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Article

A Review on Bacillus subtilis and Related Bacilli in Wastewater Treatment: Biofilm Formation, Genetic Regulation, and Industrial Pollutant Degradation

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Abstract: The global water scarcity crisis and the growing complexity of industrial effluents call for the development of resilient, self-sustaining bioremediation technology. Bacillus subtilis and its phylogenetically close representatives have been casted among the best characterisation candidates in wastewater treatment, owing to their capacity to generate robust biofilms and exhibit metabolic versatility. In this review, we will examine the molecular processes that dictate biofilm structure, with a focus on the EPS matrix, TasA amyloid fibers, and BslA hydrophobins. In this review, we discuss genetic circuitry that regulates the transition from planktonic to sessile lifestyles including the Spo0A (general response regulator)- and DegS-DegU (two-component system)-mediated systems. Additionally, It provides the degradation pathways of recalcitrant organic compounds and the immobilization of heavy metals. To meet the requirements of the relatively new industrial scale applications, we investigate the now fused fields of genetic engineering and nanotechnology. This review cites a total of 40 references in Vancouver style and provides a comprehensive overview of the current status of environmental biotechnology based on Bacillus.

Keywords: Bioremediation, Biofilm Formation, Nutrient Removal, Anaerobic Digestion

1. Introduction

The escalation of anthropogenic pollutants discharge into water bodies has become a pivotal problem of global concern, and there is an established wastewater treatment (WWT) model that needs to be followed. Classic treatment protocols typically struggle to satisfy the metabolic demands necessary to metabolize complex xenobiotics. However, some single bacteria of Bacillus subtilis group and the closely related bacilli have proven most useful in the biodegradation of water pollutants, which is, on the one hand, their physiological stability, real in industrial conditions, and, on the other hand, their ability to thrive under extreme environmental conditions. These microorganisms are especially important in continuous-flow bioremediation systems where they develop strong biofilms - communities of multicells that are resistant to high-shear forces and toxic shocks. [1, 13, 24].

Bacillus subtilis is a Gram-positive and rod-shaped bacterium that has a wide distribution in the soil and vegetation. Its capacity to generate endospores also enables it to survive under conditions exceedingly adverse to its life, including food shortage or chemical poisoning and subsequently regain metabolic activity once the situation returns to normal. In the wastewater, some of the species that are commonly used to attack organic pesticides, phosphate compounds, phenols, and synthetic dyes include B. pumilus, B. licheniformis, and B. amyloliquefaciens.. [2, 21, 25].

2. Physiology and Metabolic Diversity

Bacillus genus is distinguished by the wide metabolic range. B. subtilis is a model organism in the study of aerobic respiration, but has the apparatus to adopt an anaerobic growth with nitrate as alternative electron acceptor. This plasticity is important in the wastewater plants that are variable between oxic and anoxic conditions and permits the oxidation of carbon and denitrification to occur concurrently. [26, 28].

Other species of the genus give specialized services. B. licheniformis is a good producer of biosurfactants and alkaline proteases, which is vital in the treatment of high-strength organic waste of the food and laundry sectors [19, 27]. On the same note, it is also well known that B. amyloliquefaciens possesses high-grade secretion of amylases and lipases which help to break down complex carbohydrates and fats in municipal sludge [15, 29]. The ability of these bacilli to breakdown biogenic amines, as well as petrochemical alternatives, further brings out the industrial use of these bacilli [18, 20].

3. Biofilm Formation: The Architecture of Resilience

Of natural and engineered systems, the existing microbial life is mainly sessile. Biofilms consist of structurally organized groups that are embedded into a self-secreted matrix of extra cellular polymeric materials (EPS). This matrix is not only a physical anchor but also a chemical buffer, nutrient pool and molecular screen against predation and antimicrobial agents. The formation of tightly-knit communities is essential in wastewater reactors to ensure that the activated population density is high [3, 7, 30].

The Bacillus biofilm development cycle begins with reversible attachment, which is followed by irreversible attachment mediated by EPS production. The matrix is a complex mix of polysaccharides, proteins (e.g, TasA and BslA) and extracellular DNA (eDNA). The TasA produces amyloid fibers which give the biofilm structural rigidity and BslA also functions as a hydrophobin which forms a water-repellent surface layer which shields the inner cells against chemical stressors [2, 31, 32].

4. Genetic Regulation and Quorum Sensing

This morphological shift between a motile and a planktonic cell to a stationary and biofilm forming form is regulated by a complex genetic network. The central one is the master regulator Spo0A. Cells can use a phosphorelay system to respond to environmental signals including population density (quorum sensing) and food availability. The activation of Spo0A (phosphorylation) leads to the expression of both eps and tasA operons, and at the same time represses flagellar synthesis [10, 33, 34].

