Gembloux Agro-Bio Tech
Université de Liège

Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Monsieur MASY Thibaut,

Titulaire d'un diplôme de bioingénieur, chimie et bio-industries, à finalité spécialisée

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 14 juin 2016, à 15 heures, en l'auditorium de Chimie Analytique**,**

Passage des Déportés, 2 à 5030 **GEMBLOUX.**

Cette dissertation originale a pour titre :

« *Rhodococcus erythropolis* T902.1 as a bioaugmentation starter: mechanisms, constrains and potentialities for hydrocarbon degradation »

Le jury est composé comme suit :

Présidente : Prof. M-L FAUCONNIER : Présidente du Département AgroBioChem,
Membres : Prof. Ph. JACQUES (Promoteur), Ph. THONART (Co-promoteur), J. DESTAIN, G. LOGNAY, F. DELVIGNE, F. NGUYEN, B. HEINRICHS, T. M. VOGEL (Université Claude Bernard, Lyon, France), O. TROMME (Sanifox)

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Résumé

Since the advent of the industrial revolution in the 19th century, anthropogenic activities and lack of environmental concern gave birth to numerous contaminated areas. Amongst released pollutants, hydrocarbons (HC) are the most widespread in the environment but they can also constitute a carbon source for numerous microorganisms. Therefore, bioremediation (i.e. the use of microorganisms to degrade pollutants) appears as an ecologically- and cost-effective technique compared to chemical or physical treatments. This biotreatment technology often relies either on stimulating indigenous microorganisms already present in soil (biostimulation) or on adding specific microbial degraders (bioaugmentation) to enhance the natural attenuation of contaminants. However, there is a need for improved understanding of the causes that can lead to its failure or its low efficiency, such as diverse environmental constraints or poor adaptation ability of laboratory-cultivated microorganisms. Amongst bacteria studied, *Rhodococcus* sp. has been previously described as a potential candidate for bioaugmentation due to its ability to degrade a broad range of organic pollutants, to produce biosurfactants, which improves pollutant bioavailability, and to rapidly adapt to many environmental stresses (e.g. desiccation, low temperature, high salinity).

The main objective of this work is thus to assess the potentiality and limitations in the use of a specific strain, *Rhodococcus erythropolis* T902.1, to degrade HC (from simple n-alkanes to polyaromatic) in diverse field conditions. The factors limiting such a process have to be identified and, as much as possible, overcome.

A first bioaugmentation experiment in microcosms aimed at identifying these constrains in carbon and clay-rich soils contaminated with heating oil. This treatment was successful in strongly polluted soil, since the addition of the strain T902.1 helped in redirecting the limited quantity of available oxygen towards a higher HC degradation and also correlated with a higher proportion of degrading genes in bioaugmented soils, compared to biostimulated and control ones. However, this effect decreased with time as T902.1 development was curtailed by competition and potentially predation from the endogenous flora. In addition, HC were heterogeneously distributed and this hampered the detection of a real degradation in lowest polluted soils. As a result, inoculation should be targeted to highly polluted areas (e.g. contaminant source zones), but it requires controlling soil heterogeneity.

We thus resorted to electrical resistivity tomography (ERT), to describe this heterogeneity and to monitor bacterial HC degradation activity. This geophysical tool could discriminate lithological heterogeneities that were artificially introduced in a 2 m³ pilot. Compared to a first insufficient biostimulation phase, the introduction of *R. erythropolis* T902.1 in this pilot led to a HC depletion of almost 80% (6900 to 1600 ppm) in 3 months in the injection zone, where pollutants were less bioavailable. Simultaneously, HC mineralization and biosurfactant production were deduced from the monitoring of ERT, biological and physicochemical parameters.

In another study, *R. erythropolis* T902.1 could form stable biofilms on the materials constituting draining pavement structures, which allowed its long-term survival in a real parking lot and improved the decontamination of runoff water drained through this structure during pollution tests carried out at a pilot scale. This indicates that the strain could be widely used in other decontaminating systems and not only in soil. Furthermore, this biofilm formation could be triggered by an appropriate pre-adaptation of the cells before their injection in the polluted environment, to ensure a higher ecological robustness of the inoculum, compared to the one observed in the microcosm experiment.

A third constrain is the poor or slow degrading activity toward some recalcitrant compounds, such as polyaromatic HC (PAH). Metallic nanoparticles synthesized by a sol-gel process were considered as potential catalysts for the improvement of degradation kinetics. Iron nanoparticles boosted the bacterial catalytic activity of the strain T902.1 in liquid cultures containing biphenyl as the sole carbon source. Following results suggested that the iron encapsulated in the porous silica matrix, was progressively attracted by siderophores (heterobactins) produced by the strain. However, this hypothesis has still to be confirmed by further analyses.

From these experiments, *R. erythropolis* T902.1 globally proved to compete and improve degradation rates in highly polluted soils compared to biostimulation, even under low oxygen and nutrient contents. Notably, the production of trehalolipidic biosurfactants enhances HC bioavailability for their further uptake by the strain and surrounding microorganisms. Furthermore, this strain forms stable biofilms on several supports, which increases its lifespan and paves the way for many applications in bioremediation systems. Finally, it can also be used in synergy with sol-gel iron nanoparticles to treat recalcitrant compounds such as PAH and some chlorinated aromatics, but this combination needs to be further tested in more complex media, such as soil or waste water.