

Industrial wastewater treatment through bioaugmentation

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Abstract

Bioaugmentation of activated sludge processes through the addition of microorganisms is employed with the aim of enhancing treatment, in particular the removal of priority pollutants. With industrial wastewaters, studies have covered target pollutants including ammonia and polycyclic aromatic hydrocarbons (PAHs): compounds that are regulated around the globe. However, bioaugmentation is a technique that has been associated with doubt in regard to its ability to benefit treatment processes. Failure of bioaugmentation has been reported to be associated with numerous factors that include the growth rate being lower than the rate of washout, insufficient inoculum size and substrate availability. Limitations of bioaugmentation can be overcome through techniques that include increased inocula dosing, pre-acclimatisation of inocula in side-stream reactors, addition of nutrients and surfactants and application of sufficient acclimatisation periods. Surveys of the literature show that a key area for further research should be towards acquiring a better understanding of the degradation pathways where bioaugmentation is applied. There also remains a need to undertake bioaugmentation efficacy studies at full scale with test and control streams. Further reporting on the economic viability of the technique is also necessary.

Keywords: Bioaugmentation; industrial wastewater; nitrogen; polycyclic aromatic hydrocarbons; phenol

1. Introduction

Industrial wastewaters account for a large proportion of pollution in freshwater systems and are therefore regulated across the globe. For example, in Europe, industrial wastewaters are regulated under the Industrial Emissions Directive (IED) whilst in the United States they are regulated under the Clean Water Act (European Commission, 2015b; US.EPA, 2015). Under the IED, the compounds included in the regulation vary for each industrial process and are reported along with the associated emission limits in the best available techniques reference document (BREF) (European Commission, 2011). An activated sludge process (ASP) has been identified as the best available technique (BAT) to meet the required emission limits (**Table 1**) for a number of such wastewaters. This includes wastewaters from the milk and food industry, waste gas treatment, refinery of mineral oil and gas, iron and steel coke processing and glass manufacturing (European Commission, 2003, 2006, 2012, 2013a, 2013b, 2014, 2015a).

The suspended microbial mass in an ASP is responsible for the biodegradation of organic compounds via the metabolic reactions of the bacteria (Zhang et al., 2014a). Many industrial wastewaters contain a mixture of compounds that are recalcitrant and others that may be toxic; such wastewaters therefore have the potential to persist in effluents after an ASP. It is thus necessary to establish treatment methods which can cope with the complex mixture of compounds typically associated with industrial wastewaters. Bioaugmentation, the addition of supplementary microorganisms with their associated biodegradation capacities, may allow for the improved performance of ASPs (Semrany *et al.*, 2012).

Table 1: Industrial Emission Directive emission limits for wastewaters for which an activated sludge process is recognised as the best available technique.

Wastewater origin	BAT emission limit (mg/L)	Reference
Produced Water (Oil and gas wastewater)	Hydrocarbon oil index: 0.1 – 2.5 COD: 30 – 125 TN: 1 -25	(European Commission, 2014)
Food and Milk: e.g. Raw dairy, Cheese, Mixed dairy, palm oil mill effluent.	Oil and grease: < 10 COD: <125 BOD ₅ : <25 TN: < 10 TP: 0.4-5	(European Commission, 2006)
Glass manufacturing	COD: < 5-130 Total hydrocarbons: <15 Ammonia (as NH ₄): < 10 Phenol: < 1	(European Commission, 2012)
Coke making wastewater:	COD: < 220 BOD ₅ : <20 SCN: < 4 PAHs*: 0.05 Phenols: 0.5 TN: <15-50	(European Commission, 2013a)

*Sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene

Industrial wastewaters represent some of the most challenging waters requiring treatment and therefore offer insight into some of the more complex situations in which bioaugmentation may be implemented. Benefits may include more stable operating conditions, better flocculation characteristics, decreased start-up times, resistance to shock loads and better cold weather performance (Stephenson and Stephenson, 1992; Van Limbergen, Top and Verstraete, 1998; Guo *et al.*, 2009; Bartrolí, Carrera and Pérez, 2011; Qu *et al.*, 2011). Bioaugmentation has been reported to be unpredictable (Boon *et al.*, 2000), however, a number of factors have been highlighted as impacting successful bioaugmentation including: strain selection, addition and maintenance techniques and knowledge of the molecular biology and the capabilities of commercial products (Stephenson and Stephenson, 1992; Van Limbergen, Top and Verstraete, 1998; Thompson *et al.*, 2005; Herrero and Stuckey, 2014).

2. Strain selection

The selection of a suitable strain is essential to the success of bioaugmentation. The selected strain(s) must be able to withstand the environmental conditions imposed on them within a treatment process including; temperature, pH, dissolved oxygen, nutrient availability, toxicity and microbial pressures (Bitton, 2011). It is well recognised that, without an understanding of the conditions within the treatment process, bioaugmentation is likely to fail due to the poor survival of the inoculum and/or competition from indigenous microbial populations (Stephenson and Stephenson, 1992; More *et al.*, 2010). The selection and isolation of a strain from the indigenous population has become, progressively, the favoured approach as this increases the likelihood of success as the strain is already adapted to survival in the selected environment (Ueno *et al.*, 2007). This approach can be taken when a species is present in a treatment process but in insufficient numbers for adequate treatment. Selection of a strain from an alternative site may be the only option when a compound cannot be degraded by the species already present at location, however, success may be limited if the environmental conditions are not conducive to the survival of the inoculated strain (Thompson *et al.*, 2005).

Applications may include the use of a single strain or a combination of strains to produce a suitable consortium (Khehra *et al.*, 2005; Qu *et al.*, 2011). An individual strain may be selected for its ability to degrade a specific compound or due its role in a more complex degradation pathway. A number of strains may be used to replicate a natural community, enhance or replicate a catabolic pathway with numerous stages and/or degrade a number of target pollutants within the same wastewater (Van Limbergen, Top and Verstraete, 1998; Thompson *et al.*, 2005). Increasingly, consortia are selected for bioaugmentation applications, with degradation processes frequently built upon the combined actions of numerous species, especially for the degradation of complex xenobiotic compounds (Stroo, Leeson and Herb Ward, 2013).

The success of a consortium was demonstrated by Khehra *et al.* (2005) for the treatment of recalcitrant dyes released from the textile processing industry. In laboratory investigations, both single strains and the consortium were supplemented with 20 mg/L of dye. Whilst the individual strains were able to decolourise 3 of the 6 dyes, to varying degrees, the consortium decolourised all of the dyes. Further to this, the time required for the decolourisation was reduced from 24 hours to 8 hours. Due to the structural diversity of the dyes, the synergistic actions of the consortium proved to have a beneficial role. Similarly, the synergistic actions of a consortium previously developed by Chhatre *et al.* (1996) were recognised as important by Domde, Kapley and Purohit (2007) in the treatment of petroleum wastewater. In this application, a combination of isolates worked together to solubilise and then degrade various components of crude oil. One isolate was responsible for producing a biosurfactant followed by the emulsification of the crude oil which then made long chain aliphatics and aromatics available to the other two isolates for degradation. This combination of isolates resulted in an overall degradation rate of 65-70% and an increase in chemical oxygen demand (COD) removal from 15% without bioaugmentation to 52.2% with the consortium (Chhatre *et al.*, 1996; Domde, Kapley and Purohit, 2007).

Genetic manipulation provides further opportunities for the degradation of compounds for which a pollutant-degrading natural strain does not exist (Stroo, Leeson and Herb Ward,

2013). Microorganisms can be genetically engineered to over-express degradation genes or to exhibit increased survivability (McClure, Fry and Weightman, 1991; Nüßlein *et al.*, 1992; Stroo, Leeson and Herb Ward, 2013). Such techniques enable the possibility of designing microorganisms to assist with the treatment of pollutants which require numerous degradation steps or those required to degrade xenobiotic compounds. Knowledge of the degradation pathways involved for such compounds is limited and a naturally occurring species capable of such degradation may not exist (Stroo, Leeson and Herb Ward, 2013). Microorganisms which have been genetically modified have been investigated in groundwater aquifer microcosms (Jain *et al.*, 1987), lake waters (Awong, Bitton and Chaudhry, 1990) and ASP (McClure, Weightman and Fry, 1989; McClure, Fry and Weightman, 1991). McClure, Weightman and Fry (1989) demonstrated that genetically engineered bacteria were able to survive within a laboratory-scale ASP and did not encourage protozoa reproduction despite large numbers of bacteria being inoculated. Additionally Nüßlein *et al.* (1992) were able to confirm that microorganisms that were genetically engineered were not only capable of surviving in an ASP but were also able to maintain their genetic information and degrade the required pollutants. Such genetic adaptation may allow for the design of microorganisms which are able to assist in the degradation of pollutants which require numerous degradation steps. Further to the genetic modification of microorganisms, gene bioaugmentation, which involves the use of catabolic mobile genetic elements (MGEs), has been highlighted in regard to its applicability to bioaugmentation (Stroo, Leeson and Herb Ward, 2013). Mobile genetic elements consist of pieces of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) which can be transferred from one organism to another (Stroo, Leeson and Herb Ward, 2013).

