Structural and functional changes in biofilm during adaptation towards amaranth biodegradation

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ABSTRACT
In the field of the microbial ecology of biofilms and activated sludges, it is widely accepted that the microstructure of the communities depends on the environmental factors. Nevertheless, due to their complexity, the exact mechanisms are still unknown. In this study, we applied a stepwise increase of an azo-dye concentration as a selective factor for adaptation towards biodegradation. The degrading biofilm was developed in a lab-scale sand biofilter. It functioned in a semi-continuous regime for 623 h. The concentration of the azo-dye amaranth was increased from 10 to 55 mg L⁻¹. The effectiveness was 90% and the rate of amaranth elimination was 1.136 mg h⁻¹. The fluorescence in-situ hybridisation (FISH) revealed zones with high activity of Pseudomonas sp. Also increasing importance of the unculturable Pseudomonas sp. and the relationships in the biofilm were found. At the final stage of the experiment, a decrease of the azoreductase activity and an increase of the catechol-1,2-dioxygenase activity were established in the depth of the biofilter. The obtained results were linked with different Pseudomonas microstructures (shown by FISH). The obtained data showed that the changes in the biofilm structure occurred accordingly to the biodegradation of the toxic compound and it included the development of cooperative microbial relationships in the key genus Pseudomonas.

Introduction
In 1856 the first synthetic dye was discovered by Sir William Henry Perkin. Its color was purple and it was used on the fabrics for Queen Victoria of England and Empress Eugenie, wife of Napoleon Bonaparte [1]. By the end of the nineteenth century, more than 100 000 synthetic dyes were found with an annual production of more than 0.7 million tons [2]. Among the largest synthetic dyes consumers are the printing industry, the textile industry, the plastic production companies, the photography, the cosmetics, the pharmaceutical industry, etc [3]. Azo-dyes are the most widely used type of dyes in the textile industry [4]. Because the adsorption of all of the dye stuff to the fibers is impossible, 2-50% of the colorants remain in the wastewater [5]. The azo-dyes pose serious environmental and health risks. Some of the dyes can be seen when present in concentrations lower than 1 mg L⁻¹ [5]. When colored wastewater is present in the natural water bodies it can diminish the light penetration. This affects negatively photosynthesis and as a result - the oxygen concentrations. Another big issue is the toxicity of the dyes which have recalcitrant xenobiotic molecules [6,7].

Different physico-chemical and chemical methods for dyes removal have been developed but they have serious drawbacks such as generation of sludge, high operating/energy costs and production of byproducts [4]. One of the most promising non-biological groups of methods for dye removal is membrane-based. These technologies include the use of a porous membrane that separates the contaminants from the water [8,9]. They have simple operation and are very suitable for the practice. Their main drawbacks are related to the concentration of the pollutants that have to be treated additionally and the fouling of the membranes [10]. The biological methods for the treatment of dye contaminated wastewater are considered suitable, non-costly and flexible means for achieving highly efficient water detoxification [11]. Many organisms have the ability to degrade azo-dyes – bacteria, fungi and algae [12]. Bacteria are microorganisms known for their ability to adapt to hostile environments. That is...
why they possess impressive diversity of enzymes that
degrade most of the known xenobiotics, including the
azo-dyes [5,13].

The biological methods for the treatment of
azo-dyes contaminated wastewater are based on aer-
obic and anaerobic regimes [14]. Both stages are usu-
ally needed because of the chemistry of the azo-dyes
biodegradation. In the absence of oxygen, the azo
bond in the dyes molecules is reductively cleaved. This
leads to decolorisation and accumulation of toxic aro-
matic amines. Their full biodegradation requires the
presence of oxygen [3]. It is known that the complete
azo-dye biodegradation is achieved more easily when
microbial communities are used compared to the cases
with pure cultures [15].

In complex microbial communities, more diverse
and sophisticated degrading mechanisms can evolve.
Their nature is still poorly understood. The aim of this
study is to contribute to the elucidation of bacterial
catabolic interplay during azo-dye elimination.
Information for this is obtained by the biodegradation
parameters, the enzymological activity of the commu-
nity and conventional and FISH analysis.

