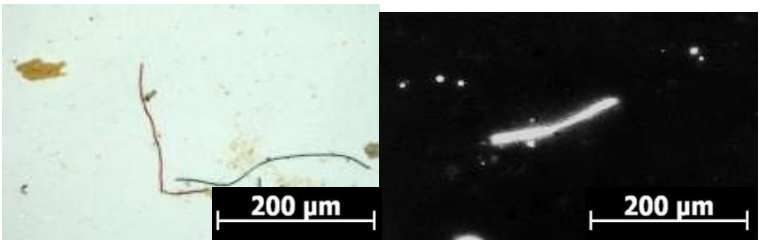


Fig. 2 Example of image collection, surface of membrane filter scanned using an optical (left) and fluorescence (right) microscope.

2.3.1. Evaluation of breakaway fibers using optical microscopy

The dried filters were scanned using an Olympus BX51M optical microscope with a total magnification of 50×. Thirty photographic images were taken of each filter, which were then evaluated using the QuickPHOTO MICRO 2.3 image analysis program. The use of this method (carrier dyeing and evaluation by optical microscope) meant that all breakaway fibers could be counted (i.e. not only nanofibers but also fibers from the supporting and fixation threads).

2.3.2. Evaluation of breakaway fibers using UV fluorescence microscopy

The dried filters were also scanned using a Carl Zeiss Axio Imager M2 fluorescence microscope with a total magnification of 50×. Thirty photographic images were taken of each filter, which were then evaluated using the Matlab (The MathWorks) image analysis program. Compared to the optical microscopy method mentioned above, where all fibers could be evaluated, this method evaluates nanofibers only as the other fibers do not glow under UV fluorescent light. Wrapping fibers were also visible but were not counted as they had visibly different dimensions.

2.3.3. Evaluation of nanofiber carrier surface using fluorescence microscopy

Evaluation of the nanofiber's carrier surface was undertaken using the same method as mentioned in section 2.3.2; though in this case it was the carrier surface itself that was evaluated and not the membrane filter. After following the same laboratory procedure outlined in section 2.2 the fibers were fixed to a glass microscopy slide in order that the same part of the carrier (nanofibers and fixation fibers on the supporting fiber) could be scanned on each occasion (Fig. 3). Any changes to the surface were evaluated using the Matlab (The MathWorks) image analysis program. After image analysis, we calculated the total area suffering some change over a given time (expressed as a percentage).

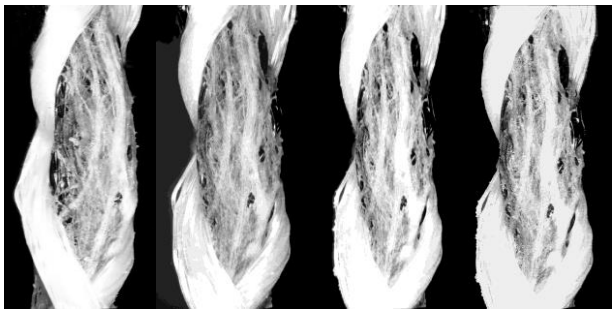
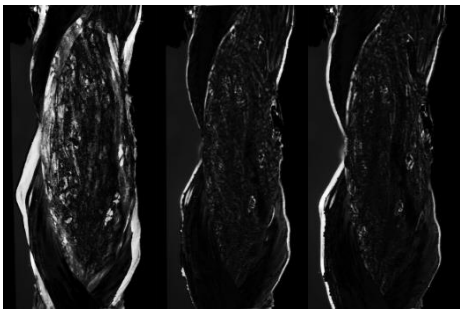
(a)  (b) 

Fig. 3 Outputs of photo-documentation of nanofiber carrier breakaway. (a) Images show the surface of the carrier and changes to the nanofiber and fixed-fiber structures over time (left to right – after 1, 2, 3 and 4 weeks of aeration). (b) Images show the results of image analysis, whereby the left image was evaluated through subtraction of Fig. 3a2 from 3a1; the middle image by subtraction of Fig. 3a3 from 3a2; and the right image by subtraction of Fig. 3a4 from 3a3 and so on for the rest of the images.

2.4. Evaluating nanofiber toxicity to *Daphnia magna*

For the toxicity test, two ca. 260 nm diameter polyurethane nanofiber samples were used (marked as PUR-260). The first sample was of an "aerated nanofiber carrier", whereby nanofibers broke away into solution spontaneously through aeration. The sample was prepared from a 75 m carrier with PUR-260 nanofiber in 1 L of distilled water. The sample was aerated for 28 days. The second sample was prepared by weighing out 100 mg of pure PUR-260 nanofiber and homogenizing the sample in a mechanical mill with the help of siliceous sand. The homogenized sample was then dissolved in 1 L of distilled water.