Despite the numerous possible ways in which genetic engineering may improve the future of bioaugmentation, current research is heavily laboratory based and success in the field cannot currently be fully assessed due to legislative restrictions resulting from concerns surrounding the risks to both the environment and human health of the uncontrolled spread of microorganisms which have been genetically engineered (Van Limbergen, Top and Verstraete, 1998). Strategies such as the use of a ‘suicide element’ and immobilisation techniques have been considered in order to reduce such risks (Liu, Huang and Wang, 2008;

Stroo, Leeson and Herb Ward, 2013). Suicide techniques, for example, may be repressed by an environmental signal such as from the pollutant to be degraded. When the signal no longer exists the suicide element is activated. This technique has been shown to be successful in preventing the spread of engineered cells (Torres, Garcia and Diaz, 2003). Legislation often ignores the ways in which molecular genetics can be used for risk mitigation, and consequently, future research will have to both inform and follow the regulations (Davison, 2005; Stroo, Leeson and Herb Ward, 2013).

Commercial inocula are now also widely available. Such products vary greatly in their make-up, cell density, advised dosing rates and the incorporation of other additives e.g. stabilisers and nutrients. Each of these factors need to be considered when selecting a suitable product (Stroo, Leeson and Herb Ward, 2013). The use of commercial inocula may offer a short-term solution to an immediate treatment issue; however, success rates may vary because such inocula are typically produced and tested under stable conditions. Such conditions do not reflect the real-life scenario relevant to many industrial wastewaters, in turn reducing the survivability of the inocula (Stephenson and Stephenson, 1992).

3. Operational considerations

The application of bioaugmentation is more likely to be successful in a treatment system with well-characterised wastewater and operational parameters. This knowledge helps to identify potential obstacles to the survival of the inoculated bacteria, including toxicity and nutrient availability (Jianlong *et al.*, 2002). Without a detailed knowledge of the system, the likelihood of a successful integration of the inoculum is reduced. Activated sludge processes can differ greatly in their configuration, although principally they consist of one or more treatment cells containing biomass which may be aerobic, anoxic or anaerobic in nature. Such treatment cells may operate under continuous flow conditions, in mixed systems or be operated under a batch or plug-flow system. The introduction and maintenance method for bioaugmentation applications should therefore be informed by the design of the treatment

system. Treatment efficiencies and pollutant concentrations, on the other hand, will inform decisions relating to dosing rates (Stephenson and Stephenson, 1992; Park *et al.*, 2008).

3.1 Dosing technique

Direct dosing involves the addition of the selected microorganisms straight into a treatment vessel. Such a technique represents the simplest method of bioaugmentation and can be advantageous in the sense that it can be applied as and when required. This can also be economically beneficial as it does not require plant modification and the associated operational costs. Problematic to this approach, however, is the reduced survival rates of the inoculated microorganisms due to a lack of acclimatisation to the environmental conditions of the host environment resulting, for example, from toxicity, pH, carbon availability, predation and competition between the indigenous and inoculated bacteria (Chong, Pai and Chen, 1997; Bouchez *et al.*, 2000; Songzhe *et al.*, 2009). The use of a side-stream reactor can help to overcome some of the aforementioned problems as it enables the acclimatisation of the inoculated microorganisms to the environmental conditions, thus increasing their survival rate in the treatment process (Parker and Wanner, 2007). The footprint of a side-stream is typically approximately 10% that of the main reactor. As the side-stream can enable process intensification, this can represent a much smaller investment cost than the cost associated with expanding a treatment works to cope with a higher capacity. Despite this, in some instances the additional land requirements may still be problematic (Salem *et al.*, 2003). The use of encapsulation techniques can assist in the incorporation of inoculated bacteria into the existing flocs (Stormo and Crawford, 1992). Bouchez *et al.* (2009) mixed the inoculum with an alginate solution, forming bead structures which were employed in the reactor. The beads allowed the inoculated bacteria to remain in the system and protected them from the intense grazing that was experienced without such encapsulation. The beads were observed to break into fragments by day 8, but such fragments were incorporated into flocs of the indigenous sludge, allowing their successful incorporation into the system. Another recent strategy that has been showed successful treating high strength pyridine wastewater is through the addition

of aerobic granules of pure strains formed in a sequencing batch reactor (SBR) to the main treatment reactor (Shen *et al*, 2009; Liu *et al*, 2015).

3.2 Dosing location

The success of bioaugmentation has been shown to be influenced significantly by the location at which the selected microorganisms are dosed. Dosing location should be selected based on a careful consideration of the environmental conditions that the selected microorganisms require in comparison to those they will face. Determination of the most suitable location may be more critical in industrial wastewaters, which frequently contain single or multiple pollutants known for their toxic effects. The impact of the identification of the correct location was demonstrated by Jianlong *et al.* (2002) during the treatment of coke-making wastewater. The wastewater, characterised by the presence of multiple toxic compounds, was treated through an ASP with an anaerobic, anoxic and aerobic reactor. *Burkholdiera pickettii*, a quinoline degrading species, was shown to have a beneficial role at any location; nevertheless, its positive impact was higher when *Burkholdiera pickettii* was added to the aerobic reactor. The provision of a suitable food source and the lower toxicity, as a result of the degradation of co-occurring compounds in previous treatment cells to smaller compounds, resulted in higher degradation efficiencies. Similar conclusions were drawn for the removal of 2-4-dichlorophenol in a laboratory-scale ASP. A mixed culture was developed through the enrichment of sludge taken from two wastewater treatment plants. The mixed culture was then added to a separate reactor with a carrier system of plastic lace strings (Quan, Shi, Liu, Wang, *et al.*, 2004). Removal was higher, at 90.3%, when the bioreactor was located after the aeration cell than when the bioreactor was situated before the aeration cell (86.2% removal). It had been assumed that locating the bioreactor before the aeration cell would allow the removal of 2-4-dichlorophenol, which in turn would improve the removal efficiency of other pollutants as a result of the lowered toxicity of the wastewater. Despite this, the 2-4-dichlorophenol removal decreased when the bioreactor was placed before the aeration cell as a lack of easily degradable compounds resulted in a decrease in the removal of the targeted 2-4-dichlorophenol. When the bioreactor was placed after the aeration cell, the

bioaugmented culture was able to specialise in the removal of 2-4-dichlorophenol, increasing the treatment efficiency.

3.3 Dosing size and regime

Dosing characteristics and regimes vary considerably between the different applications of bioaugmentation. The first characteristic that requires consideration is the initial inoculum size, which should be sufficiently large enough to overcome initial predation pressures whilst not so large as to result in a disturbance to the ecosystem equilibrium. Ramadan, El-Tayeb and Alexander (1990) reported that p-nitrophenol containing wastewater required a high initial dose (4.3×10^4 cells per mL) in order to overcome predation pressures. In contrast, Bouchez *et al.* (2000) reported a disturbance of the ecosystem balance resulting from increased pressures due to a large inocula dose Secondly, the use of maintenance doses may be necessary in order to maintain the population of the inoculated bacteria which may decrease over time as a result of routine sludge wastage or inherently low survival rates. The need for a maintenance dose varies from application to application. Boon *et al.* (2003) noted that bioaugmentation was not a permanent process when investigating the removal of 3-chloroaniline whilst Martín-Hernández, Suárez-Ojeda and Carrera (2012) reported that maintenance doses were not necessary when the initial dose was high enough to overcome initial predation pressures.

4. Bioaugmentation failures and associated improvement techniques

Successful reports concerning bioaugmentation have also been associated with reports of unsuccessful bioaugmentation attempts. Fundamental to the success of any application is the ability of the inoculated bacteria to survive and prosper. Numerous factors have been cited for the failure of bioaugmentation (**Table 2**) including the growth rate of the microorganism being lower than the rate of washout (Boon *et al.*, 2000), an insufficient inoculum size (Ramadan, El-Tayeb and Alexander, 1990), an insufficient substrate (Goldstein, Mallory and

Alexander, 1985; Bouchez *et al.*, 2000; Tyagi, da Fonseca and de Carvalho, 2011; Martín-Hernández, Suárez-Ojeda and Carrera, 2012), predation by protozoa (Goldstein, Mallory and Alexander, 1985; Boon *et al.*, 2000; Bouchez *et al.*, 2000), competition between the inoculated and indigenous bacteria (Stephenson and Stephenson, 1992; Bouchez *et al.*, 2000; More *et al.*, 2010), the presence of other inhibiting substances (Goldstein, Mallory and Alexander, 1985; Bouchez *et al.*, 2000; Tyagi, da Fonseca and de Carvalho, 2011), the availability of alternative substrates (Goldstein, Mallory and Alexander, 1985; Chitra *et al.*, 1995; Quan, Shi, Liu, Wang, *et al.*, 2004; Mahin *et al.*, 2011), the need for an acclimatisation period (Stephenson and Stephenson, 1992) and extremes in environmental factors such as temperature and pH (Tyagi, da Fonseca and de Carvalho, 2011). An understanding of the root cause of the failure of the bioaugmentation process is important to ensure the advancement of bioaugmentation applications.