Materials and methods

Work hypothesis

In our previous studies a characteristic pattern of
development of the biodegradation activity of the
microbial communities with gradual unlocking of dif-
ferent biodegradation mechanisms (key enzyme activ-
ities) was found. Similar data were also obtained by
other research groups [16,17]. For the present study
as a model process, we used the biodegradation of
the azo-dye amaranth (C.I. Acid Red 27). It is known
that the biodetoxification of the azo-dye amaranth is
in two phases. The first phase is the reduction of the
azo-bond by the enzyme azoreductase (AzoR). The
second phase includes the cleavage of the benzene
ring of the aromatic amines generated during the first
phase. This step is performed mainly by ortho-
meta-mechanisms, respectively with the participation
of the enzymes catechol-1,2-dioxygenase and
catechol-2,3-dioxygenase.

We hypothesised that the processes of development
of the biodegradation potential are tightly related not
only to the key enzymes activities but also to the
formed microorganism’s relationships which are formed
in the community. We selected the genetic technique
FISH (fluorescence in-situ hybridization) for investigat-
ing the micro-distribution of a key genus of microbial
degraders (g. Pseudomonas). The main advantage of

Experimental design

In the experiments, a lab-scale down-flow sand biofil-
ter was used (Figure 1). The working volume of the
model biofilter was 192 cm$^3$. The depth of the biofilter
layer was 3 cm. In our experiments, the biofilter layer
was divided into three parts: a first upper part, where
the concentration of the substrates was the highest,
a second middle part, where their concentration was
lowered by the microorganisms and a third bottom
part, where the biodegradation mainly affected the
intermediates passed from the upper two layers. The
period of functioning was 26 days. To ensure a con-
stant flow of the wastewater, the biofilter was con-
nected to a peristaltic pump. The height of the sand
layer was 30 mm, the flow of the wastewater varied
between 520 – 790 ml day$^{-1}$, COD was 550-600 mgO$_2$
L$^{-1}$ and the concentration of the total organic carbon
(TOC) was 230 – 245 mg L$^{-1}$.

We applied a gradual increase of the inflow concen-
tration of amaranth from 10 mg L$^{-1}$ up to 55 mg
L$^{-1}$. The dosing was controlled by a peristaltic pump
connected to a relay which additionally decreased the
flow of the wastewater entering the biofilter. The ama-
ranth concentration was determined for each portion
of model wastewater passing through the pump.

The detoxification process was separated into three
time stages: an early stage (0 h – 191 h), a late stage
(191 h – 455 h) and a final stage of the process (455 h
– 623 h). During the three stages, the amaranth concen-
tration was increased while considering the activity
of the biofilm. This was monitored using the following
indicators: the rate and the effectiveness of decolori-
sation, the quantity of the biomass dropping from the
biofilm and the activities of the key enzymes. The

Figure 1. Design of the model biofilter.
The elimination rate of amaranth was calculated with the following formula (2):

\[ V(\text{mg h}^{-1}) = C_{\text{res}} \times Q \]  

where \( C_{\text{res}} \) is the concentration difference of amaranth in the influent and the effluent in mg mL\(^{-1}\), \( Q \) is the flow rate in mL h\(^{-1}\).

**Enzyme activities**

For determining the key enzyme activities cell-free extract was used (obtained by the method of Ref. [19] in modification by Ref. [20]).

For the study of the AzoR activity/EC 1.7.1.6 we used the method by Ref. [21]. Catechol-1,2-dioxigenase activity (C12DO)/EC 1.13.11.1 was defined by the method of Ref. [22]. The accumulation of the product of the reaction cis, cis-muconic acid was monitored at 260 nm. The succinate dehydrogenase activity (SDH)/EC 1.3.5.1 was determined by the method described by Ref. [23] by measuring the accumulation of fumarate at 455 nm. All the three enzyme activities were indicators for the rate of the key stages from the biodegradation of the amaranth: AzoR activity was an indicator for the reduction rate of the azo-bond, C12DO was an indicator for the rate of benzene rings cleavage and detoxification and SDH activity provided information for the metabolic activity of the microorganisms as well as for performing full biodegradation of the azo-dye.

The total protein content was determined by the micro-biuret method using bovine serum albumin as a standard.

