DEVELOPMENT OF NANOFIBER CARRIER FOR MONITORING OF BIOMASS AT A CONTAMINATED SITE

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Abstract

The main goal of this work is to develop an effective method for biomass sampling to monitor the microbial diversity at contaminated sites.

Within this work, various nanofiber carriers suitable for sampling of biomass and simultaneously for use in molecular-genetic analysis were developed. Two different shapes of a carrier with three different nanofiber densities (including carrier without nanofibers) were prepared and submerged into wells with different contamination (chlorinated aliphatic hydrocarbons and BTEX). Carriers were sampled in one-month interval for one year. To compare the biomass growth on carriers, groundwater samples were also taken.

DNA from samples was isolated by FastDNA SPIN Kit for Soil and measured on Qubit 2.0 fluorometer. Extracted DNA was used as a template for real-time PCR amplification. 16S rDNA gene was used as a total bacterial community marker. Specific genes as *vcrA* and *catechol-2,3-dioxygenase* were measured to detect ongoing reductive dechlorination, BTEX degradation respectively.

Long-term tests of carriers revealed their suitability for biomass sampling as real-time PCR values measured on carriers oscillated closed to the groundwater samples. The small variance in detected values indicated long-term stability of biofilm present on carriers. Moreover, use of carriers is not time-consuming, very easy to use and no sample preparation is required prior to DNA extraction. The developed nanofiber carriers can be, therefore, effectively used for biomass sampling.

Keywords: Biomass carriers, monitoring, nanofibers, real-time qPCR, sampling

1. INTRODUCTION

Soil and groundwater pollution is a serious environmental problem. To solve this issue, natural microbial processes can be used and supported towards contaminant biodegradation. Characterisation, including proper sampling, of the present microbial community is, therefore, a crucial factor in understanding and controlling remediation processes. It is clear, that sampling must not impact the situation at a site and should be technically and economically feasible at the same time. To characterise microbial community at a contaminated site, soil or water samples can be withdrawn.

Although soil samples typically provide good results, their sampling is limited by a low reproducibility and time and financial demand. Hence, groundwater samples are currently used for the molecular-genetic analysis. A disadvantage of water samples is usually low biomass concentration requiring the filtration of large volume of sample through a membrane filter. It is, however, time-consuming and strongly depending on the sample properties [1].

Above mentioned problems can be avoided by sampling of biomass in form of biofilm which is formed on suitable carriers. Such carriers have to be made of materials with good colonization of microbial cells, high biocompatibility, chemical and physical stability and convenient morphology. For the molecular-genetic analysis, it is crucial that the carrier material does not interfere with DNA extraction. As nanofiber carriers meet the above-mentioned conditions, they were used for carrier's preparation. Nanofibers have a high specific

surface, an interconnection structure and surface roughness on the nano and micro scale. Surface with these properties made of the hydrophobic material is preferentially colonized by bacterial cells [2].

2. MATERIALS AND METHODS

2.1. Nanofiber carriers

Polyurethane nanofibers (Larithane 1083) were prepared by the needleless electrospinning from a free liquid surface using a high voltage source. Polyurethane was chosen because it proved great microbial colonization in previous experiments. The nanofibers were deposited on polyester silk support thread (SLOTERA, 167f36x1x1) with surface densities 3 dtex (0.34 $g \cdot m^{-2}$) and 10 dtex (1.12 $g \cdot m^{-2}$). Such prepared thread was arranged into two different shapes, planar (**Figure 1a**) and circular (**Figure 1b**). As reference carriers thread without nanofibers were prepared.

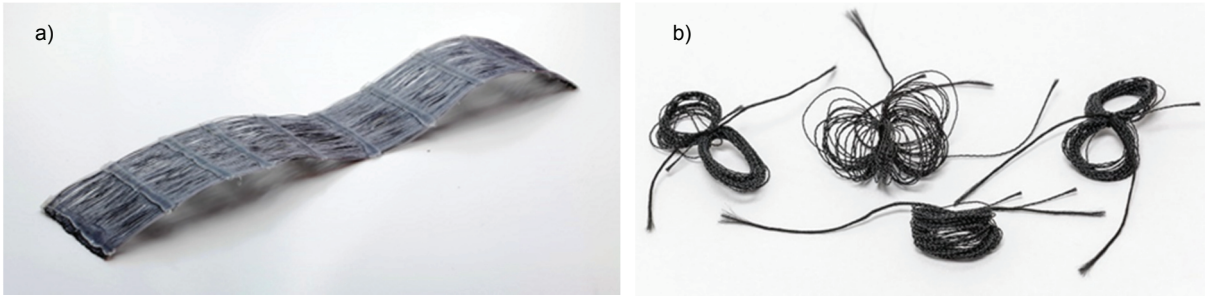

Figure 1 - Tested nanofiber carriers: a) planar arrangement, b) circular arrangement