Grazing was held responsible for the failure of *M. aerodenitrificans* becoming established in an aerobic nitrifying sequencing batch reactor by Bouchez *et al.* (2000). The added bacteria were associated with the liquid phase of the reactor and were not incorporated into bacterial flocs. As a result of their suspended nature they were targeted by grazing protozoa, which have grazing rates proportional to the fast rates of decline seen in the system. Ramadan, El-Tayeb and Alexander (1990) also saw a decline in the inoculated bacterial abundance which coincided with the multiplication of protozoa in the treatment of p-nitrophenol (PNP)-containing wastewaters. Similarly, an overgrowth of protozoa as a result of bioaugmentation was reported by Songzhe *et al.* (2009) during the removal of ammonia from marine aquaculture wastewaters. Furthermore, a rapid decline of the denitrifier *Pseudomonas stutzeri* TR2 was again associated with probable predation during the treatment of piggery wastewater (Ikeda-Ohtsubo, Miyahara, Kim, *et al.*, 2013). Songzhe *et al.* (2009) concluded that a form of protection, e.g. encapsulation from grazing, was necessary. An alternative approach investigated related to the ability of heat treatment to protect the inoculated bacteria from predation (Ikeda-Ohtsubo, Miyahara, Yamada, *et al.*, 2013) and results showed that adapting the reactor conditions overcame the predation problems. When the temperature of the treatment reactor was reduced to 35°C the predators were able to proliferate and during this period, there was a rapid tenfold increase in their associated genes. When the temperature

was increased to 40-44°C there was no increase in the number of genes representing predators and therefore *Pseudomonas stutzeri* TR2 was protected from predation.

Contrary to reports concerning the negative effects of grazing on bioaugmentation, Yu, Peng and Ren (2011) demonstrated that grazing did not have a significant impact on nitrogen removal. Nitrification efficiencies were monitored in a bioaugmentation system in which all protozoa were inhibited and compared to one in which protozoa were not inhibited. Although initially protozoa numbers increased rapidly in the non-inhibited reactor, their numbers then declined gradually over the duration of the study and complete nitrification was ultimately possible in both reactors. The increased time requirement, from 71 days with protozoa inhibition to 76 days without protozoa inhibition, was not considered to be significant.

Nutrient limitation is a particularly important factor in the treatment of industrial wastewaters which frequently lack the essential nutrients required for microbial development (Burgess, Quarmby and Stephenson, 1999). Nutrient limitations have been held responsible for failed bioaugmentation attempts due to the competition between the indigenous and inoculated bacteria. Ramadan, El-Tayeb and Alexander (1990) demonstrated that the supplementation of nutrients could increase the likelihood of a successful bioaugmentation outcome as the addition of nitrogen and phosphate allowed for low densities of inoculum to remove p-nitrophenol (PNP), potentially increasing the inoculum growth rates and resistance to higher protozoa numbers. Such nutrient addition is referred to as biostimulation. Biostimulation, however, can also include the addition of other stimulants such as surfactants. Nikolopoulou, Pasadakis and Kalogerakis (2013) demonstrated that the presence of a biosurfactant could increase degradation rates in oil-contaminated sites by enhancing the solubility of the hydrocarbons. Under such treatment systems it is important, however, to have adequate controls in place in order to be able to assess to what degree the improvement is a result of the biostimulation rather than a result of the bioaugmentation itself. Due to its complementary action, biostimulation has therefore become a technique that is frequently reported for use alongside bioaugmentation (Wenderoth *et al.*, 2003; Olaniran, Pillay and Pilay, 2006; Tyagi,

da Fonseca and de Carvalho, 2011; Nikolopoulou, Pasadakis and Kalogerakis, 2013; Shoji *et al.*, 2014; Sun *et al.*, 2014).

Industrial wastewaters are frequently characterised by changing load rates which result in fluctuating concentrations of the target compounds. Some failures of the bioaugmentation process have been linked to long periods of starvation in the target pollutant. One means with which to tackle this problem is to select an initial dose which is high enough to allow a proportion of the bacteria to persist in the treatment system until the load rate increases again. This approach was investigated by Martín-Hernández, Suárez-Ojeda and Carrera (2012) during the treatment of p-nitrophenol in a laboratory-scale sequencing batch reactor. Using a dose rates of 2% and 5% respectively, it was found that the higher initial dose rate allowed the inoculated bacteria to survive the 20 day period of starvation and maintain subsequent treatment. Importantly, the dose rate of 5% was still practical in terms of its application to full-scale treatment works. In contrast Duque *et al.* (2011) found that periods of substrate inhibition did not cause failure during the treatment of 2-fluorophenol in a rotating biological contactor.

For some bioaugmentation applications failure lies in the inadequate adaptation of the inoculum to the host environment. Chong, Pai and Chen (1997) reported that a mixed culture, designed to treat petroleum wastewater, was unable to proliferate in the system, yielding no benefit to the water treatment under pH shock conditions and complete failure under continuous high pH conditions. The failure was linked to biomass washout as a result of growth retardation or death of the inoculated population. Biomass washout, as a result of poor reactor conditions, including an inadequate carrier system and violent air bubbling, was also reported by Park *et al.* (2008) in the treatment of cyanide wastewater. Additionally, Songzhe *et al.* (2009) reported that inoculated bacteria were unable to form an adequate biofilm due to interaction with other indigenous bacteria resulting in biomass washout and the failure of bioaugmentation. The likelihood of inadequate adaptation is increased with industrial wastewaters and this highlights the requirement for understanding the treatment conditions and adaptation techniques.

Problem	Technique to overcome problem
Predation (Overgrowth of protozoa) (Goldstein, Mallory and Alexander, 1985; Ramadan, El-Tayeb and Alexander, 1990; Songzhe <i>et al.</i> , 2009; Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	High initial doses (Ramadan, El-Tayeb and Alexander, 1990) Protection from grazing (Songzhe <i>et al.</i> , 2009) Heat treatment (Ikeda-Ohtsubo, Miyahara, Yamada, <i>et al.</i> , 2013)
Competition for nutrients between indigenous and inoculated bacteria (Ramadan, El-Tayeb and Alexander, 1990; Yu <i>et al.</i> , 2005; Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	Supplementation of nutrients (biostimulation) (Ramadan, El-Tayeb and Alexander, 1990)
Insufficient inoculations (Ramadan, El-Tayeb and Alexander, 1990)	Repeated inoculations (Boon <i>et al.</i> , 2003) Continual inoculations (Abeyasinghe <i>et al.</i> , 2002)
Poor biofilm formation (Park <i>et al.</i> , 2008; Songzhe <i>et al.</i> , 2009)	Immobilisation/encapsulation (Stormo and Crawford, 1992; Quan, Shi, Liu, Lv, <i>et al.</i> , 2004)
Wash-out (Chong, Pai and Chen, 1997; Park <i>et al.</i> , 2008)	High initial doses (Ramadan, El-Tayeb and Alexander, 1990) Immobilisation/encapsulation (Stormo and Crawford, 1992; Quan, Shi, Liu, Lv, <i>et al.</i> , 2004)
Decline of inoculated bacteria due to toxins (Goldstein, Mallory and Alexander, 1985)	Protection from adverse environmental conditions (Songzhe <i>et al.</i> , 2009) Allow acclimatisation period (Stephenson and Stephenson, 1992) Use autochthonous bioaugmentation (Ueno <i>et al.</i> , 2007)
Alternative substrates available (Goldstein, Mallory and Alexander, 1985; Chitra <i>et al.</i> , 1995; Quan, Shi, Liu, Lv, <i>et al.</i> , 2004; Mahin <i>et al.</i> , 2011)	Detailed understanding of ecological background (Songzhe <i>et al.</i> , 2009)
Large inoculations disturbing balance of ecosystem (Bouchez <i>et al.</i> , 2000)	Careful consideration of dose rate
Periods of starvation (Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	Higher dose rate to allow survival in the system for longer time periods (Martín-Hernández, Suárez-Ojeda and Carrera, 2012)

5. Applications of bioaugmentation to pollutants regulated by the Industrial Emissions Directive

A wide variety of wastewaters are regulated under the IED, all of which could potentially benefit from the application of bioaugmentation. An improved understanding of the capabilities of bioaugmentation could therefore offer widespread opportunities for industrial wastewater treatment. Industrial wastewaters can encompass a wide variety of pollutant compounds, although typically some commonalities exist between the different wastewaters. Nitrogen compounds are common to many types of wastewater, particularly those from the milk and food industries as well as coke processing activities. The levels of ammonia in coke-making wastewater can vary from 123 mg/L up to 4,500 mg/L (Ganczarczyk, 1983; Gould, 1986). Ammonia concentrations vary between sites due to variations in the operational conditions and also temporally at a single site due to variations in production levels (Marañón *et al.*, 2008). High concentrations of ammonia are also characteristic of dairy wastewaters. As with coke-making wastewaters, they are subject to both spatial and temporal variations due to difference in the products produced and the treatment methods in place. Furthermore, these wastewaters are often produced intermittently (Vidal *et al.*, 2000).

