# Evaluation of a stable 1,4-dioxane-degrading microbial consortium in the presence of co-occurring compound: Role of microbial coexistence and interplay

# 博 士 学 位 論 文

## Doctoral Dissertation

**Evaluation of a stable 1,4-dioxane-degrading microbial consortium in the presence of co-occurring compound: Role of microbial coexistence and interplay (共存化合物存在下で安定な 1,4-ジオキサン分解コンソーシアムの評価－微生物共存と相互作用の役割－)**


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**Summary**

Biodegradation is found to be a promising, viable and environment friendly approach to remove 1,4-dioxane (1,4-D) from contaminated groundwater and industrial wastewater. In recent years, substantial progress has been achieved in developing biological treatment methods for 1,4-D-contaminated environments, while significant efforts have particularly been given to isolate, identify and characterize 1,4-D-degrading microbial strains. As a result, a plethora of potential 1,4-D-degraders have been isolated and identified. However, successful removal of 1,4-D from the contaminated environments is still challenging since 1,4-D is usually found to be mixed with diverse organic compounds/contaminants inhibiting the biodegradation activity of potential 1,4-D-degraders. Moreover, application of laboratory tested efficient 1,4-D-degrading strain may fail to exhibit degradation activities in contaminated environments owing to the existence of other indigenous microbes and associated microbial interactions. Therefore, this study comprehensively evaluated the efficacy and functioning of an indigenous 1,4-D-degrading microbial consortium (N112) in the presence of various structurally different co-occurring compounds in order to develop an efficient and robust biological 1,4-D treatment system for possible environmental and industrial applications.

Firstly, the 1,4-D degradation efficacy of the consortium N112 as well as its microbial community structure and functional gene compositions were investigated in the absence or presence of eight structurally different co-occurring organic compounds including glucose, lactic acid (LA), ethylene glycol (EG), tetrahydrofuran (THF), phenol, tetradecane (TD), toluene, and 1,1,1-trichloroethane (1,1,1-TCA). This is first study that established a stable 1,4-D-degrading microbial consortium consisting of more than 10 bacterial genera and investigated how it responds and functions in the presence of additional carbon sources of diverse nature. The results demonstrated that the consortium can efficiently degrade 1,4-D in the presence of tested substrates although substrate-specific inhibition occurred, indicating the role of stable coexistence and interactions of

diverse bacterial genera. Substrate-specific changes in the microbial community structure were also observed, while group 5 soluble di-iron monooxygenases (SDIMOs), especially group 5C (*thm/dxm*), were predominantly found as the key functional genes regardless of the treatment applied. The results indicated that 1,4-D-degraders of the consortium N112 remain viable and possess group 5C SDIMOs possibly catalyzing the degradation of 1,4-D. The results demonstrated that the consortium N112 possesses great potentiality to be used for the treatment of 1,4-D-contaminated industrial wastewater containing additional organic load.

Afterward, the effort was given to isolate and identify the key players of the consortium N112 i.e., the 1,4-D-degraders and non-degraders as the results of previous chapter suggested the possible coexistence of multiple degraders in N112. As a result, three metabolic 1,4-D-degrading strains belonging to the genera *Pseudonocardia*, *Afipia* and *Dokdonella* and six non-degraders belonging to the genera *Sphingomonas*, *Sphingopyxis*, *Chryseobacterium*, *Bosea*, *Nitrobacter* and *Cupriavidus* were isolated and identified. Two 1,4-D-degradaing strains (*Dokdonella* sp. TS32 and *Afipia* sp. TS43) were identified as the novel degraders, whereas this study for the first time reported the potentiality of the genus *Dokdonella* in the biodegradation of 1,4-D. Further experimental and molecular investigation revealed that all the isolated 1,4-D-degrading strains are capable of utilizing various organic compounds and possess identical SDIMO genes encoding group 5C *thmA/dxmA*, supporting the findings obtained during 1,4-D biodegradation by the consortium N112. Among the degraders, the novel strain TS32 (*Dokdonella* sp.) exhibited higher 1,4-D biodegradation efficiency in the absence or presence of tested co-occurring compounds although found to be less efficient as compared to the consortium N112, indicating the potentiality of microbial consortium over pure cultures.

Finally, synthetic consortia-based experiments were conducted to understand why microbial consortium degrades 1,4-D more efficiently as compared to pure 1,4-D-degrading strains. Synthetic

consortia composed of degraders (SCD) resulted in lower 1,4-D biodegradation efficiency, regardless of the two- or three-species SCD, when compared with the pure cultures. These results were found to be contrasting with the existing knowledge suggesting that the co-culture of indigenous degraders accelerates the biodegradation of the target compound. Further biodegradation experiments based on the simplex lattice mixture design (SLMD) revealed that possible competitive interactions among degraders resulted in inefficient degradation of 1,4-D. However, synthetic consortia composed of degraders and non-degraders (SCDN) resulted in efficient biodegradation of 1,4-D, even in the presence of co-occurring compounds, indicating that the complementary role of non-degraders coexisting with the degraders in the system. These results thereby experimentally proved how the coexistence and interplay of non-degraders with degraders contributes to the efficient biodegradation of target compound under complex environments. Moreover, the SCDN exhibited equal or higher 1,4-D degradation efficiency even in the presence of co-occurring compound when compared with the consortium N112, which indicate its potentiality and robustness for efficient biodegradation of 1,4-D like recalcitrant organic pollutants.

This study concluded that stable microbial consortia consisting of degraders and non-degraders are highly efficient and robust to remove recalcitrant organic pollutants under ‘real world’ conditions. Therefore, for the remediation of contaminated sites using synthetic biotechnological approach, the non-degraders should be given equal priority as like as the degraders in order to achieve desired outcome.