2.2. Site description

The nanofiber carriers were fixed in sampling tubes (**Figure 2a**) and submerged into contaminated wells. Wells V1 and V2 were contaminated mainly by chlorinated ethenes and well V3 by BTEX as a result of previous industrial production. Wells V1 and V2 were on the same groundwater flow with direction from V2 to V1. From each well six carriers, 3 planar (nanofiber density 0, 3 and 10 dtex) and 3 circular (nanofiber density 0, 3 and 10 dtex) arrangements, were monthly sampled for molecular-genetic analysis (**Figure 2b**). The groundwater sample was also taken to compare with carriers. The monitoring period lasted for one-year.

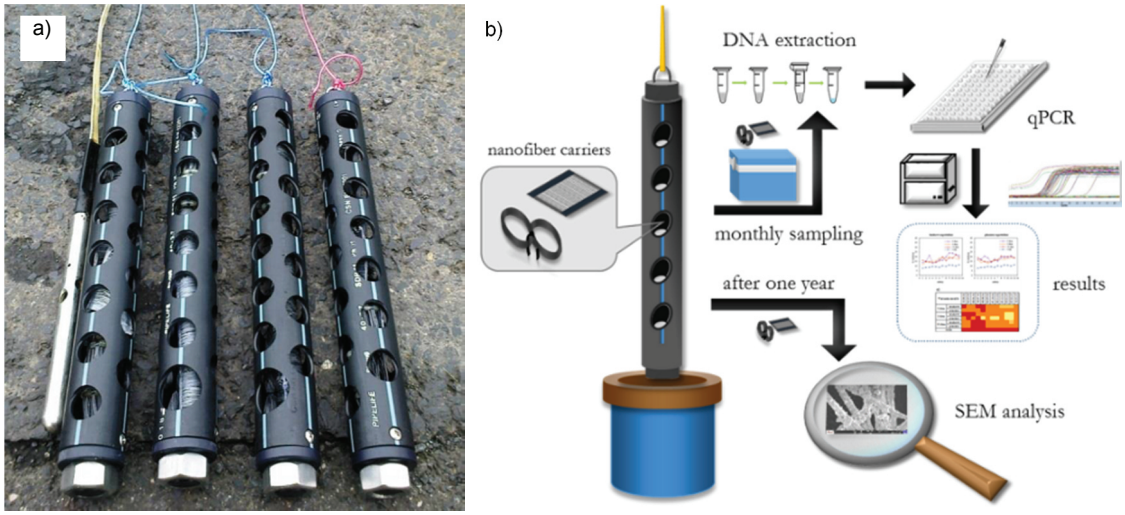

Figure 2 - a) Sampling tubes with nanofiber carriers; b) scheme of monitoring procedure

2.3. Molecular-genetic analysis

DNA was isolated by FastDNA SPIN Kit for Soil. Extracted DNA was used as a template for real-time quantitative polymerase chain reaction (qPCR) using SYBR Green I Master (Roche). The qPCR analysis was used to detect the total bacterial biomass and the presence of key enzymes and microbial consortia involved in the biodegradation of present contaminants. All primers used are displayed in **Table 1**.