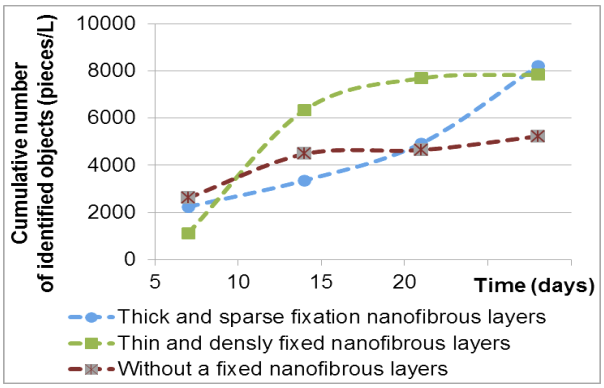
Toxicity testing ("acute immobilization of Daphnia, VP 23/E") was undertaken by an accredited laboratory (The Research Institute of Organic Synthesis a.s., Center for Ecology, toxicology and analytics, Rybitví 296, Pardubice 533 54, Czech Republic. Testing laboratory Nr. 1057 - accredited through the Czech Institute for Accreditation (ČIA) under code ČSN EN ISO/IEC 17025). The methodology used is accredited under ČSN EN ISO 6341 (1997) Water quality - Determination of mobility of *Daphnia magna* (Cladocera, Crustacea), acute toxicity test. Test *Daphnia* were obtained from laboratory breeding by acyclic parthenogenesis (source - Research Institute of Organic Synthesis a.s.) and fed using a standard seaweed mixture. Ten *Daphnia* specimens were used for each concentration and for the control in the preparatory test, and 20 *Daphnia* for each concentration and control in the limit test. Limit tests were undertaken in triplicate. Immobilization of *Daphnia* was observed after 24 and 48 hours of the test.

3. RESULTS AND DISCUSSION

3.1. Evaluation of breakaway fibers

Breakaway fibers were measured as "number of released fibers captured on the filter's surface" per 1 L of water (in Fig. 4, the parameter is expressed as a cumulative value). Fiber breakaway from the carriers changed over time (under these specific conditions). Differences in the methodology used (optical vs. fluorescence microscopy) and in image analysis undertaken tended to result in different numbers of objects (fibers) detected, the optical microscope capturing all fibers, and especially the supporting and fixation fibers. Fluorescence microscopy, on the other hand, captured nanofibers only due to their reaction to UV light. Nanofibers were dominant in water suspension.

(a)



(b)

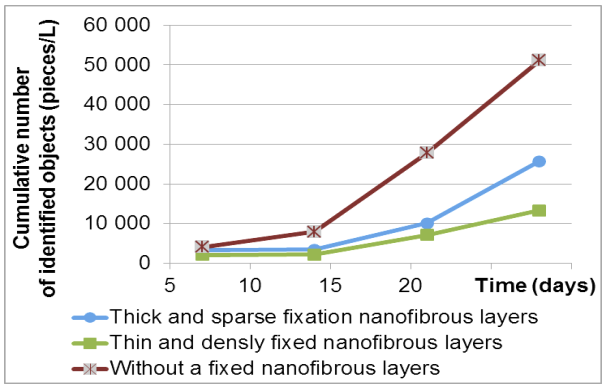


Fig. 4 Quantification of fiber breakaway. (a) The cumulative number of all fibers present in suspension (especially larger fibers) evaluated using an optical microscope; and (b) the cumulative number of nanofibers only, evaluated using a fluorescence microscope.

Under optical microscopy, all carrier types indicated a relatively large breakaway of fibers immediately after the start of the experiment (Fig. 4a). During the following two weeks, the number of (micro)fibers breaking away from the "thin and densely fixed" carrier increased rapidly; the maximum rate being reached sometime around the 15th day of aeration. After the first two weeks, fiber breakaway appeared to stabilize, with few or no more large fibers breaking away. In comparison, the release of fibers from yarn with a "thick but sparse" nanofiber fixation layer gradually increased over time. This was mainly due to a gradual release of the thick fixation fibers themselves (see Figs. 3 & 4). The fiber release process continued throughout the course of the experiment, and appeared to show an increasing trend after the first two weeks. Release of fibers from yarn with no nanofiber fixation was lower, because there is a small amount of (micro)fibers (no fixation fibers were present). While the initial release of fibers from yarn with no nanofiber fixation was highest, breakaway appeared to stabilize after two weeks and total fiber release remained relatively stable thereafter.

Based on fluorescence microscopy (Fig. 4b), yarn with no nanofiber fixation showed highest breakaway values. Total number of fibers released by the carrier with no fixed nanofiber layer was approximately twice that for "thick but sparse" nanofiber fixation, while the total number of fibers released by the carrier with a "thin and densely" fixed nanofiber layer was approximately half that for thick but sparse nanofiber fixation. Even by the end of the 4th week, however, many nanofibers were still to be seen on the filter. No nanofiber breakaway stabilization was observed during the experiment. The results suggest that, while nanofiber breakaway gradually declines due to fixation of the nanofiber layer, breakaway continues from the fixation fibers themselves, and especially from the "thick and sparse" fixation type.

