**Efficacy in Aquatic Microcosms of a Genetically Engineered Pseudomonad Applicable for Bioremediation**

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**Abstract.** A genetically engineered microorganism (GEM), *Pseudomonas sp.* B13 FRI (pFRC20P) (abbreviated FR120), has previously been engineered to simultaneously mineralize mixtures of methylated and chlorinated benzoic acids and phenols through a modified *ortho* cleavage pathway. In this study, its performance was investigated both in different types of aquatic microcosms and in pure culture to determine (1) if under simulated in situ conditions the genetically engineered pathway effectively removes mixtures of model pollutants simultaneously, quickly, and completely; (2) where the optimum pollutant concentration range for this activity lies; and (3) how physical, chemical, and biological factors in the microcosms influence degradation rates. Growth and degradation parameters of FR120 in pure culture were determined with 3-chlorobenzoate (3CB), 4-methylbenzoate (4MB), and equimolar mixtures of both as carbon sources. These substrates were degraded simultaneously, albeit with different degradation velocities, by FR120. The optimum growth concentrations for 3CB and 4MB were 3.0 mM and 2.1 mM, respectively, and the inhibition constants (K_i) were 11 mM (3CB) and 6 mM (4MB). The pathway was induced at low concentrations of substrate (> 1 μM). The first order degradation constants (k_i) were determined with respect to substrate concentration, cell density, and temperature. In aquatic microcosms inoculated with FR120, first order degradation constants and half lives of target chemicals were calculated based on the total amount of aromatics recovered. Half lives ranged from 1.3 days to 3.0 days, depending on the target chemical and the type of microcosm. Degradation constants determined in pure culture were extrapolated to the densities of FR120, substrate concentrations, and temperature occurring in the microcosm experiments, and used to calculate theoretical half

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lives. In water microcosms, theoretical and observed half lives corresponded well, indicating that FR120 functioned optimally in this environment. In whole core sediment microcosms, and especially at low cell densities, the observed degradation activity was in some cases considerably higher than expected from pure culture degradation rates. This suggests that environmental conditions in the sediment were more favorable to the degradation of substituted aromatics than those in pure culture. The physiological characteristics of FR120 and its performance in aquatic microcosms make it a good candidate for bioremediation at sites contaminated with mixtures of chlorinated and methylated aromatics.

**Introduction**

Major advances have been made or are under way to engineer organisms with novel catabolic abilities that may have applications to degrading environmental pollutants [6, 23, 24]. Some of the resulting genetically engineered microorganisms (GEMs) are able to survive in microcosms simulating sewage, groundwater, and sediment environments where the novel catabolic pathway is expressed, i.e., degradation of the target compounded by the GEMs takes place [20, 22]. Before the release of a GEM into the environment can take place, a risk assessment analysis addressing safety issues is needed [6, 11, 18, 26]. Moreover, the effectiveness of a remediation strategy applying the GEM needs to be compared to other possible remediation strategies. As a basis for such a cost/benefit evaluation we here determined quantitatively the degradation efficacy of a GEM under simulated in situ conditions in microcosms.

*Pseudomonas sp.* B13 FR1 (pFRC20P) [5, 12, 16, 24] (called FR120 in the following text) has a catabolic pathway designed by patchwork assembly of genes from three different bacterial strains (*Pseudomonas* sp. B13, *Pseudomonas putida* mt-2 (pWWO), and *Alcaligenes eutrophus* JMP 134) to degrade simultaneously mixtures of chlorinated and methylated aromatic compounds [24] (Fig. 1). In natural bacterial assemblages, these mixtures can cause induction of both the ortho and meta cleavage pathways for degradation of aromatics, resulting in misrouting of intermediates, poisoning of key enzymes, accumulation of detrimental products, and possible death of the microbial community [14, 15]. Mixtures of chlorinated and methylated benzoic acids occur as pollutants from wood and chemical industries [10]; chlorinated benzoic acids are also intermediates in PCB degradation [1]. In the present study, 3-chlorobenzoate (3CB) and 4-methylbenzoate (4MB) were used as model pollutants. 3CB can be metabolized by the nonengineered parent organism, *Pseudomonas sp.* B13; degradation of 4MB is possible via the constructed catabolic pathway in FR120.

Most industrial pollutants are discharged into aquatic ecosystems, e.g., lakes and rivers, either directly or via runoff from contaminated areas. Many end up in sediments where they may persist for long periods of time. Sediments therefore represent major potential sites for bioremediation. They exhibit pronounced gradients of physical, chemical, and biological parameters. The energy flow within the sediment ecosystem is strongly influenced by protozoan grazing and benthic invertebrates [27]. Sediments cannot be understood distinct from the overlying site