**Microbiological assays**

The abundance of the key microbial groups in the biofilm was studied by using the plate count techniques [24]. The azo-degraders (AzOD) were cultivated on Nutrient agar (HiMedia Laboratories) with 50 mg L\(^{-1}\) amaranth. The colonies with decolorisation of the medium were count. The aerobic heterotrophs were cultivated on Nutrient agar (HiMedia Laboratories), and the bacteria

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**Quantity of carbon-containing pollutants (TOC, COD)**

The chemical oxygen demand (COD) was measured according to the standard procedure [18]. The total organic carbon (TOD) was measured with TOC-V\(_{\text{CPN}}\) Analyzer (Shimadzu Corp.).
from genus *Pseudomonas* were cultivated on Glutamate Starch *Pseudomonas* Agar (HiMedia Laboratories). The ratio of the two key groups - *Pseudomonas* sp. and AzoD were calculated on the base of aerobic heterotrophs (cultivated on Nutrient agar (HiMedia Laboratories)) with the following equations [3,4]:

\[
\text{Ratio of culturable } Pseudomonas (\%) = \left( \frac{N_{Ps}}{N_{AH}} \right) \times 100 \tag{3}
\]

where \(N_{Ps}\) is the number of *Pseudomonas* sp. in CFU g\(^{-1}\) and \(N_{AH}\) is the number of aerobic heterotrophs in CFU g\(^{-1}\).

\[
\text{Ratio of culturable AzoD(\%) = } \left( \frac{N_{AzoD}}{N_{AH}} \right) \times 100 \tag{4}
\]

where \(N_{AzoD}\) is the number of azo-degrading bacteria in CFU g\(^{-1}\) and \(N_{AH}\) is the number of aerobic heterotrophs in CFU g\(^{-1}\).

**FISH (fluorescence in-situ hybridisation)**

Samples were taken from the three layers of the biofilm (upper, middle and bottom). The samples were fixed in 4% paraformaldehyde. The dehydration and permeabilisation were carried out according to Ref. [25]. A fluorescent signal was obtained by a 5'-labeled oligonucleotide probe (5'- GCC GGC CTA GCC TTC -3') with fluorescent dye Cy3. As a negative control, the non-sense probe NON338 (5'- ACT CCT ACG GGA GGC AGC -3') was used [26]. The hybridisation was performed with 20% formamide. After the hybridisation, the samples were counterstained with DAPI (4',6-diamidino-2-phenylindole) (AppliChem GmbH). The images were taken with a fluorescent microscope Leica Microsystems DFC310FX, under 400 times magnification. The digital processing of the images was done with the software daim (27). The ratio of the hybridised *Pseudomonas* sp. to the total quantity of the microorganisms was calculated on the base of the images with DAPI.

**Statistical analysis**

All data are mean values from three independent repetitions. The results were assessed according to Student & Fisher [28].

**Results**

The present study demonstrated spatial rearrangement in the biofilm community degrading azo-dye in heavily contaminated wastewater. The gradual increase of the amaranth concentration was used as a selective factor for the development of amaranth degrading biofilm. The increasing toxicity provoked the adaptive changes in the biofilm. The microorganisms from the key genus *Pseudomonas* participated in a cooperative relationship and formed the necessary for this kind of relations specific spatial distribution. Studied with FISH this effect looked like clustering of cells with strong fluorescence which indicated a high metabolic activity. They formed 'hot spots', where the detoxification catabolic processes were very active.

**The early stage of the wastewater treatment**

At the early stage of the functioning of the lab-scale biofilter (0h – 191h), the inflow concentration of amaranth increased from 10 mg L\(^{-1}\) to 30 mg L\(^{-1}\). The effectiveness of elimination of the model xenobiotic was 88.35%. COD of the effluent was 372.74 mgO\(_2\) L\(^{-1}\), and TOC – 116.02 mg L\(^{-1}\). At this early stage, the biofilm community showed initial adaptation towards amaranth biodegradation. This was proved by the increase of the effectiveness of elimination of amaranth from 44.44% up to 97.56%. The rate of biodegradation increased more than 5 times and the removed quantity of the azo-dye increased from 4.68 mg L\(^{-1}\) to 33.48 mg L\(^{-1}\). Simultaneously during the studied period, there was an increase of the xenobiotic load in the wastewater with 20 mg L\(^{-1}\).