3.2. Evaluation of nanofiber surface carrier using fluorescence microscopy

Due to its higher observed rate of nanofiber breakaway (see Figs 4a & b), only the "thick and sparse" nanofiber fixation was used for this evaluation.

Our data suggest that the shearing forces during air filtration result in a gradual slackening of the fixed fibers, i.e. release of fixation. Changes in fixation fiber structure (movement or breakaway) were around 8.5 % at the beginning of the experiment; with structural changes gradually declining to around 1 % after 28 days (Fig. 5). Fixation fiber release declined gradually during the experiment, with a possible 'leveling off' in the rate of change (movement or breakaway) beginning at around day 20.

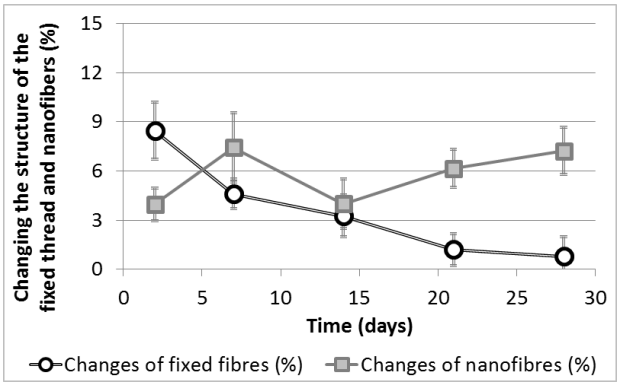


Fig. 5 Results of nanofiber carrier surface structure evaluation.

Changes in nanofiber structure (movement or breakaway) followed an altogether different pattern. The rate of modification fluctuated between 4 and 7 % throughout the experiment, i.e. it stayed relatively stable throughout. Changes in nanofiber structure were still occurring at approximately 7 % even after 4 weeks.

Overall, the results of breakaway evaluation indicate that, while nanofibers can be used for slow-growing microorganisms, there is cause for concern regarding fiber breakaway, though more tests are needed to confirm our results. We suggest that nanofiber carriers should be modified prior to actual application, i.e. the carrier should be 'fixed' by washing with running water to remove free fibers and the output water separated from the reactor through filtration or sedimentation, e.g. in the settling tank. Realistically, it can be assumed that nanofiber layers will be colonized by microorganisms immediately after installation into biological reactors; hence the resulting number of breakaway fibers will be much lower (i.e. the bacterial biofilm will prevent breakaway).

3.3. Evaluation of nanofiber toxicity to *Daphnia magna*

A byproduct of the use of nanofibers as bacterial biomass carriers is the release of breakaway (nano)fibers into the surrounding aqueous environment. Previous studies [10, 11] suggest that there is no toxicity risk to microorganisms from such nanofibers; however, there is a risk of toxic action in aquatic animals, and especially higher organisms, through bioaccumulation. As yet, nanofiber decomposition in biological systems has not been precisely defined, partly as a consequence of an absence of appropriate methods [7]. Similarly, there is no appropriate toxicity protocol for evaluation of polymer nanofiber toxicity. The methodology presented in this paper, therefore, represents the first theoretical experimental procedure.

No statistically significant immobilization of *D. magna* was observed for the "aerated nanofiber carrier" sample; hence EC50 values could not be determined.

Similarly, no statistically significant immobilization of *D. magna* was observed for the "100 mg of pure PUR-260 nanofiber" sample; hence EC50 values could also not be determined.

Toxicity analysis, therefore, revealed no significant direct effect of nanofibers on higher organisms (*D. magna*). Note, however, that the experimental procedure was not able to assess the extent to which NPs (nanofibers) bioaccumulate in organisms; a factor that could be crucial in the food chain. This factor, therefore, requires further verification.

4. CONCLUSIONS

Synthetic polymeric nanofibers are generally non-biodegradable. Consequently they have high potential for bioaccumulation reasoning for concern as regards their application. A lack of knowledge regarding nanofiber ecotoxicity, and the absence of adequate analytical methods, further adds to the uncertainty. Reliance on a standard biological toxicity test alone is insufficient; any evaluation of potential toxic effects of nanofibers will require the use of multiple animal species, and especially those that are usually found in or around wastewater treatment plants or their receiving waters.

Our results also indicate that nanofiber breakaway continues after more than one month. Use of appropriate methodology, such as binding nanofibers to the supporting surface with an appropriate fixed yarn, appears to prevent large-scale nanofiber breakaway into receiving waters; however, there is still a need to examine the potential for nanofibers bioaccumulation.

ACKNOWLEDGEMENTS

This research was supported by a state subsidy of the Czech Republic within the ALFA project "Modified biomass carriers for wastewater treatment - TA01021764". The research reported in this paper was supported in part by Project OP VaVpI of the Centre for Nanomaterials, Advanced Technologies and Innovation CZ.1.05/2.1.00/01.0005.

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