Nitrogen is a key target pollutant as it can cause the eutrophication of receiving waters. Nitrifying bacteria grow more slowly than the general heterotrophic community and are less resistant to toxicity. Consequently, nitrifying bacteria may be outcompeted. Supplementation through bioaugmentation may therefore be beneficial to treatment systems characterised by a high nitrogen loading. As the removal of nitrogen occurs in a two-step process involving the oxidation of ammonia to nitrite and the subsequent oxidation of nitrite to nitrate, nitrifying treatment processes require process stability in order to allow the two steps to remain synchronised and to prevent accumulation of the more toxic nitrogen species nitrite. Abeysinghe *et al.* (2002) investigated the ability of bioaugmentation to support the nitrification process when operating under stress conditions. At a solids retention time of two days, the treatment system operated near washout conditions, but the addition of 45 and 67.5 mg/L of ammonia oxidisers, allowed effluent ammonia concentrations to be reduced from 4.5 mg/L to <1 mg/L. The application of bioaugmentation can therefore be an effective and

convenient tool to support industrial treatment systems which frequently operate under stress conditions.

Obbard and Shan (2001) also reported the use of bioaugmentation to support the treatment of prawn aquaculture ponds which are characterised by high nitrogen loading rates but which experience high levels of nitrifier washout as a result of the regular exchange of pond water exchange employed to prevent the build-up of toxins in such ponds. Inert media have been reported to enhance treatment by increasing bacterial populations through biofilm formation (Stephenson *et al.*, 2013). This technology has been selected in order to tackle the problem of washout, with indigenous nitrifiers immobilised onto porous clay pellets, allowing the total ammonical nitrogen to be reduced from 3 mg/L to 0.5 mg/L, the latter being below the required concentration necessary for optimum prawn growth (1.33–1.53 mg/L) (**Table 3**). The treatment of high nitrogen loads through bioaugmentation was reported by Onyia *et al.* (2001) for palm oil wastewater (**Table 3**). Palm oil wastewaters are characterised by organic nitrogen loads of 180–1,820 mg/L and the treatment of this type of wastewater is time intensive, with more than 11 days required in order to achieve 50% nitrification. However, the addition of 15 mg/L of a mixed nitrifying culture increased this efficiency to 100% within seven days.

Carrier materials have also been employed to support bioaugmentation. In the treatment of petrochemical wastewater, Ma *et al.* (2009) used a carrier system of polyurethane foam to encourage the inoculated bacteria to form a biofilm (**Table 3**). The resulting biofilm prevented the washout of the inoculated bacteria as well as the gradual decrease in their numbers as a result of predation. Additionally, the inoculated bacteria were provided with organic substrates and inorganic trace elements to support their growth. Consequently, the bioaugmented reactor showed better performance with decreased start-up times (20 days compared to 30 days without bioaugmentation), a higher resistance to shock loads of COD, higher treatment efficiencies of refractory organic compounds (reduced to 21 compared to 46 without bioaugmentation) and a reduction of effluent ammonia concentrations (4.1 mg/L compared to 12.4 mg/L).

418 **Table 3: Examples of bioaugmentation applied to compounds present in industrial**
 419 **wastewaters.**

Compound	Scale	Application	Conclusions
Nitrogen			
(Onyia <i>et al.</i> , 2001)	Laboratory	Palm oil effluent	15 mg/L of mixed cultures led to 100% increase in nitrification. Reduced HRTs led to 20% reduction in land requirement.
(Obbard and Shan, 2001)	Laboratory	Prawn aquaculture wastewaters	Immobilised bacteria allowed total ammonical nitrogen reduced from 3 mg/L to 0.5 mg/L allowing ponds to remain at optimal conditions.
(Ma <i>et al.</i> , 2009)	Full-scale	Petrochemical wastewaters	Immobilisation prevented washout of nitrifiers. National discharge limits met in 20 days compared to 30 days. Effluent ammonia concentrations fell from 12.4 mg/L to 4.1 mg/L.
Aromatic compounds			
(Qu <i>et al.</i> , 2011)	Laboratory	Synthetic alkaline wastewaters	Addition of <i>Pseudomonas</i> sp. JY-2 allowed improved start-up times (90% removal compared to 65% after 1.5 days) and increase long-term treatment efficiency (90% compared to 80%).
(Fang <i>et al.</i> , 2013)	Laboratory	Coal gasification wastewater	Bioaugmentation increased removal efficiencies from 66% to 80% despite high variation in levels of phenol (500-3000 mg/L).
(Duque <i>et al.</i> , 2011)	Laboratory	2-fluorophenol wastewaters	2-fluorophenol degrading species FP1 allowed treatment of waters subjected to shock loads of up to 50 mg/L of 2-fluorophenol.
(Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	Laboratory	p-nitrophenol (PNP) wastewaters	Bioaugmentation allowed immediate removal of shock loads of PNP. Without bioaugmentation PNP removal took 4 days to reach 100% and then failed after 8 days.
(Straube <i>et al.</i> , 2003)	Laboratory and pilot-	PAH contaminated soil	Bio-surfactant producer <i>Pseudomonas aeruginosa</i> strain 64 increased PAH

	scale		degradation from 23% to 34%. Bioaugmentation and biostimulation increased degradation to 87%. Biostimulation alone increased degradation to 86%.
(Sun <i>et al.</i> , 2014)	Pilot	Former coke works contaminated soil	Total PAH levels fell by 24% in the control, 35.9% with bioaugmentation, and 59% with biostimulation. Bioaugmentation was responsible for the increased removal of heavy molecular weight molecules.

420

421 Bioaugmentation has also been used for the treatment of aromatic compounds including
422 phenols and polycyclic aromatic hydrocarbons (PAHs) which are present in a wide variety of
423 industrial wastewaters, including those from agrochemical, pharmaceutical, petrochemical,
424 coal gasification, coke processing, insecticide and hydrocarbon wastewaters among others
425 (**Table 3**). Aromatic compounds are regulated under the IED and are also listed as Priority
426 Substances within the European Union (European Union, 2013).

427

428 Coal gasification wastewater is subject to a high variability of phenol concentration, from
429 500–3,000 mg/L as a result of fluctuations in the pre-treatment performance. Such variability
430 can be problematic in regard to biological treatment due to the changing substrate levels and
431 the subsequent decline in bacterial numbers during periods of limited food supply. However,
432 system stability is of increasing importance as emission limits continue to be lowered. The
433 addition of phenol-degrading bacteria by Fang *et al.* (2013) (**Table 3**) allowed phenol
434 treatment efficiencies to increase from 66 to 80% and further increased the resistance to
435 fluctuating loads. Ammonia removal also improved (5 to 25%), although fluctuating
436 ammonia load rates required a higher recovery time. Resistance to shock loading of phenolic
437 compounds was also seen to improve due to bioaugmentation in the work of Duque *et al.*
438 (2011) for the removal of 2-fluorphenol. Interestingly, Duque *et al.* (2011) promoted biofilm
439 formation in a rotating biological contactor (RBC) through batch application of the inoculum.
440 This technique provided a means via which the system was able to stabilise and consequently
441 long-term maintenance was not required. This allowed for improved resistance to shock loads
442 and increased resistance to periods of starvation (**Table 3**). Although improved resistance to
443 shock loads of p-nitrophenol was also observed by Martín-Hernández, Suárez-Ojeda and

Carrera (2012), resistance to starvation periods was determined as a function of the size of the initial inoculum dose (**Table 2**).

The stable removal of both pyridine and quinoline from coke-making wastewater was observed after the inoculation of a laboratory-scale sequencing batch reactor filled with modified zeolite (Zhang *et al.*, 2014b). Removal of both compounds was maintained at 100% whereas removal efficiencies could vary from 0 to 93% without bioaugmentation. This was attributed to an improved bacterial diversity, which increased the resistance to shock loadings. The interaction of species in a mixed culture of four species (*Paracoccus* sp. BW001, *Shinella zoogloeoides* BC026 and *Pseudomonas* sp. BC001) was believed to be responsible for the success of bioaugmentation for the removal of pyridine and quinoline in coke-making wastewaters (Bai *et al.*, 2010).

Polycyclic aromatic hydrocarbons (PAHs) can be found in oil and gas wastewaters as well as coke-making wastewaters and are typically difficult to treat as they accumulate in the suspended solids of ASP, reducing their bioavailability to microbial degradation (Douben, 2003). Examples of bioaugmentation to enhance removal of PAHs typically focus on the treatment of contaminated soils and groundwater (Vogel, 1996; Straube *et al.*, 2003; Yu *et al.*, 2005; Jacques *et al.*, 2008; Silva *et al.*, 2009; Teng *et al.*, 2010). Useful knowledge may be gained from these applications, however, since PAHs are mainly associated with the suspended solids in ASPs.

