Intro

In ground we can find an entire world of microorganism, the native community. They are composed of a vast amount of microorganism constantly interacting between each other in ways that are not always fully understand. But one of the most important part of this community is that they not only interact with one another but are able to interact with other organism, for example they can help the grow of plants [mycorrhiza][1]. This community is usually resilient to outside event but can be perturbed by the effect of pollution.

Toluene is a solvent widely used in nowadays industry, for example as a paint solvent or as extraction solvent in the cosmetic industry. Part of the BTEX group composed of monoaromatic volatile molecule who are all toxic and ecotoxic. Being volatile most of the pollution caused by it will end in the atmosphere, but small amount of it can be found in the ground or water in case of spillage[2].

When such event happens one way to treat those pollution is the use of bioremediation. There are several different technics of bioremediation but they all have in common is their aim: Helping the native community overcome the effect of a pollution event and promoting it’s regrow. The technic that interested us in this experiment was the Bioaugmentation, the addition of a microorganism to a native community which can degrade the targeted pollutant[3].

The microorganism we choose for it was the *Pseudomonas Putiida* and *Pseudomonas Veronii*, both able to metabolize toluene. In our experiment we wanted to know if this ability to metabolize toluene could not only have a positive effect on a native community by lowering the concentration of toluene but also, maybe, by giving the community access to metabolite by product.

To be able to measure the grow of our cell we used a flux cytometry. This technic consists of passing cells one by one through a capillary and hit each individual cell with a laser. The machine will them measure the scattered light, this way we can measure the size of the cell [time during which the light is scattered] and the density of our cell. We can also recognise cells if they are marked, the machine can measure the fluorescence emitted by the cell. Thanks to this we can differentiate two different groups of cells if they are marked by different marker. In our case *Pseudomonas Putiida* and *Pseudomonas Veronii* are tagged with m\_Cherry, a red fluorescent molecule, and we will stain the entire culture with syto 9. This way we will be able to recognise the cells which are only marked with a green fluorescent marker [in our case the sand native community] and the cells marked with both syto 9 or m\_Cherry [*Pseudomonas putiida* and/or *Pseudomonas Veronii*].

It is important to note that for a flux cytometry we must use two fluorescents marker who do not emit similar wave length. If we would have chosen two marker that emit similar fluorescence or overlap with each other, the machine could confuse the two markers with one another.

The other crucial point in the use of flux cytometry is that we must dilute our culture before measuring it. Otherwise, if the concentration of cells is too high, the result could be wronged by a jammed capillary or a cluster of cell.

The culture medium used for this experiment was in a liquid medium. Each flask containing a medium composed of a buffer and hunter solution (on a marquer lesquel?) and the addition of either a mixed carbon source or a tiny recipient containing toluene. We chose to not directly include the toluene in the medium to avoid his toxic effect on our initial culture, by adding toluene in a culture by diffusion will give it as a carbon source for the cells without immediately killing them due to his toxicity.

The other advantage of a culture medium is that we can directly take a sample and dilute it without needing specific preparation befor staining it.

Source

[1]Margaret L. Ronsheim, *The Effect of Mycorrhizae on Plant Growth and Reproduction Varies with Soil Phosphorus and Developmental Stage* ; The American Midland Naturalist.

[2]Scottish environment protection agency, <http://apps.sepa.org.uk/spripa/Pages/SubstanceInformation.aspx?pid=89> (14/11/2017)

[3] 1.Bing Zhao, Chit Laa Poh, 2008, “Insights into environmental bioremediation by microorganisms through functional genomics and proteomics”, Proteomics, 8:874–881

[4] I.H.Nam, Y.S. Chang, et al., 2003, “A novel catabolic activity of P. veronii in biotransformation of pentachlo