OPINION

Improved biological treatment of medium-strength wastewater at low temperatures via diversity manipulation of psychrophilic bacterial consortiums

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Ackroyd P. Improved biological treatment of medium-strength wastewater at low temperatures via diversity manipulation of psychrophilic bacterial consortiums J. Environ. Microbiol. 2022;4(5):1-2.

ABSTRACT

In order to create effective bioaugmentation formulations for improved wastewater treatment at low temperatures or under temperature-variable settings, psychophilic bacteria are excellent biocatalysts. Here, we investigated the impact of an artificial bacterial consortia's microbial diversity on the biomass gross yields (measured through OD600) and removal efficiency of soluble chemical oxygen demand (mg sCOD removed/mgs COD introduced) in synthetic, medium-strength wastewater using various biodiversity indices [based on Species Richness (SR), Phylogenetic Diversity (PD), and Functional Diversity (FD)]. We created artificial consortia from one to six 4 °C-isolated bacterial strains using combinatorial biodiversity experiments.In monocultures, tricultures, pentacultures, and hexacultures, respectively, increased species richness led to greater COD removal efficiency (i.e., 0.266 0.146, 0.542 0.155, 0.742 0.136, 0.822 0.019) and higher biomass gross yields (i.e., 0.065 0.052, 0.132 0.046, 0.173 0.049). Consideration of the metabolic profile (functional diversity) or evolutionary linkages also revealed this favourable relationship between biodiversity, COD elimination, and biomass gross output (phylogenetic diversity). The choice of a specific, top-performing species (*Pedobacter sp.)* as well as complementary usage of carbon resources by consortium members may be responsible for the beneficial effect of biodiversity on SOD removal efficiency (i.e., complementarity effects). Compared to SR and FD diversity metrics, PD diversity metrics among the biodiversity indices explained a greater variation in SOD removal. Our findings highlight the significance of adopting phylogenetically varied consortia with improved degrading capacity rather than single pure cultures for more successful bioaugmentation. Additionally, in psychrophilic circumstances, PD could be applied as an assembly rule to direct the composition of mixed cultures for wastewater bioaugmentation.

Key Words: *Bioaugmentation; Psychophilic; Pentacultures; Hexacultures; Monocultures; Phylogenetically*

INTRODUCTION

In aerobic wastewater treatment systems, wastewater temperature is a critical factor determining microbial functions. The van't Hoff-Arrhenius equation predicts that biological activity and reaction rates will decrease as the temperature of wastewater falls below 10°C, which will lead to a decrease in the efficiency of wil removal. The use of psychrophiles in bioaugmentation has been suggested as a method to improve the efficiency of specific cleaning processes of domestic and municipal wastewater at low temperatures (for example, carbon removal through oxidation and biomass growth) and make up for mesophilic bacteria's diminished activities. Psychrophiles are cold-adapted microorganisms that can survive at or below the freezing point of water, with an upper critical temperature of roughly 20°C and an optimal growth temperature below 15°C. Using cold-adaptive features to make up for the negative effects of low temperatures on biochemical response rates, they have the capacity to demonstrate high metabolic activity at low and moderate temperatures. Under frigid temperatures, the use of psychrophilic strains in bioaugmentation procedures has already been studied. For instance, a cold-adapted Arthrobacter psychrolactophilus was able to develop in a synthetic wastewater at 10°C, causing the turbid medium to completely clear out and resulting in the effective hydrolysis of proteins, carbohydrates, and lipids.

In another study, fed-batch cultivation at 10°C allowed psychrophilic bacteria and yeasts to completely breakdown phenol, a characteristically aromatic hazardous contaminant typically found in wastewater. By controlling propagule pressure and establishing exogenous microorganisms in the invaded ecosystems, the choice of strong and functionally active bioaugmentation cultures is crucial to maximising invasion success. Bioaugmentation formulations typically go through five life stages from product development to final application, including I capture, II production, III establishment, IV function, and V downstream consequences. Because the injected

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Received: 05-Oct-2022, Manuscript No PULJEM-23-6084; Editor assigned: 07-Oct-2022, Pre-QC No. PULJEM-23-6084 (PQ); Reviewed: 12-Oct-2022, QC No. PULJEM-23-6084 (Q); Revised:14-Oct-2022; Manuscript No. PULJEM-23-6084 (R); Published: 19-Oct-2022, DOI: 10.37532/puljem.22.4 (5).1-2

strains have a dismal survival rate, bioaugmentation initiatives have frequently failed. Both biotic and abiotic variables are responsible for the hostile impact of the invaded ecosystem circumstances on the active injected microbial invaders. The degree of propagule pressure, the rivalry between the inoculant and native populations, or predation by protozoa and bacteriophages are examples of biotic variables. Strain selection criteria based on distinctive traits that offer ecological advantages in the environment are another. Temperature, pH, substrate availability, and the presence of hazardous substances are examples of abiotic variables. Recent research has focused on both the characteristics of the invaders that enable effective invasion and the characteristics of the resident population that define its susceptibility to invasion.

From an invader-centric standpoint, using polycultures rather than isolated single strains (pure cultures) and formulating microbial inoculants based on eco-physiological characteristics are two different ways to improve bioaugmentation and boosting. Due to consortiums' increased functional redundancy, diversity, and stability, in particular, improved pollutant removal efficiency and more reliable procedures can be ensured. Indeed, more diversified groups can assimilate a larger percentage of the available resources and benefit more from specialty opportunities. Additionally, they demonstrate a stronger capacity to survive particular disturbances (resistance) or recover their pre-perturbation structural and functional baseline.

In this work, the removal of Soluble Chemical Oxygen Demand (CODs) and biomass gross yields in synthetic, medium-strength wastewater at 4°C were examined in relation to the effects of an increasing variety of synthetic, psychrophilic consortiums. Diverse carbon sources were available as growth substrates in the developed synthetic wastewater, increasing the variability of the resources available to heterotrophic microbes (i.e., high niche dimensionality). Using a combinatorial approach, we isolated six bacterial strains for their capacity to extracts COD from synthetic wastewater at 4°C, and then we put together synthetic psychrophilic communities with increasing levels of richness. To evaluate the interaction between biodiversity and the operation of the microbial ecosystem, the elimination of SOD and the gross outputs of biomass were examined over time.