Straube *et al.* (2003) and Sun *et al.* (2014) both considered the role of bioaugmentation and biostimulation for the removal of PAHs from soil (**Table 3**). Biostimulation was applied in order to overcome environmental limitations. Straube *et al.* (2003) demonstrated the ability of the bio-surfactant-producer *Pseudomonas aeruginosa* strain 64 to stimulate the autochthonous PAH degraders in soil samples. After 11 months, bioaugmentation led to an increase in PAH degradation from 23 to 34%. Biostimulation in combination with bioaugmentation however led to an increase in the PAH degradation to 87%. At pilot scale,

after 16 months, PAH removal increased from 12% in the control to 87% with bioaugmentation and biostimulation, although, 86% removal could in fact be achieved with biostimulation alone. Sun *et al.* (2014) found comparable results to Straube *et al.* (2003) when researching the impact of bioaugmentation and biostimulation on former coke works. Over a 3 month period the total PAH levels fell by 24% in the control, 35.9% with bioaugmentation and by 59% with biostimulation. The combination of bioaugmentation and biostimulation only brought about small improvements in comparison to biostimulation alone. The removal of heavy molecular weight PAHs, however, was noticeably higher with bioaugmentation than with biostimulation alone. This is significant due to the increased resistance of heavy molecular weight PAHs to degradation.

6. Discussion and Conclusions

The consistent and stable removal of priority pollutants from industrial wastewater is essential. Whilst close system monitoring and process control are important factors in achieving stable operation and meeting emission limits, operational regimes also need to be economically viable. Even with optimal process control, the inherent variability of industrial wastewaters can still result in emission variability. Compliance with increasingly stringent emission limits therefore requires the application of additional techniques to both meet the required limits and respond to transient treatment issues. Whilst achieving effluents of increasingly high quality is important in the long term, it is equally important that techniques are developed to re-establish treatment promptly after transient events have occurred. Bioaugmentation should be considered as one such technique.

Compliance with nitrogen effluent standards affects a wide variety of industries including palm oil effluent, aquaculture wastewaters, coke making wastewaters and petrochemical wastewaters. Nitrification is well known for its process instability due to the requirement for the close linking of the bacterial species responsible for different parts of the removal process (Philips, Laanbroek and Verstraete, 2002). Low growth rates of nitrifying bacteria and uncoupling of the nitrification chain can be problematic in any treatment, but those of an

industrial nature are much more susceptible to disruption as a result of their characteristic variations in loading and the frequent presence of toxic compounds. Bioaugmentation has been shown to offer the potential to stabilise nitrification and in particular to deal with transient treatment problems. Abeysinghe *et al.* (2002) demonstrated the ability of bioaugmentation to improve ammonia removal during stress conditions. Similarly, Ma *et al.* (2009) demonstrated the improved capability of a bioaugmented ASP-treating petrochemical wastewater to deal with shock loadings of COD. Recovery from shock loading was also 50% faster. Compliance can also be problematic for priority pollutants which are persistent and toxic, as the biomass not only requires acclimation but it can still be negatively impacted by a sudden shock load of the toxic compound. As with nitrogen, bioaugmentation has been demonstrated to have some success in the treatment of such compounds. Qu *et al.* (2011) observed improved long-term stability of treatment systems for treating aromatic compounds. The addition of *Pseudomonas* sp. JY-2 led to 90% removal efficiencies compared to 80% without bioaugmentation, with the additional benefit of decreased start-up times. Both Duque *et al.* (2011) and Fang *et al.* (2013) also observed an improved resistance of treatment systems to fluctuating phenol levels with the application of bioaugmentation.

Despite the benefits which have already been reported, caution must be applied to the findings of the numerous reported investigations. For instance, under the stress conditions reported by Abeysinghe *et al.* (2002), daily dosing was required to maintain sufficient levels of the microorganisms. Bioaugmentation was therefore capable of dealing with transient issues, but would be uneconomic for the long-term maintenance of an unstable treatment system. Similarly, although Ma *et al.* (2009) demonstrated improved nitrogen removal efficiencies, bioaugmentation was conducted in a system with immobilisation and then compared against a conventional reactor. The reduced washout, which was the main benefit of the former system, could therefore potentially have been achieved through the use of carrier media alone, simply supporting biofilm formation. It is important therefore that the purpose of bioaugmentation is clearly defined before success is determined e.g. whether a short-term solution technique or long-term benefits are desired.

A significant benefit of bioaugmentation is its ability to treat on demand. Direct dosing can provide an immediate solution to a wide array of failing treatment systems. Where space is an issue and treatment systems are already operating at their maximum capabilities, bioaugmentation may be the only way by which to maintain effluent compliance without resorting to the halting of upstream operations. Direct dosing may make use of commercial products, but these have been associated with a tendency to fail to produce the reported benefits of the product and/or require higher dosing rates than suggested by the manufacturer (Stephenson and Stephenson, 1992). These products may be able to offer a short-term solution to an immediate problem, but because of the problems associated with inadequate adaption of the microorganisms to the environment and the high dosing levels required, they may not be able to meet the requirements for long-term use. As the economic costs associated with treatment processes become more pertinent, the use of ‘one-off’ dosing may become less viable. The use of side-stream technologies is becoming increasingly common due to their advantages in terms of bacterial adaptation and use in long-term bioaugmentation applications (Krhutková *et al.*, 2006; Smith *et al.*, 2008; Yu, Peng and Pan, 2012).

Despite some positive reports of the impact of bioaugmentation on process performance, there are still substantial areas that require further research. Firstly, one of the most important aspects requiring research involves the development of an increased understanding of degradation pathways, in the absence of which the possibility of finding a suitable species to inoculate a given compound is reduced. The area of strain development has previously been highlighted for its importance (Thompson *et al.*, 2005). It is not only important to consider which strain(s) may be required, but also the requirements of that the strain to operate successfully. Under some circumstances the use of biostimulation may be necessary in order to provide nutrients, or other critical components such as biosurfactants, for the decontamination process to be successful. The synergistic action of a consortium was highlighted by Khehra *et al.* (2005) whilst the importance of the combined action of a biosurfactant and a pre-adapted consortium was reported by Nikolopoulou *et al.* (2013). More research in this field may support the degradation of wastewaters containing polycyclic aromatic hydrocarbons, where complex compounds of different molecular weights are present simultaneously. Developments in genetics may also assist in the development of

strains suitable to target xenobiotic compounds for which removal is currently limited; however, concerns around the release of genetically modified bacteria have significantly impacted progress in this area (Davison, 2005). Van Der Gast *et al.* (2003) also reported that treatment performance was more reproducible for a constructed consortium than an undefined community.

The success of bioaugmentation is increasingly being linked to the effective incorporation of the inoculated strain into the host environment, the success of which is influenced by issues ranging from strain selection and the introduction strategy through to the ability of the strain to survive within the environment to which it is introduced (Herrero and Stuckey, 2014; Thompson *et al.*, 2005). The importance of having a detailed knowledge of the treatment system has been emphasised through numerous applications (Goldstein, Mallory and Alexander, 1985; Bouchez *et al.*, 2000; Songzhe *et al.*, 2009; Martín-Hernández, Suárez-Ojeda and Carrera, 2012). An understanding of the conditions in a treatment process offers a way in which to prevent an inoculum being negatively influenced by environmental factors such as pH and temperature, as well as exposure to toxic compounds, allowing for the selection of a dosing strategy or location for introduction of the strain to minimise its exposure to negative conditions. Such detailed knowledge can also help inform possible solutions to any problems that may arise. Industries such as dairy processing, where each site encompasses different process operations, would particularly benefit from this approach. As bioaugmentation methodologies can vary greatly, the technique allows for the individuality of different treatment processes to be recognised and catered for.

Appropriate dosing rates also lack sufficient research. Although many references have been made to over-dosing and/or under-dosing, huge variations can be seen in dose rates that have been successful between applications which appear to be very similar. In the treatment of pyridine and quinoline in laboratory-scale SBRs, both treating wastewater from the same site and achieving a 99% removal rate, Bai *et al.* (2010) reported a dose rate of 0.007–0.0200 g/L in comparison to a dose of 0.223 g/L for Zhang *et al.* (2014b). Of the three species used in each study, two of the species applied were the same in both applications. Whether the

relatively large difference in dose rate can be accounted for by the third species is unknown. Research is also contradictory in the need for repeated inoculations through maintenance dose rates. Both Boon *et al.* (2003) and Abeysinghe *et al.* (2002) reported the need for repeated inoculations via maintenance doses whilst Martín-Hernández, Suárez-Ojeda and Carrera (2012) reported that this was unnecessary if the initial dose rate was sufficiently high to overcome initial survival pressures. High dosing rates have equally been criticised as they have been linked to disturbances in the balance of an ecosystem (Bouchez *et al.*, 2000). For this reason, it is important that investigations take place which consider a variety of different dosing regimens for identical wastewater treatment facilities.

The complexity of industrial wastewaters increases the challenge of identifying the most effective techniques, as many interacting processes can take place simultaneously. Despite this, industries should take the opportunity to learn from previous bioaugmentation successes and failures in order to gain from the benefits that may be obtained from bioaugmentation. Research has already increased our understanding of the complex interactions between the introduced microorganisms and the host environment, leading to improved application success. Many of the problems that have arisen in the field of bioaugmentation have been overcome through process development (Error! Reference source not found. 2).