Table 1 - Primers used in the study

Primer	Gene	Sequence (3‘ → 5‘)	Amplicon length (bp)	Reference
U16SRT	16S rDNA	**F**: ACTCCTACGGGAGGCAGCAGT **R**: TATTACCGCGGCTGCTGGC	180	[3]
vcrA	vinyl chloride reductase	**F**: CCCTCCAGATGCTCCCTTTA **R**: ATCCCCTCTCCCGTGTAACC	139	[4]
DHC-RT	*Dehalococcoides mccartyi*	**F**: GGGAGTATCGACCCTCTCTG **R**: CGTTYCCCTTTCRGTTCACT	191	[5]
DEF/G	catechol-2,3-dioxygenase	**F**: CGACCTGATC(AT)(CG)CATGACCGA **R**: T(CT)AGGTCA(GT)(AC)ACGGTCA	239	[6]
bssA	benzylsuccinate synthase	**F**: GACATGACCGACGCSATYCT **R**: TCGTCGTCRTTGCCCCAYTT	794	[7]

16S rDNA gene was used as a total bacterial community marker. Specific genes were used for detection of ongoing reductive dechlorination (*vcrA,* DHC-RT) or BTEX degradation (*bssA*, DEF/G). The gene encoding vinyl chloride reductase was detected by primer *vcrA*. Primer DHC-RT was used to quantify 16S rDNA gene of *Dehalococcoides mccartyi* which is capable of complete reductive dechlorination of PCE to ethene [4]. Primer *bssA* was used for quantification of gene encoding benzylsuccinate synthase involved in anaerobic toluene degradation [7]. The catechol-2,3-dioxygenase gene was quantified using primer DEF/G as it is responsible for aerobic degradation of aromatic hydrocarbons by cleaving the aromatic ring [6].

2.4. SEM analysis

The long-term stability of nanofibers on support thread was evaluated by scanning electron microscope (SEM) Carl Zeiss ULTRA Plus. Carriers from last sampling run, i.e. carriers after 12-month exposition of real condition at the contaminated site, were subjected to this analysis.

3. RESULTS AND DISCUSSION

3.1. Effect of carrier characteristics

The analysis of the samples from well V1 showed higher biomass growth on carriers with nanofibers than on carriers without nanofibers at the 1st sampling (**Figure 3**). This confirms the assumption that nanofibers support bacterial surface colonization. A sharp decline in Ct values[1] detected between the 3rd and 4th sampling was probably caused by presence of substances inhibiting DNA isolation. These inhibitors apparently adhered to the carrier surface after the first sampling and released after the 3rd sampling. After this decline, the Ct values detected on carriers stabilised and reached similar levels as the Ct values obtained in groundwater samples. Moreover, stable biofilm on nanofibers carriers was observed over monitoring period.

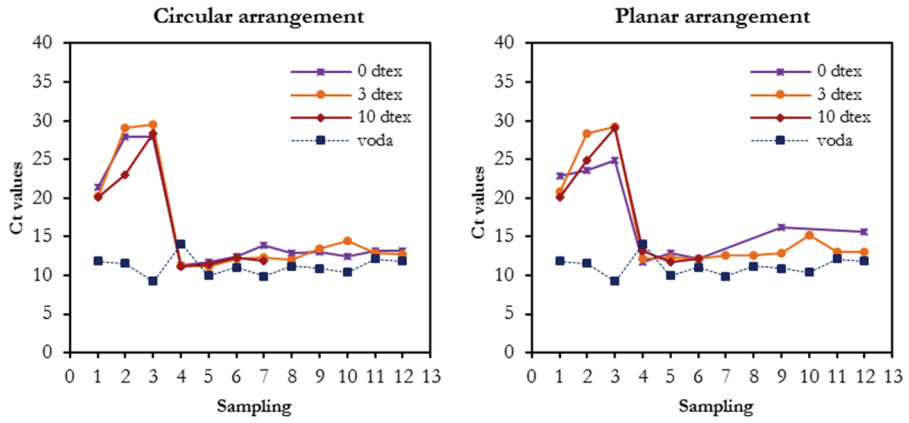

Figure 3 - Evolution of mean Ct values of primer U16SRT (total bacterial community) in the well V1

[1] Note: Higher Ct values mean lower amount of amplified product, thus target DNA, analysed and vice versa.

No sharp decline in Ct values was observed in the well V2 which was upstream from V1 (**Figure 4**). In this well, the stable biofilm was observed on carriers with nanofibers during a few samplings unlike carriers without nanofibers. Circular arrangement of carriers with nanofiber surface density 3 dtex showed most stable development of Ct values of all carriers tested.

