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Review

# Bioaugmentation and its application in wastewater treatment: A review

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## HIGHLIGHTS

- Community ecology and dynamics in bioreactors in WWTPs are complex processes.
- Progress in ecology, molecular biology, immobilization, bioreactor design are reviewed.
- Emerging research facilitates novel applications in bioaugmented bioreactors.
- Bioaugmentation should ultimately be assessed by data from field implementation.

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## ABSTRACT

Bioaugmentation (the process of adding selected strains/mixed cultures to wastewater reactors to improve the catabolism of specific compounds, e.g. refractory organics, or overall COD) is a promising technique to solve practical problems in wastewater treatment plants, and enhance removal efficiency. The potential of this option can now be enhanced in order to take advantage of important advances in the fields of microbial ecology, molecular biology, immobilization techniques and advanced bioreactor design. Reports on bioaugmentation in WWT show the difficulties in evaluating the potential parameters involved, leading frequently to inconclusive outcomes. Many studies have been carried out on the basis of trial-and-error approaches, and it has been reported that reactors bioaugmented with pure cultures often fail to perform as well as the pure cultures under laboratory conditions. As an interesting technical challenge, the feasibility of bioaugmentation should ultimately be assessed by data from field implementation, and this review highlights several promising areas to explore in the future.

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## 1. Introduction

Advanced bioreactor design and operation in wastewater treatment plants (WWTPs) is essential in order to develop proper environmental conditions so that the most desirable microorganisms can be selected for and maintained under adequate physiological conditions. This extends to the contact with the stream to be treated, controlling mass transfer and reactor performance to achieve the treatment goal at full scale. Until recently, biological reactors in wastewater treatment have been considered as "black boxes", their functionality depending only on empirical knowledge, and hence they are difficult to predict and control. Even now their performance can only be evaluated by applying material balances. Now advanced bioreactor design can take advantage of compartmentalisation and the use of membranes to confine the key microorganisms in the process to a bioreactor (Barber and Stuckey, 1999; Vyrides et al., 2010). However, it is also known that effective operation of biological treatment systems relies on highly active microorganisms carrying out the process, and therefore on the ratio of resistant/sensitive microorganisms to the contaminant being treated (depending on both its chemical nature and concentration). Microorganisms can degrade a wide variety of organic contaminants and can adapt to many different inhospitable environments. However, there are a variety of pollutants, both man made (xenobiotic) and natural, that are not easy to degrade biologically, even over long periods of time -these are referred to as "refractory or recalcitrant".

In principle, poor bioreactor performance may be due to the lack of a sufficient number of a specific microorganism harbouring a key metabolic route to transform the target contaminant into less harmful end products. However, despite functional redundancy (i.e. different strains that can carry out the same or similar functions), and metabolic versatility being common in environmental bioprocesses, it is more likely that the specific target contaminant (or mixture of compounds) can only be degraded by a very specific mixture of microorganisms (a consortium) harbouring the key metabolic pathways, and cooperating in a synergistic way. The importance of microbial consortia is such that, without their combination, many biodegradation processes could not be explained thermodynamically on the basis of free energy in a single chemical reaction, explaining ultimately the shift in fermentation patterns (Melandri, 1997; Jorgensen and Gallardo, 1999).

Despite non-specific bioaugmentation being used for years in agriculture and wastewater treatment to reinforce the process, it is still considered a procedure with unpredictable outcomes. Even now bioaugmentation is considered less predictable and controllable than other removal techniques which result in the direct destruction of contaminants (Boon et al., 2000). The approach of bioaugmentation focuses on taking advantage of microbial consortia designed for the specific physico-chemical properties of the bioprocess, (Van der Gast et al., 2004) since this approach was shown to be more efficient than using undefined inocula. Potentially higher efficiencies could occur in systems such as membrane bioreactors which stop bacteria being "washed out", in contrast to the natural environment where the environmental conditions can be manipulated to enhance survival and prolong the activity of the exogenous population (El Fantroussi and Agathos, 2005).

Bioaugmentation of wastewater treatment has not been reviewed specifically for a number of years, while the current knowledge base has changed rapidly. Metagenomics (or Ecogenomics – the application of genomics to ecological and environmental sciences – (for a review see Maphosa et al., 2010), other molecular methods, microscopy and flow cytometry- based methods are providing an enormous source of information for monitoring, detection, quantification and characterisation of microorganisms. This opens up the possibility of exploring unculturable microorganisms, and exploiting biodiversity as a means to increasing biodegradation, even through genetic engineering (though the impact of genetically modified microorganisms on the environment is still unknown). The question is to determine not only the systematic and taxonomical structure of the microbial consortia taking part in environmental bioprocesses (community structure), but also to learn how these biological systems respond to changes in the influent (understanding the biochemistry and toxicity mechanisms involved), how the microbial population dynamics evolve (community ecology and assembly), and in which way these changes can be related to bioprocess efficiency (community function).

## 2. Community ecology and assembly

It should be noted that community assembly in biological reactors is a very complex process since different microorganisms comprising a multitude of cells work together to treat the effluent, interacting by means of different types of cell signalling such as quorum sensing and horizontal gene transfer (Verstraete, 2007).

At the moment a complex community cannot be engineered rationally (Curtis et al., 2003), since is still poorly understood (Sloan et al., 2006). Theoretical ecologists such as MacArthur and Wilson (1967) and May (1974) assumed microbial communities to be dynamic systems which were nonlinear, and predicted community stability or instability. Lawrence and McCarty (1970) developed equations based on biochemistry and microbial kinetics that enabled key process variables to be predicted in two different biological systems, aerobic and anaerobic. It has been postulated that dynamic population behaviour is probably innate to microbial systems. Curtis et al. (2003) also suggest that it is possible "steady-state" conditions never really exist in bioprocesses which have been engineered, even in processes with constant inputs or no apparent variations in growth conditions. Immigration and chance are important in shaping the behaviour seen in prokaryotic communities (Sloan et al., 2006). Interestingly, it has been reported that biological communities in similar econiches may have contrasting dynamic behaviour depending on the specific chemical, physical, or biological conditions which the culture operates under (Ayala-del-Río et al., 2004).

In order to advance functional organisation, the usefulness of Pareto–Lorenz diagrams have been applied to measure microbial diversity graphically to represent the structure of a bacterial community. The Pareto 80/20 principle applied in macroeconomics has been established where 20% of the participants normally acquire 80% of all the energy (labour) flux (Dejonghe et al., 2001). By using Pareto–Lorenz distribution patterns, Mertens et al. (2005) showed that only a small group of ammonia oxidising bacteria (AOB) played a dominant role in nitrification, while the less dominant species remaining were postulated to embody a reserve of AOB which could grow up to replace the species which dominated