For the field of bioaugmentation to move forward, it is now essential for key gaps in the research to be addressed. Overall, when considering whether bioaugmentation is successful, the aim of the bioaugmentation process must first be considered i.e. short-term solution to a treatment issue or the long-term improvement of a system. Current research has been limited by the focus on laboratory-scale investigations, synthetic wastewaters and the failure to have adequate controls in place. Understanding in the field would be enhanced significantly by operating parallel studies with control and test process streams. Full-scale investigations have been limited in extent and such investigations have also lacked controls (Parker and Wanner, 2007).

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625

626 **References**

627 Abeysinghe, D. H., De Silva, D. G. V., Stahl, D. a and Rittmann, B. E. (2002) ‘The
628 effectiveness of bioaugmentation in nitrifying systems stressed by a washout condition and
629 cold temperature.’, *Water environment research: a research publication of the Water*
630 *Environment Federation*, 74(2), pp. 187–99.

631 Awong, J., Bitton, G. and Chaudhry, G. R. (1990) ‘Microcosm for Assessing Survival of
632 Genetically Engineered Microorganisms in Aquatic Environments’, *Applied and*
633 *environmental microbiology*, 56(4), pp. 977–83.

634 Bai, Y., Sun, Q., Zhao, C., Wen, D. and Tang, X. (2010) ‘Bioaugmentation treatment for
635 coking wastewater containing pyridine and quinoline in a sequencing batch reactor’, *Applied*
636 *Microbiology and Biotechnology*, 87(5), pp. 1943–1951. doi: 10.1007/s00253-010-2670-8.

637 Bartrolí, A., Carrera, J. and Pérez, J. (2011) ‘Bioaugmentation as a tool for improving the
638 start-up and stability of a pilot-scale partial nitrification biofilm airlift reactor.’, *Bioresource*
639 *technology*, 102(6), pp. 4370–5. doi: 10.1016/j.biortech.2010.12.084.

640 Bitton, G. (2011) *Wastewater Microbiology*. 4th edn. Wiley-Blackwell.

641 Boon, N., Goris, J., Vos, P. De, Verstraete, W. and Top, E. M. (2000) ‘Bioaugmentation of
642 Activated Sludge by an Indigenous 3-Chloroaniline-Degrading *Comamonas testosteroni*
643 Strain , I2 gfp’, *Applied and Environmental Microbiology*, 66(7), pp. 2906–13. doi:
644 10.1128/AEM.66.7.2906-2913.2000.Updated.

645 Boon, N., Top, E. M., Verstraete, W., Steven, D. and Siciliano, S. D. (2003)
646 ‘Bioaugmentation as a Tool To Protect the Structure and Function of an Activated-Sludge
647 Microbial Community against a 3-Chloroaniline Shock Load.’, *Applied and environmental*
648 *microbiology*, 69(3), pp. 1511–19. doi: 10.1128/AEM.69.3.1511.

649 Bouchez, T., Patureau, D., Dabert, P., Juretschko, S., Doré, J., Delgenès, P., Moletta, R. and
650 Wagner, M. (2000) 'Ecological study of a bioaugmentation failure.', *Environmental*
651 *microbiology*, 2(2), pp. 179–90.

652 Bouchez, T., Patureau, D., Delgenès, J. P. and Moletta, R. (2009) 'Successful bacterial
653 incorporation into activated sludge flocs using alginate', *Bioresource Technology*. Elsevier
654 Ltd, 100(2), pp. 1031–1032. doi: 10.1016/j.biortech.2008.07.028.

655 Burgess, J. E., Quarmby, J. and Stephenson, T. (1999) 'Role of micronutrients in activated
656 sludge-based biotreatment of industrial effluents', *Biotechnology Advances*, 17, pp. 49–70.

657 Chhatre, S., Purohit, H., Shanker, R. and Khanna, P. (1996) 'Bacterial consortia for crude oil
658 spill remediation', *Water Science & Technology*, 34(10), pp. 187–193.

659 Chitra, S., Sekaran, G., Padmavathi, S. and Chandrakasan, G. (1995) 'Removal of phenolic
660 compounds from wastewater using mutant strain of *Pseudomonas pictorum*', *Journal of*
661 *general and applied microbiology*, 41(3), pp. 229–37.

662 Chong, N.-M., Pai, S.-L. and Chen, C.-H. (1997) 'Bioaugmentation of an activated sludge
663 receiving pH shock loadings', *Bioresource Technology*, 59(2–3), pp. 235–240. doi:
664 10.1016/S0960-8524(96)00138-1.

665 Davison, J. (2005) 'Risk mitigation of genetically modified bacteria and plants designed for
666 bioremediation', *Journal of Industrial Microbiology and Biotechnology*, 32(11–12), pp. 639–
667 650. doi: 10.1007/s10295-005-0242-1.

668 Domde, P., Kapley, A. and Purohit, H. J. (2007) 'Impact of Bioaugmentation with a
669 Consortium of Bacteria on the Remediation of Wastewater-Containing Hydrocarbons.',
670 *Environmental science and pollution research international*, 14(1), pp. 7–11.

671 Douben, P. E. T. (2003) *PAHs: An Ecotoxicological Perspective*. England: John Wiley &
672 Sons.

673 Duque, A. F., Bessa, V. S., Carvalho, M. F. and Castro, P. M. L. (2011) 'Bioaugmentation of
674 a rotating biological contactor for degradation of 2-fluorophenol.', *Bioresource technology*.
675 Elsevier Ltd, 102(19), pp. 9300–3. doi: 10.1016/j.biortech.2011.07.003.

676 European Commission (2003) 'Integrated Pollution Prevention and Control Reference
677 Document on Best Available Techniques in Common Waste Water and Waste Gas Treatment
678 / Management Systems in the Chemical Sector'. Available at:
679 http://eippcb.jrc.ec.europa.eu/reference/BREF/cww_bref_0203.pdf.

680 European Commission (2006) *Integrated Pollution Prevention and Control Reference*
681 *Document on Best Available Techniques in the Food, Drink and Milk Industries*. Available
682 at: http://eippcb.jrc.ec.europa.eu/reference/BREF/fdm_bref_0806.pdf.

683 European Commission (2011) *Reference Documents: Best Available Techniques Reference*
684 *Document (BREFs)*. Available at: <http://eippcb.jrc.ec.europa.eu/reference/>.

685 European Commission (2012) *(BAT) conclusions under Directive 2010/75/EU of the*
686 *European Parliament and of the Council on industrial emissions for the manufacture of*
687 *glass*.

688 European Commission (2013a) *Best Available Techniques (BAT) Reference Document for*
689 *Iron and Steel Production, Industrial Emissions Directive 2010/75/EU*. doi: 10.2791/97469.

690 European Commission (2013b) 'Best Available Techniques (BAT) Reference Document for
691 the Manufacture of Glass'.

692 European Commission (2014) *Best available technique conclusions, under Directive*
693 *2010/75/EU of the European Parliament and of the Council on industrial emissions, for the*
694 *refining of mineral oil and gas*. Available at: [http://eur-lex.europa.eu/legal-](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL_2014_307_R_0009)
695 [content/EN/TXT/?uri=OJ:JOL_2014_307_R_0009](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL_2014_307_R_0009).

696 European Commission (2015a) *Best Available Techniques (BAT) Reference Document for the*
697 *Refining of Mineral Oil and Gas*.

698 European Commission (2015b) *The Industrial Emissions Directive*. Available at:
699 <http://ec.europa.eu/environment/industry/stationary/ied/legislation.htm> (Accessed: 11
700 November 2015).

701 European Union (2013) *Annex 1 List of priority substances in the field of water policy*.
702 Available at:

703 <http://www.europarl.europa.eu/sides/getDoc.do?type=TA&language=EN&reference=P7-TA->
704 2013-298 (Accessed: 3 December 2013).

705 Fang, F., Han, H., Zhao, Q., Xu, C. and Zhang, L. (2013) 'Bioaugmentation of biological
706 contact oxidation reactor (BCOR) with phenol-degrading bacteria for coal gasification
707 wastewater (CGW) treatment.', *Bioresource Technology*. Elsevier Ltd, 150, pp. 314–20. doi:
708 10.1016/j.biortech.2013.09.119.

709 Ganczarczyk, J. J. (1983) 'Second-Stage Activated Sludge Treatment of Coke-Plant
710 Effluents', *Water Research*, 13, pp. 337–342.

711 Van Der Gast, C. J., Whiteley, A. S., Starkey, M., Knowles, C. J. and Thompson, I. P. (2003)
712 'Bioaugmentation strategies for remediating mixed chemical effluents', *Biotechnology*
713 *Progress*, 19(4), pp. 1156–1161. doi: 10.1021/bp020131z.

714 Gerrard, A. M. and Stephenson, T. (1990) 'Some economic targets for inoculum enhanced
715 activated sludge processes', *Process Safety and Environmental Protection*, 68b, pp. 269–272.

716 Goldstein, R. M., Mallory, L. M. and Alexander, M. (1985) 'Reasons for possible failure of
717 inoculation to enhance biodegradation', *Applied and environmental microbiology*, 50(4), pp.
718 977–83.