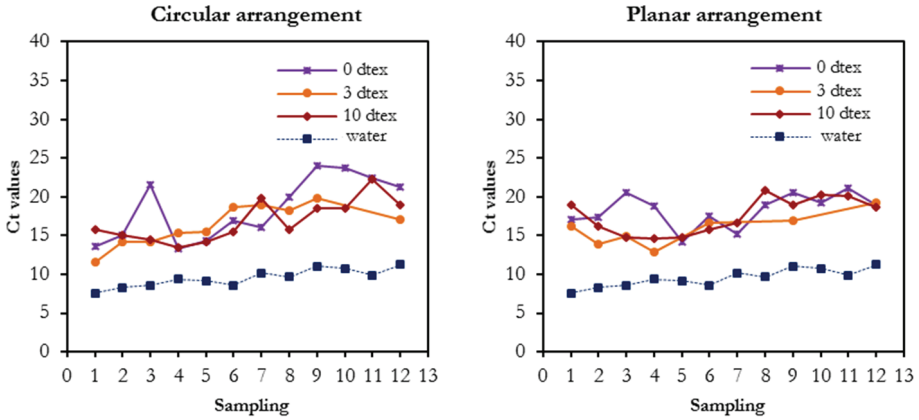

Figure 4 - Evolution of mean Ct values of primer U16SRT (total bacterial community) in the well V2

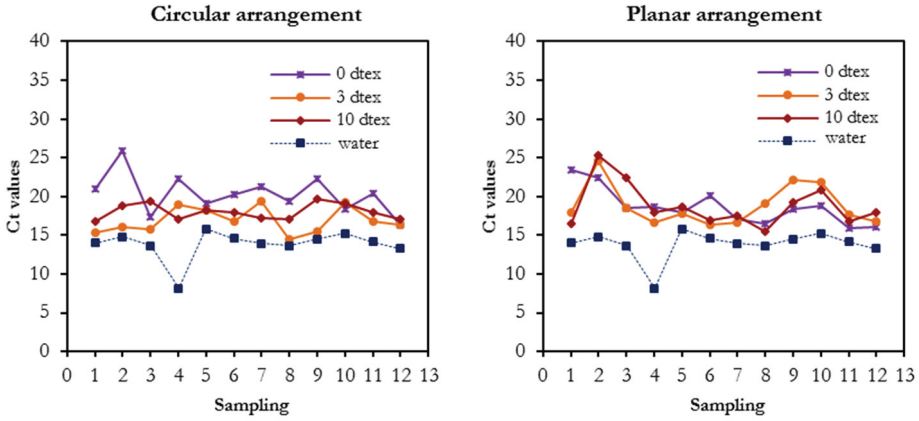

Figure 5 - Evolution of mean Ct values of primer U16SRT (total bacterial community) in the well V3

In the well V3, which was contaminated by BTEX, the biofilm stability was observed on the circular arrangement of carriers with nanofibers. The Ct values of other carriers oscillated within 5 cycles of groundwater Ct values. Evolution of bacterial biomass on circular carriers (10 dtex) was comparable with groundwater.

