Development of a new paradigm in biochemical engineering: predicting the genetic regulation of aromatic pollutants degradation

Argyro Tsipa[1,2], Michalis Koutinas[3], Athanasios Mantalaris[1], Efstratios Pistikopoulos[2]

1. Biological Systems Engineering Laboratory, Imperial College London South Kensington Campus, London SW7 2AZ, UK
2. Centre of process Systems Engineering, Imperial College London South Kensington Campus, London SW7 2AZ, UK
3. Department of environmental science & technology, Cyprus University of Technology, Cyprus

Keywords: toluene degradation, genetic regulation, chromosome, TOL plasmid

In this work, we study *Pseudomonas Putida* which is a metabolically versatile soil bacterium. Its significance lies not only on the fact that it can metabolise numerous industrially important aromatic compounds (Pieper *et al*., 2004) but on its biotechnological potential by producing a series of fine and bulk chemicals, resulting in a growing interest of studying specific metabolic pathways at the genetic expression and regulation levels (Ballerstedt *et al*., 2007). This bacterium has several strains, but we focus on mt-2 because it degrades efficiently **toluene**. This strain can harbour TOL plasmid which specifies a metabolic pathway for toluene and m-xylene (Timmis, 2002). Toluene and m-xylene are aromatic pollutants belonging to the BTEX (Benzene-Toluene-Ethylbenzene-Xylene) group and their **degradation** leads to Krebs cycle intermediates which are essential for the biomass growth. In *P. Putida* mt-2, toluene oxidative catabolism occurs **through the chromosomal and TOL plasmid (pWW0) metabolic pathways**. TOL plasmid is a paradigm of global and specific genetic regulation due to the interactions between DNA-bending proteins, a set of sigma factors and the regulators encoded in it (Aranda-Olmero *et al*.,2005). Our objective is to investigate the gene expression and regulation of toluene in *P. Putida* mt-2 though the above metabolic pathways.

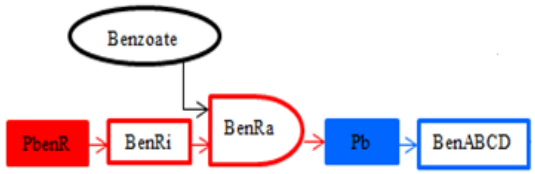
In TOL plasmid, the entrance of toluene activates xylR and xylS genes which encode the regulatory proteins XylR and XylS respectively. The activation of these proteins is the precursor of the expression of upper and meta operon of TOL plasmid. When expressed, these operons encode the necessary catalytic enzymes for the further degradation of toluene to Krebs cycle intermediates. The four transcriptional units mentioned, are controlled by four promoters, Pr, Ps, Pu, and Pm respectively (Ramos *et al*., 1997).

In particular, Pr promoter boosts the expression of xylR gene which encodes XylR protein. The induction of toluene to the bacterial cell culture system provokes a series of regulatory events. XylR becomes a down-regulator for Pr promoter and up-regulator for the activation of Pu and Ps promoters. These promoters boost the expression of upper operon and xylS gene respectively. Afterwards, the enzymes encoded by the upper operon, catalyse the oxidative reaction of toluene to benzoate. In addition, XylS protein is encoded by xylS gene. Both XylS protein and benzoate activate Pm promoter which boosts the expression of meta operon. The enzymes encoded by the meta operon enable the further catabolism of benzoate to Krebs cycle intermediates.

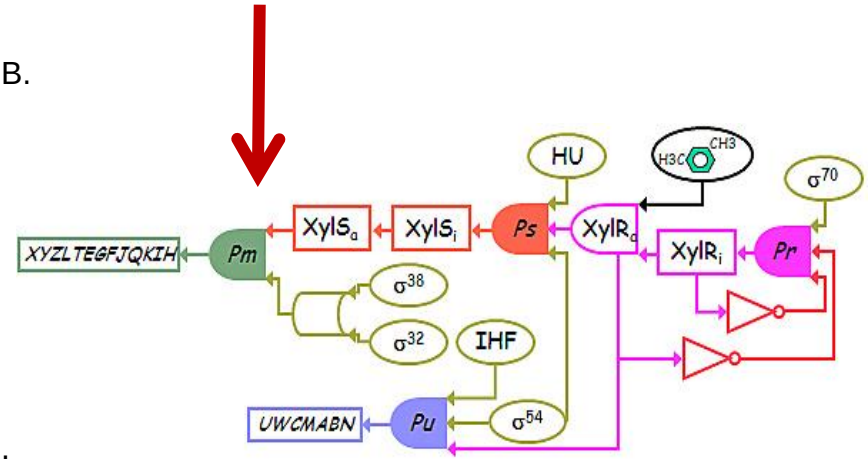
Besides that, benzoate is the compound which activates the chromosomal pathway. In this pathway the transcriptional unit is benR gene which is driven by PbenR

promoter. More specifically, PbenR promoter boosts the expression of benR gene which encodes BenR protein. With the appearance of benzoate in the biosystem, BenR becomes one of the up-regulators of Pm promoter of TOL plasmid's meta operon. Therefore **a cross-talk between the two pathways is observed** (Cowles *et al.*, 2000).
A more detailed genetic representation can be found in figure 1.In this figure the genetic information are presented in a genetic circuit which is like an electric circuit based on biological information (Weiss *et al*., 2003).