The DegS- DegU two component system is also important. It controls social activities such as swarming motility as well as the formation of extra cellular enzymes. Low concentrations of phosphorylated DegU favor motility but large concentrations of DegU-P attract the cell to a sessile lifestyle and enhances the release of proteases needed to digest the high-molecular-weight organic matter within the wastewater [4, 35].

5. Biodegradation of Organic Pollutants

The recalcitrant organic compounds present in industrial wastewater are usually synthetic azo dyes and organophosphate pesticides. The Bacillus species possess specialized enzymes, including azoreductases and laccases, which have the ability to break down the chemical bonds of these pollutants which are stable. As an illustration, B. subtilis has been demonstrated to decolorize Methyl Orange and Congo Red dye with high efficacy by decomposing the chromophores into intermediates, which are less toxic [11, 36, 37].

There is also a high environmental threat by hydrocarbons and detergents. Bacillus salmalaya and other strains with the biosurfactant properties increase the bioavailability of the hydrophobic oils, which can be more effectively microbially uptaken and mineralized. The process finds application especially when handling refinery effluents and cleaning oil spills within the aquatic environments [16, 17, 38].

6. Remediation of Heavy Metals and Inorganic Hazards

The heavy metals are not degradable like organic pollutants, but should be transformed or sequestered. Bacillus biofilms use various forms of metal remediation:

1. Biosorption: Metal ions, including Pb^{2+}, Cd^{2+} and Cu^{2+}, are sorbed to the negatively charged functional groups (carboxyl and hydroxyl) on the EPS matrix [13, 39].
2. Bio-precipitation: Bacteria release phosphates or carbonates which react with the metal ions to precipitate insoluble minerals [14, 40].
3. Reductive Dissolution: Both B. megaterium and B. flexus are able to reduce toxic Cr^{VI} to the less toxic and less mobile Cr^{III} and help the elimination of toxic Cr^{VI} in groundwater [1, 13].

7. Industrial Case Studies and Environmental Impact

Bacillus species are colonized in moving bed biofilm reactors (MBBR) by the use of plastic carriers to maximize carbon and phosphorus removal. In aquaculture, where the nitrogenous waste (ammonia and nitrite) may rise to toxic levels, Bacillus consortia is applied as a probiotic to control the quality of water and prevent pathogen growth [8, 15, 41].

These microbial consortia have been successfully used to treat pollutants such as heavy metals and toxic chemicals in field applications in such states as Telangana and Andhra Pradesh, India. B. subtilis promotes widespread aerobic biofilm, which is an important contributor to such purification of the local tanneries and chemical plants [8, 42].

8. Innovations: Genetic Engineering and Nanotechnology

Biotechnology and materials science will be the synergies of Bacillus-based wastewater treatment in the future. Genetic engineering enables the production of so-called designer strains which overexpress certain degradative enzymes, or which are more resistant to biofilm in response to extreme pH or salinity [19, 20, 43].

In addition to this nanotechnology is being employed to boost bioremediation. As an example, microencapsulation of Bacillus cells in polymers of alginate modified with TiO_2 or SiO_2 nanoparticles can afford a dual-action treatment: physical photocatalysis and biological degradation. Although certain nanoparticles prevent biofouling, even at low doses, concentrations can in fact activate bacterial growth and EPS formation, and improve the overall treatment capacity of the biofilm [21, 22, 44].

9. Discussion and Challenges

Although the benefits of Bacillus biofilm usage are obvious, there are still a number of issues. Biofilms are much more antibiotic and biocide-resistant than planktonic cells (up to 1,000-fold), which may result in the presence of antibiotic-resistant bacteria (ARB) within treatment plants [1, 18]. Also, biofilms have uncontrollable proliferation, which may cause biofouling of piping and membrane systems, which also require the formulation of precise biocontrol mechanisms [40, 45].

10. Conclusion

The Bacillus subtilis and its associated bacilli is an extremely flexible and robust biological system in the treatment of contemporary wastewater. With the help of their complex biofilms, which are controlled by sophisticated genetic circuitry, they are capable of degrading an extensive range of organic contaminants and sequestration of dangerous heavy metals. The functionality of these biological systems can be enhanced to a significant level by incorporation of the latest genetic tools and nanotechnology. With the stringent water standards set by the world, the bioremediation based on Bacillus will also be a part of the sustainable environmental management approach.

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