719 Gould, F. J. (1986) *Biological effluent treatment using pure oxygen*, *The Coke Oven*
720 *Managers' Yearbook*.

721 Guo, J., Ma, F., Chang, C., Cui, D., Wang, L., Yang, J. and Wang, L. (2009) 'Start-up of a
722 two-stage bioaugmented anoxic–oxic (A/O) biofilm process treating petrochemical
723 wastewater under different DO concentrations', *Bioresource Technology*. Elsevier Ltd,
724 100(14), pp. 3483–3488. doi: 10.1016/j.biortech.2009.02.059.

725 Herrero, M. and Stuckey, D. C. (2014) 'Bioaugmentation and its application in wastewater
726 treatment: A review.', *Chemosphere*. Elsevier Ltd, 44. doi:
727 10.1016/j.chemosphere.2014.10.033.

728 Ikeda-Ohtsubo, W., Miyahara, M., Kim, S.-W., Yamada, T., Matsuoka, M., Watanabe, A.,
729 Fushinobu, S., Wakagi, T., Shoun, H., Miyauchi, K. and Endo, G. (2013) 'Bioaugmentation
730 of a wastewater bioreactor system with the nitrous oxide-reducing denitrifier *Pseudomonas*

731 stutzeri strain TR2.’, *Journal of bioscience and bioengineering*. Elsevier Ltd, 115(1), pp. 37–
732 42. doi: 10.1016/j.jbiosc.2012.08.015.

733 Ikeda-Ohtsubo, W., Miyahara, M., Yamada, T., Watanabe, A., Fushinobu, S., Wakagi, T.,
734 Shoun, H., Miyauchi, K. and Endo, G. (2013) ‘Effectiveness of heat treatment to protect
735 introduced denitrifying bacteria from eukaryotic predatory microorganisms in a pilot-scale
736 bioreactor.’, *Journal of bioscience and bioengineering*. Elsevier Ltd, 116(6), pp. 722–4. doi:
737 10.1016/j.jbiosc.2013.05.024.

738 Jacques, R. J. S., Okeke, B. C., Bento, F. M., Teixeira, A. S., Peralba, M. C. R. and Camargo,
739 F. A. O. (2008) ‘Microbial consortium bioaugmentation of a polycyclic aromatic
740 hydrocarbons contaminated soil’, 99, pp. 2637–2643. doi: 10.1016/j.biortech.2007.04.047.

741 Jain, R. K., Sayler, G. S., Wilson, J. T., Houston, L. and Pacia, D. (1987) ‘Maintenance and
742 Stability of Introduced Genotypes in Groundwater Aquifer Material’, *Applied and*
743 *environmental microbiology*, 53(5), pp. 996–1002.

744 Jianlong, W., Xiangchun, Q., Libo, W., Yi, Q. and Hegemann, W. (2002) ‘Bioaugmentation
745 as a tool to enhance the removal of refractory compound in coke plant wastewater’, *Process*
746 *Biochemistry*, 38, pp. 777–781.

747 Khehra, M. S., Saini, H. S., Sharma, D. K., Chadha, B. S. and Chimni, S. S. (2005)
748 ‘Comparative studies on potential of consortium and constituent pure bacterial isolates to
749 decolorize azo dyes.’, *Water research*, 39(20), pp. 5135–41. doi:
750 10.1016/j.watres.2005.09.033.

751 Krhutková, O., Novák, L., Pachmanová, L., Benáková, A., Wanner, J. and Kos, M. (2006) ‘In
752 situ bioaugmentation of nitrification in the regeneration zone: practical application and
753 experiences at full-scale plants’, *Water Science & Technology*, 53(12), pp. 39–46. doi:
754 10.2166/wst.2006.404.

755 Van Limbergen, H., Top, E. M. and Verstraete, W. (1998) ‘Bioaugmentation in activated
756 sludge: current features and future perspectives’, *Applied Microbiology and Biotechnology*,
757 50(1), pp. 16–23. doi: 10.1007/s002530051250.

758 Liu, X., Chen, Y., Zhang, X., Jiang, X., Wu, S., Shen, J., Sun, X., Li, J., Lu, L., Wang, L.
759 (2015) ‘Aerobic granulation strategy for bioaugmentation of a sequencing batch reactor

760 (SBR) treating high strength pyridine wastewater.’, *Journal of Hazardous Materials*. Elsevier
 761 Ltd, 15(295), pp. 153-60. doi: 10.1016/j.jhazmat.2015.04.025.

762 Liu, C., Huang, X. and Wang, H. (2008) ‘Start-up of a membrane bioreactor bioaugmented
 763 with genetically engineered microorganism for enhanced treatment of atrazine containing
 764 wastewater’, *Desalination*, 231, pp. 12–19.

765 Ma, F., Guo, J., Zhao, L., Chang, C. and Cui, D. (2009) ‘Application of bioaugmentation to
 766 improve the activated sludge system into the contact oxidation system treating petrochemical
 767 wastewater.’, *Bioresource technology*. Elsevier Ltd, 100(2), pp. 597–602. doi:
 768 10.1016/j.biortech.2008.06.066.

769 Mahin, A. A., Chowdhury, A. Z., Alam, M, K., Aktar, Z. and Fakhruddin, A. N. M. (2011)
 770 ‘Phenol Biodegradation by Two Strains of *Pseudomonas putida* and Effect of Lead and Zinc
 771 on the Degradation Process’, *International Journal of Environment*, 1, pp. 27–33.

772 Marañón, E., Vázquez, I., Rodríguez, J., Castrillón, L., Fernández, Y. and López, H. (2008)
 773 ‘Treatment of coke wastewater in a sequential batch reactor (SBR) at pilot plant scale.’,
 774 *Bioresource Technology*, 99(10), pp. 4192–8. doi: 10.1016/j.biortech.2007.08.081.

775 Martín-Hernández, M., Suárez-Ojeda, M. E. and Carrera, J. (2012) ‘Bioaugmentation for
 776 treating transient or continuous p-nitrophenol shock loads in an aerobic sequencing batch
 777 reactor.’, *Bioresource technology*, 123, pp. 150–6. doi: 10.1016/j.biortech.2012.07.014.

778 McClure, N. C., Fry, J. C. and Weightman, A. J. (1991) ‘Survival and catabolic activity of
 779 natural and genetically engineered bacteria in a laboratory-scale activated-sludge unit.’,
 780 *Applied and environmental microbiology*, 57(2), pp. 366–73.

781 McClure, N. C., Weightman, A. J. and Fry, J. C. (1989) ‘Survival of *Pseudomonas putida*
 782 UWC1 containing cloned catabolic genes in a model activated-sludge unit.’, *Applied and*
 783 *environmental microbiology*, 55(10), pp. 2627–2634.

784 More, T. T., Yan, S., Tyagi, R. D. and Surampalli, R. Y. (2010) ‘Review: Potential use of
 785 filamentous fungi for wastewater sludge treatment.’, *Bioresource technology*. Elsevier Ltd,
 786 101(20), pp. 7691–7700. doi: 10.1016/j.biortech.2010.05.033.

787 Nikolopoulou, M., Pasadakis, N. and Kalogerakis, N. (2013) 'Evaluation of autochthonous
788 bioaugmentation and biostimulation during microcosm-simulated oil spills.', *Marine*
789 *pollution bulletin*. Elsevier Ltd, 72(1), pp. 165–73. doi: 10.1016/j.marpolbul.2013.04.007.

790 Nublein, K., Maris, D., Timmis, K. and Dwyer, D. F. (1992) 'Expression and transfer of
791 engineered catabolic pathways harbored by *Pseudomonas* spp . introduced into activated
792 sludge microcosms.', *Applied and environmental microbiology*, 58(10), pp. 3380–86.

793 Obbard, J. and Shan, H. (2001) 'Ammonia removal from prawn aquaculture water using
794 immobilized nitrifying bacteria', *Applied Microbiology and Biotechnology*, 57(5–6), pp. 791–
795 798. doi: 10.1007/s00253-001-0835-1.

796 Olaniran, A. O., Pillay, D. and Pilay, B. (2006) 'Biostimulation and bioaugmentation
797 enhances aerobic biodegradation of dichloroethenes', *Chemosphere*, 63, pp. 600–608. doi:
798 10.1016/j.chemosphere.2005.08.027.

799 Onyia, C. O., Uyu, A. M., Akunna, J. C., Norulaini, N. A. and Omar, A. (2001) 'Increasing
800 the fertilizer value of palm oil mill sludge: bioaugmentation in nitrification.', *Water science*
801 *and technology: a journal of the International Association on Water Pollution Research*,
802 44(10), pp. 157–62. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11794647>.

803 Park, D., Lee, D. S., Kim, Y. M. and Park, J. M. (2008) 'Bioaugmentation of cyanide-
804 degrading microorganisms in a full-scale cokes wastewater treatment facility.', *Bioresource*
805 *Technology*, 99(6), pp. 2092–6. doi: 10.1016/j.biortech.2007.03.027.

806 Parker, D. and Wanner, J. (2007) 'Review of methods for improving nitrification through
807 bioaugmentation', *Water Practice*, 1(5), pp. 1–16.