The results obtained from the cultivation studies showed that the quantity of the azo-degrading bacteria and the bacteria from genus *Pseudomonas* was highest in the middle layer of the biofilter (1.59 \times 10^7 CFU mL\(^{-1}\)). In the middle part of the biofilter, *Pseudomonas* sp. were 257.28% more than those in the upper part and 66.04% more than the ones in the bottom biofilter part. At this stage of the process, *Pseudomonas* sp. were almost 92% of the aerobic heterotrophs (Table 1). This was probably related to the higher toxicity in the upper layer and the relatively low adaptation level of the community. At the same time, it possessed a high potential for adaptation (demonstrated by the high part of the *Pseudomonas* sp). The three investigated enzyme activities were found at their highest values in the upper layer of the biofilter (AzoR – 57.65% more than the other layers; SDH – 127.27%; C12DO – 158.60%) since the concentrations of the substrates there were the highest (Table 1).

**The Middle stage of wastewater treatment**

In the middle treatment stage (191h – 455h) under the increased concentrations of amaranth (from 30 mg
The effectiveness of elimination of the azo-dye increased up to 90.60%. The rate of elimination of amaranth was 2.4 times higher than the one in the early stage. The COD of the effluent was 392.78 mgO₂ L⁻¹ and TOC – 115.85 mg L⁻¹. The plate count techniques showed the highest quantity of microorganisms from the key groups – AH, AzoD, genus Pseudomonas in the upper layer of the biofilter (Table 1). The aerobic heterotrophs were more with 98.71% compared to the middle layer and 366.26% than the bottom layer. The difference in the abundance of the key groups was more clearly distinguishable for the microorganisms from the genus Pseudomonas. In the upper layer, they were 3.28 times more compared to the middle and 10.29 times more compared to the bottom layer. The highest activity of the key enzymes was registered in the middle layer of the biofilter. Their values hadn’t changed much compared to the previous time period (AzoR – 7.40 μmol min⁻¹ mg protein⁻¹; SDH – 0.73 μmol min⁻¹ mg protein⁻¹; C12DO – 2.94 μmol min⁻¹ mg protein⁻¹) (Table 1). A role for this probably played the inhibition in the upper layer due to the increased concentrations of the toxic substance and insufficiently developed cooperative relationships among the microorganisms.

The digital analysis of the images obtained with FISH also showed a decrease of the microorganisms of genus Pseudomonas in the middle (with 13.22%) and the lower layer (with 33.52%) and their increase in the upper sand layer (with 4.36%) (Figure 2, 455 h). The plate count techniques showed a considerable decrease of the part of genus Pseudomonas from the community in all the three studied layers (20.21% – 106.17%) (Figure 2, 455 h). Consequently, the increased quantity of the microorganisms from this genus registered within the upper layer was due to the unculturable microorganisms from this group of bacteria.

At the end of the experiment (455 h – 623 h), the rate of amaranth removal increased by 14.63%, but the effectiveness of its removal slightly decreased to 89.76% due to reaching the highest concentrations of xenobiotics (55 mg L⁻¹) and its toxic effect (Table 1). Despite this, the distribution of the culturable microorganisms had not changed significantly and was still considerably lower than what’s registered during the early stage, as was mentioned.

The late stage of wastewater treatment

At this final stage of the experiment, when both the concentration of the model xenobiotic and its elimination rate were highest, another evidence for the significance of the unculturable microorganisms was found. The ratio of culturable Pseudomonas sp. and azo-degraders had been further lowered to only 3.72% and 0.89% respectively. The decrease was 75.27% for the microorganisms from genus Pseudomonas and with 12.60% for the azo-degrading bacteria (Table 2). At the same time an additional increase in the part of the genus Pseudomonas up to 42.57% has been registered by the FISH method. The obtained data about the genus Pseudomonas in the azo-degradation process highlights the importance of some factors (as the environment and the inter-microbial relationships) which are key for the development of the bacteria in the complex communities and cannot be studied with the standard microbiological methods.

The results from the enzyme studies showed a gradual decrease for AzoR activity from the upper (6.04 μmol min⁻¹ mg protein⁻¹) to the bottom layer (1.78 μmol min⁻¹ mg protein⁻¹) and a gradual increase for C12DO from the upper (0.88 μmol min⁻¹ mg protein⁻¹) to the bottom sand layer (2.72 μmol min⁻¹ mg protein⁻¹). In parallel with the active azo-bond reduction, the microorganisms in the biofilm developed their ability to

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**Figure 2.** Pseudomonas sp. ratio to the total quantity of the microorganisms calculated on the basis of: FISH (a); plate count techniques (b).
In this early stage of treatment, the zones of clusters of microorganisms, showed by FISH analysis, had a small area and were found mainly in the upper and the lower layer. At this stage of the process, the biodegradation and the microbiological parameters showed that the adaptation processes were in their beginning.