3.2. Long-term monitoring

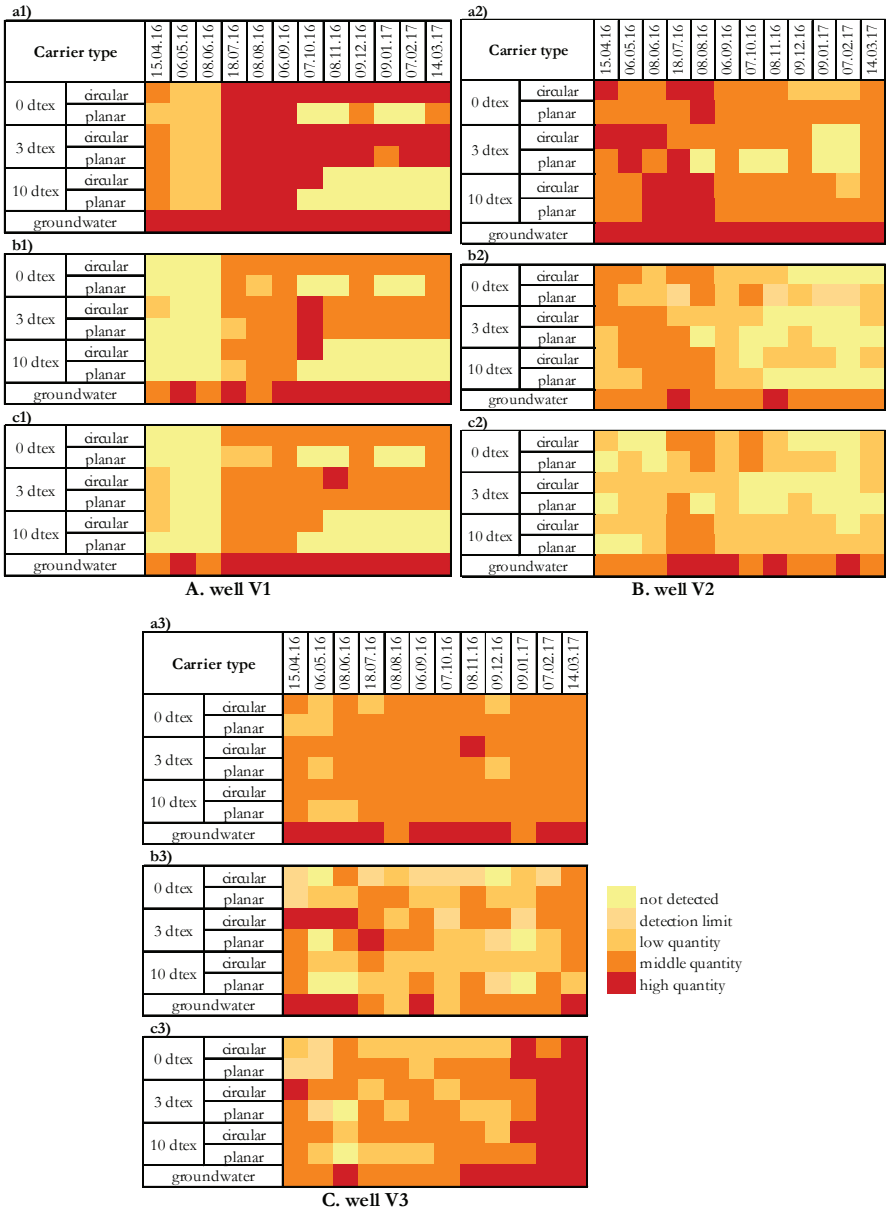

Figure 6 - Heat-maps of Ct values detected in: A. well V1 (a1 - primer U16SRT; b1 - primer DHC-RT; c1 - primer *vcrA*); B. well V2 (a2 - primer U16SRT; b2 - primer DHC-RT; c2 - primer *vcrA*); C. well V3 (a3 - primer U16SRT; b3 - primer *bssA*; c3 - primer DEF/G)

The qPCR results of 12-month monitoring are interpreted through heat-maps. The Ct values of the primer were, therefore, divided into five intervals, each indicated by appropriate colour. In the Ct values of specific primers DHC-RT and *vcrA* in the well V1 (**Figure 6b1, c1**), the same sharp decline as in total bacterial biomass (**Figure 6a1**) was observed. Small variances of Ct values of specific primers (DHC-RT, *vcrA*) indicate stable biofilm containing specific bacterial population responsible for reductive dechlorination. **Figure 6a1** shows high and stable level of total bacterial biomass in the well V2. In this well, the Ct values of primer DHC-RT, which was used for detection of the bacterial strain *Dehalococcoides mccartyi*, were high since the 1st sampling

(**Figure 6b2**). While Ct values of *vcrA*, used for detection of gene of vinyl chloride reductase, were highest after 3rd sampling (**Figure 6c2**). This evolution of specific primers of Ct values indicated ongoing reductive dechlorination of the present chlorinated ethenes. In the well V3, high amount of total bacterial biomass was observed throughout the monitoring (**Figure 6a3**). The presence of specific genes of aerobic (catechol-2,3-dioxygenase, primer DEF/G) and anaerobic (benzyl succinate synthase, primer *bssA*) biodegradation of BTEX was confirmed by analysis of specific bacterial population (**Figure 6b3, c3**).

3.3. Stability of nanofibers

After 12-month exposure to actual conditions at the contaminated site, no significant damage of nanofiber layer was observed though SEM, thereby confirming the stability of nanofiber layer (**Figure 7**).