A.



B.



.

Figure 1: Genetic circuit of: A. the specific chromosomal pathway, B. the TOL plasmid metabolic pathway (Koutinas et al., 2011) and the up-regulation of Pm promoter of TOL plasmid by BenR protein of chromosome, i: inactivated form of protein, a:activated form of protein. :input, :ouput, :AND, :NOT

From experimental point of view, we measured the behaviour over time of the five promoters, since they are the driven power of the key transcriptional units' of our biosystem. The activity was measured via RT-PCR. Different concentrations of toluene were used, ranging between 0.4-1.3mM. In addition to that, toluene consumption and biomass growth were measured over time. The macro- and genetic- scale results in 1mM of toluene induction are presented in figure 2 below.

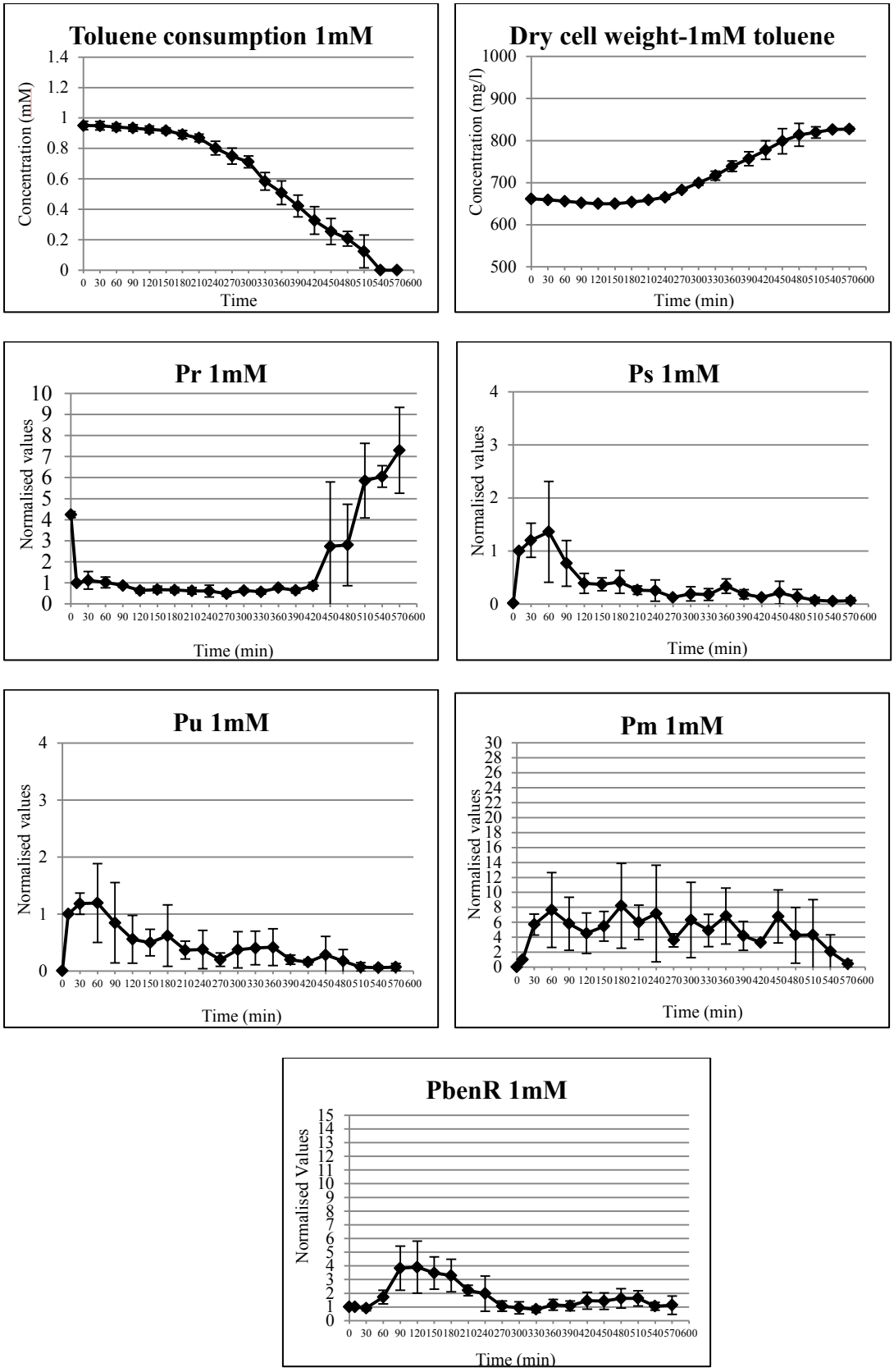


Figure 2: 1mM toluene consumption, dry cell weight (biomass growth), and the normalised levels of mRNA of Pr, Ps, Pu, Pm and PbenR promoters over the time until the depletion of toluene in the cell culture.

The results confirm the theoretical background. Pr's activity is kept in a basal level since is not directly influenced by the substrate entry, while Ps and Pu activity starts to increase when toluene is induced to the biosystem. Pr's activity increase towards the end of the process, can be attributed to the end of XylR down-regulation. After toluene's catabolism to benzoate, Ps and Pu activity decreases significantly. Pm's activation occurs with a delay as toluene has to be catabolised to benzoate in order Pm to be activated. Moreover, PbenR's activity is in a basal level until benzoate appears in the cell culture. Then, its activity starts to increase reaching a peak and decrease thereafter reaching the basal level. Pm's activity although reaching a peak and decreasing thereafter is kept in high levels compared to the other promoters' activity until the end of the process since the enzymes encoded by the meta operon are responsible for the further catabolism of benzoate to Krebs cycle intermediates. In addition, the differentiation of **Pm** among the promoters' behaviour in terms of the level of activation can be attributed to the fact that it is **activated by XylS and BenR protein**, pointing out the effect of the interaction between TOL plasmid and chromosome.