The data from the middle stage of functioning (191 h – 455 h) demonstrated that the adaptation of the biofilm progressed. The highest numbers of the key biodegraders (g. *Pseudomonas* and AzoD) shifted from the middle to the upper layer where they could already cope with the high amaranth concentration. The standard cultivation techniques showed that while the number of the two mentioned groups increased, their part of the community decreased (Figure 2).

On the contrary, the digital image analysis which estimates *Pseudomonas* spp. *in-situ* showed that their part increased. Therefore, the significance of the unculturable bacteria of genus *Pseudomonas* increased. This indicated that they had a more important role at this stage when the community was well adapted to the biodegradation of the toxic compound.

A similar increase in the quantity of the bacteria engaged in the azo-detoxification process was demonstrated by Ref. [34] for a consortium with attached growth. It was demonstrated also by Ref. [35] for another azo-dye degrading consortium that the community is dominated by *Pseudomonas* sp. The approach for adaptation of the bacteria in the azo-dye treatment applied by us was used also by Ref. [36].

At this stage, the FISH study of the biofilm community in the three sand layers of the biofilter showed
carry out the next step from the catabolic pathway of the amaranth, more precisely – the cleavage of the benzene ring of the aromatic amines by C12DO. For the activity of C12DO, oxygen was needed and its diffusion was facilitated under the decreased density of the clusters in the bottom layer.

### Discussion

In the presented study, an azo-degradation process was modelled. At the first stage of the functioning of the sand biofilter, an initial adaptive reaction was registered – a gradual increase of the efficiency and the rate of amaranth removal. The bacteria from the genus *Pseudomonas* were in the largest numbers in the middle part of the sand carrier. They represented almost 92% of the aerobic heterotrophs there. The highest activities of the main key enzymes from the amaranth biodegradation pathway were found in the upper part of the biofilter most probably because of the highest concentrations of the substrate (amaranth and the intermediate catechol-related metabolites) there.

Our findings are in accordance with the information of the target genus found by other studies. It is well known that the bacteria from the genus *Pseudomonas* are very active biodegraders of the azo-dyes [29–32]. This is why their amount was found to increase when amaranth was added to the wastewater. Also the approach including the use of complex microbial communities instead of pure cultures is more close to the real wastewater treatment practice. That is why the biofilm derived from activated sludge was used in this and other similar studies [33,34].

| Table 1. Technological, microbiological and enzymological parameters of the downflow sand biofilter in: early stage of the model process (0 h - 191 h), late stage of the model process (191 h – 455 h) and end of the experiment (455 h – 623 h). |
|-----------------------------------------------|
| **Technological parameters**                  |
| **Inflow amaranth concentration (mg/L)**      |
| **Efficiency of amaranth removal (%)**         |
| **Rate of amaranth removal (mg/h)**            |
| **Microbiological parameters**                 |
| **Aerobic heterotrophs** (CFU g⁻¹)            |
| **Pseudomonas sp.**                            |
| **Azo-degraders**                             |
| **Enzymological parameters**                  |
| **AzoR**                                      |
| **SDH**                                       |
| **C12DO**                                     |
| **Table 2. Key azo-degrading microorganisms’ ratio to the total quantity of the microorganisms calculated on the base of FISH and standard culture techniques.** |
| **191 h**                                     |
| **455 h**                                     |
| **623 h**                                     |
| **Pseudomonas ratio (FISH)**                  |
| **Pseudomonas ratio (plate count techniques)**|
| **Azo-degraders ratio (plate count techniques)**|

In this early stage of treatment, the zones of clusters of microorganisms, showed by FISH analysis, had a small area and were found mainly in the upper and the lower layer. At this stage of the process, the biodegradation and the microbiological parameters showed that the adaptation processes were in their beginning.