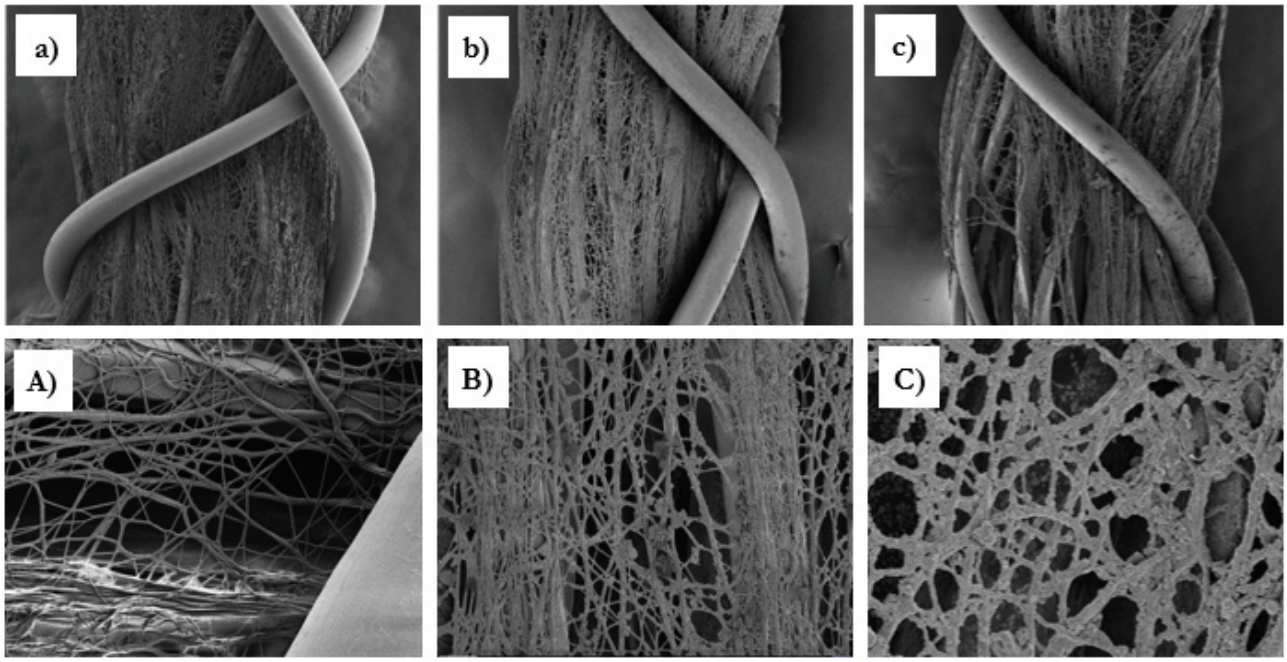

Figure 7 - SEM micrographs of thread with nanofibers 10 dtex: before the experiment (a - 100×, A - 1 000×), from well V2 (b - 100×; B - 1 000×) and from well V3 (c - 100×; C - 2 500×)

4. CONCLUSION

This work showed that nanofiber layer supported microbial attachment and biofilm stability on carriers. The best results were obtained for circular carriers 3 dtex. Specific bacterial population detected on carriers indicated ongoing bioremediation processes, i.e. reductive dechlorination and aerobic and anaerobic BTEX biodegradation. Although any significant differences between carrier shapes in microbial growth were not observed, circular carriers need no pre-treatment prior to DNA extraction in contrast to the planar arrangement. Long-term stability of nanofiber layer on support thread was also confirmed. Developed nanofiber carriers are also very easy to use, therefore, this is a promising tool for effective monitoring of contaminated sites.

ACKNOWLEDGEMENTS

Project No. TA04021210 „Microbial colonization of the fiber surface for analytical and diagnostic practice and technical applications“ (TAČR), project No. LO1201 and grant No. SGS 21176/115 is gratefully acknowledged. The authors acknowledge the assistance provided by the Research Infrastructures NanoEnviCz (Project No. LM2015073) supported by the Ministry of Education, Youth and Sports of the Czech Republic and the project Pro-NanoEnviCz (Reg. No.

CZ.02.1.01/0.0/0.0/16_013/0001821) supported by the Ministry of Education, Youth and Sports of the Czech Republic and the European Union - European Structural and Investments Funds in the frame of Operational Programme Research Development and Education.

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