The experiments indicate that there is a close relationship between the key genes and the catabolism of toluene. Therefore, it would be extremely interesting to study the link between the intracellular information and macroscale observations. Towards this direction, a hybrid growth kinetic model has already been developed (Koutinas et al., 2011) using the same microorganism and studying m-xylene degradation in TOL plasmid. Our intention is to upgrade the existing model by integrating the chromosomal genetic information obtained through toluene entry in the biosystem. If we achieve capture of the experimental data by the upgraded model then its predictive capability will be tested revealing the connection of accounting genetic regulation to aromatic pollutants degradation.

References

Aranda-Olmedo, I., Marin, P., Ramos, J.L., and Marques, S., 2006. Role of the ptsN gene product in catabolite repression of the Pseudomonas putida TOL toluene degradation pathway in chemostat cultures. Appl. Environ. Microbiol. 72, 7418–7421.

Ballerstedt, H., Volkers, R.J.M., Mars, A.E., Hallsworth, J.E., dosSantos, V.A.M., Puchalka, J., vanDuuren, J., Eggink, G., Timmis, K.N., de Bont, J.A.M., Wery,J., 2007. Genomotyping of Pseudomonas putida strains using P. putida KT2440-based high-density DNA microarrays: implications for transcriptomics studies. Appl. Microbiol. Biotechnol. 75, 1133–1142.

Cowles C.E., Nichols N.N. and Harwood C.S., 2000. BenR, a XylS Homologue, Regulates Three Different Pathways of Aromatic Acid Degradation in Pseudomonas putida. Journal of Bacteriology, 182 (22), 6339-6346.

Koutinas, M., Kiparissides, A., Silva-Rocha, R., Lam, M., Martins Dos Santos, V., de Lorenzo, V., Pistikopoulos, E., Mantalaris, A., 2011. Linking genes to microbial growth kinetics – an integrated biochemical systems engineering approach. Metabolic Engineering, 13 (4), 401-413.

Pieper, D.H., Santos, V.A.P., and Golyshin, P.N., 2004. Genomic and mechanistic insights into the biodegradation of organic pollutants. Curr Opin Biotechnol 15: 215–224.

Ramos, J.L., Marques, S., Timmis, K.N., 1997. Transcriptional control of the Pseudomonas TOL plasmid catabolic operons is achieved through an interplay of host factors and plasmid-encoded regulators .Annu. Rev. Microbiol. 51, 341–372.

Timmis, K.N., 2002. Pseudomonas putida: a cosmopolitan opportunist par excellence. Environ.Microbiol.4,779–781.

Weiss, R., Basu, S., Hooshangi, S., Kalmbach, A., Karig, D., Mehreja, R., Netravali, I., 2003. Genetic circuit building blocks for cellular computation, communications, and signal processing. Nat. Comput. 2, 47–84.