The data from the middle stage of functioning (191 h – 455 h) demonstrated that the adaptation of the biofilm progressed. The highest numbers of the key biodegraders (g. *Pseudomonas* and AzoD) shifted from the middle to the upper layer where they could already cope with the high amaranth concentration. The standard cultivation techniques showed that while the number of the two mentioned groups increased, their part of the community decreased (Figure 2).

On the contrary, the digital image analysis which estimates *Pseudomonas* spp. *in-situ* showed that their part increased. Therefore, the significance of the unculturable bacteria of genus *Pseudomonas* increased. This indicated that they had a more important role at this stage when the community was well adapted to the biodegradation of the toxic compound.

A similar increase in the quantity of the bacteria engaged in the azo-detoxification process was demonstrated by Ref. [34] for a consortium with attached growth. It was demonstrated also by Ref. [35] for another azo-dye degrading consortium that the community is dominated by *Pseudomonas* sp. The approach for adaptation of the bacteria in the azo-dye treatment applied by us was used also by Ref. [36].

At this stage, the FISH study of the biofilm community in the three sand layers of the biofilter showed
well-formed zones with a high metabolic activity of the microorganisms (Table 3). The upper sand layer was the first that had contact with the amaranth and the microorganisms in this layer were in the environment with the highest concentrations of the toxic substance. They needed to cooperate so that they formed dense clusters with high metabolic activity, where: 1) the concentration of the oxygen was probably lower, which favours the activity of the AzoR (the highest AzoR activity was registered), 2) the spatial proximity of the cells facilitated the development of synergetic, symbiotic and co-metabolic relationships, which led to increased biodegradation, 3) due to the increased density of the clusters and the high biodegradation rate the local residual concentrations of the xenobiotic were low, which lowered also the toxicity.

Other authors also highlighted that the bacteria (and especially those from genus *Pseudomonas*) were more efficient in the azo-dye removal when they inhabited consortia than in the case when they were used in pure cultures [37,38]. Ref. [39] mentions specifically that the increased biodegradation capacity of the complex consortia was due to the combined action of the bacteria and the enzymes that they excrete and also to the adaptation processes carried out in such microbial communities.

At the final stage of the experiment, the biological community maintained its high biodegradation capacity (89.76% efficiency at 55 mg L\(^{-1}\) amaranth). The azo-degradation activity of the microbial community was distributed in the following way: upper layer – a maximal concentration of amaranth, a maximal activity of AzoR, a minimal activity of C12DO (since the concentration of the aromatic amines, obtained from the azo-dye was low); middle layer – the concentration of the azo-dye decreased in the upper layer (the substrate for AzoR was in lower concentration and respectively, AzoR activity was lower; at the same time, the aromatic amines generated in the upper layer pass with the current of the water to the middle layer and induced increased activity of C12DO in this sand layer); bottom layer – the azo-dye was almost fully depleted, which had reflected in minimal AzoR activity, but the concentration of the aromatic intermediate products was the highest in this layer (due to their accumulation from the upper and the middle layers), which was the reason for the registering maximal activity of C12DO.

We used this model azo-dye treatment process to provoke adaptation changes in a biofilm community. This enabled us to estimate some aspects of its development related to efficient treatment of azo-dye contaminated waters. Most of the studies in the scientific databases are focused only on the bacteria performing the azo-degradation [32,40–42] or on the technology of the azo-removal [33,43–45]. However, often the ecological aspects of the bacterial communities (spatial distribution within the biofilms and sludges, cooperation, and adaptation) are essential for the development of successful technology. The efficient management of the mentioned ecological aspects, on which the presented study was focused, continues to be an interdisciplinary novelty with a significant importance in real practice.

Table 3. FISH of the biofilm from the three layers in the end of the process.

|            | Upper layer | Middle layer | Bottom layer |
|------------|-------------|--------------|--------------|
| Fluorescent images | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| DAPI (stain for all organisms) | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| FISH (Peudomonas sp.) | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |
Conclusions
In this study, microstructural changes were found among the bacteria from genus *Pseudomonas* in an azo-dye degrading biofilm. Microhabitats with high activity had been formed. They were related to the increasing biodegradation capacity of the sand biofilter driven by the increasing amaranth concentration. The activities of the key enzymes were linked with the structure of the microbial community and the elimination of the xenobiotic.

Data availability statement
The authors confirm that the data supporting the findings of this study are available within the article.

Disclosure statement
No potential conflict of interest was reported by the authors.

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