808 Philips, S., Laanbroek, H. J. and Verstraete, W. (2002) 'Origin, causes and effects of
809 increased nitrite concentrations in aquatic environments', *Re/Views Environm Science &*
810 *Bio/Technol*, 1(2), pp. 115–141. doi: 10.1023/A:1020892826575.

811 Qu, Y., Zhang, R., Ma, F., Zhou, J. and Yan, B. (2011) 'Bioaugmentation with a novel alkali-
812 tolerant *Pseudomonas* strain for alkaline phenol wastewater treatment in sequencing batch
813 reactor', *World Journal of Microbiology and Biotechnology*, 27(8), pp. 1919–1926. doi:
814 10.1007/s11274-011-0653-2.

815 Quan, X., Shi, H., Liu, H., Lv, P. and Qian, Y. (2004) 'Enhancement of 2,4-dichlorophenol
 816 degradation in conventional activated sludge systems bioaugmented with mixed special
 817 culture.', *Water research*, 38(1), pp. 245–53. doi: 10.1016/j.watres.2003.09.003.

818 Quan, X., Shi, H., Liu, H., Wang, J. and Qian, Y. (2004) 'Removal of 2,4-dichlorophenol in a
 819 conventional activated sludge system through bioaugmentation', *Process Biochemistry*,
 820 39(11), pp. 1701–1707. doi: 10.1016/S0032-9592(03)00307-8.

821 Ramadan, M. A., El-Tayeb, O. M. and Alexander, M. (1990) 'Inoculum size as a factor
 822 limiting success of inoculation for biodegradation.', *Applied and Environmental*
 823 *Microbiology*, 56(5), pp. 1392–6.

824 Salem, S., Berends, D. H. J. G., Heijnen, J. J. and Van Loosdrecht, M. C. M. (2003) 'Bio-
 825 augmentation by nitrification with return sludge.', *Water research*, 37(8), pp. 1794–804. doi:
 826 10.1016/S0043-1354(02)00550-X.

827 Semrany, S., Favier, L., Djelal, H., Taha, S. and Amrane, A. (2012) 'Bioaugmentation:
 828 Possible solution in the treatment of Bio-Refractory Organic Compounds (Bio-ROCs)',
 829 *Biochemical Engineering Journal*. Elsevier B.V., 69, pp. 75–86. doi:
 830 10.1016/j.bej.2012.08.017.

831 Shen, J., He, R., Yu, H., Wang, .L, Zhang, J., Sun, X., Li, J., Han, W., Xu, L. (2009)
 832 'Biodegradation of 2,4,6-trinitrophenol (picric acid) in a biological aerated filter (BAF)',
 833 Elsevier Ltd, 100(6), pp. 1922-30. 10.1016/j.biortech.2008.10.018.

834 Shoji, T., Sueoka, K., Satoh, H. and Mino, T. (2014) 'Identification of the microbial
 835 community responsible for thiocyanate and thiosulfate degradation in an activated sludge
 836 process', *Process Biochemistry*. Elsevier Ltd, 49(7), pp. 1176–1181. doi:
 837 10.1016/j.procbio.2014.03.026.

838 Silva, Í. S., Santos, E. C., Menezes, C. R., Faria, A. F., Franciscan, E., Grossman, M. and
 839 Durrant, L. R. (2009) 'Bioremediation of a polyaromatic hydrocarbon contaminated soil by
 840 native soil microbiota and bioaugmentation with isolated microbial consortia', *Bioresource*
 841 *Technology*. Elsevier Ltd, 100(20), pp. 4669–4675. doi: 10.1016/j.biortech.2009.03.079.

842 Smith, R. C., Saikaly, P. E., Zhang, K., Thomatos, S. and Oerther, D. B. (2008) 'Ecological
 843 engineering of bioaugmentation from side-stream nitrification.', *Water science and*

844 *technology : a journal of the International Association on Water Pollution Research*, 57(12),
845 pp. 1927–33. doi: 10.2166/wst.2008.628.

846 Songzhe, F. U., Hongxia, F. A. N., Shuangjiang, L. I. U., Ying, L. I. U. and Zhipai, L. I. U.
847 (2009) ‘A bioaugmentation failure caused by phage infection and a weak biofilm formation
848 ability’, *Journal of environmental sciences*, 21, pp. 1153–1161.

849 Stephenson, D. and Stephenson, T. (1992) ‘Bioaugmentation for enhancing biological
850 wastewater treatment.’, *Biotechnology Advances*, 10, pp. 549–59.

851 Stephenson, T. and Gerrard, A. M. (1990) ‘A simple economic comparison of inoculum
852 enhanced activated sludge processes’, *I.Chem.E. Symposium*, 116, pp. 85–94.

853 Stephenson, T., Reid, E., Avery, L. M. and Jefferson, B. (2013) ‘Media surface properties
854 and the development of nitrifying biofilms in mixed cultures for wastewater treatment’,
855 *Process Safety and Environmental Protection*, 91(4), pp. 321–324. doi:
856 10.1016/j.psep.2012.07.002.

857 Stormo, K. E. and Crawford, R. L. (1992) ‘Preparation of encapsulated microbial cells for
858 environmental applications’, *Applied environmental microbiology*, 58, pp. 727–730.

859 Straube, W. L., Nestler, C. C., Hansen, C. C., Ringleberg, L. D., Pritchard, P. H. and Jones-
860 Meehan, J. (2003) ‘Remediation of Polyaromatic Hydrocarbons (PAHs) through
861 Landfarming with Biostimulation and Bioaugmentation’, *Acta Biotechnology*, 23(2–3), pp.
862 179–196.

863 Stroo, H. F., Leeson, A. and Herb Ward, C. (2013) *Bioaugmentation for Groundwater
864 Remediation*. New York: Springer Science.

865 Sun, G. D., Jin, J. H., Xu, Y., Zhong, Z. P., Liu, Y. and Liu, Z. P. (2014) ‘Isolation of a high
866 molecular weight polycyclic aromatic hydrocarbon-degrading strain and its enhancing the
867 removal of HMW-PAHs from heavily contaminated soil’, *International Biodeterioration and
868 Biodegradation*. Elsevier Ltd, 90, pp. 23–28. doi: 10.1016/j.ibiod.2014.01.020.

869 Teng, Y., Luo, Y., Sun, M., Liu, Z., Li, Z. and Christie, P. (2010) ‘Effect of bioaugmentation
870 by *Paracoccus* sp . strain HPD-2 on the soil microbial community and removal of polycyclic

871 aromatic hydrocarbons from an aged contaminated soil', *Bioresource Technology*. Elsevier
872 Ltd, 101(10), pp. 3437–3443. doi: 10.1016/j.biortech.2009.12.088.

873 Thompson, I. P., van der Gast, C. J., Ciric, L. and Singer, A. C. (2005) 'Bioaugmentation for
874 bioremediation: the challenge of strain selection.', *Environmental microbiology*, 7(7), pp.
875 909–15. doi: 10.1111/j.1462-2920.2005.00804.x.

876 Torres, B., Garcia, J. and Diaz, E. (2003) *Plasmids as tools for containment*. In Funnell B,
877 Phillips G (eds) *Plasmid Biology*. Washington: ASM Press.

878 Tyagi, M., da Fonseca, M. M. R. and de Carvalho, C. C. C. R. (2011) 'Bioaugmentation and
879 biostimulation strategies to improve the effectiveness of bioremediation processes.',
880 *Biodegradation*, 22(2), pp. 231–41. doi: 10.1007/s10532-010-9394-4.

881 Ueno, A., Ito, Y., Yumoto, I. and Okuyama, H. (2007) 'Isolation and characterization of
882 bacteria from soil contaminated with diesel oil and the possible use of these in autochthonous
883 bioaugmentation', *World Journal of Microbiology and Biotechnology*, 23(12), pp. 1739–
884 1745. doi: 10.1007/s11274-007-9423-6.

885 US.EPA (2015) *Summary of the Clean Water Act*. Available at: [https://www.epa.gov/laws-](https://www.epa.gov/laws-regulations/summary-clean-water-act)
886 [regulations/summary-clean-water-act](https://www.epa.gov/laws-regulations/summary-clean-water-act) (Accessed: 20 August 2016).

887 Vidal, G., Carvalho, A., Méndez, R. and Lema, J. M. (2000) 'Influence of the content in fats
888 and proteins on the anaerobic biodegradability of dairy wastewaters', *Bioresource*
889 *Technology*, 74(3), pp. 231–239. doi: 10.1016/S0960-8524(00)00015-8.

890 Vogel, T. M. (1996) 'Bioaugmentation as a soil bioremediation approach.', *Current opinion*
891 *in biotechnology*, 7(3), pp. 311–6.

892 Wanner, J., Cech, J. S., Kos, M. and Barchanek, M. (1996) 'Municipal effluent standards in
893 the Czech Republic', *Water Science & Technology*, 33(12), pp. 1–10.

894 Wanner, J., Kos, M. and Novák, L. (2009) 'Intensification of Prague Central WWTP – Ten
895 years of practical experiences with the in-situ bioaugmentation nitrification method',
896 *Technology*, 4(1), pp. 1–8. doi: 10.2166/WPT.2009.016.

897 Wenderoth, D. F., Rosenbrock, P., Abraham, W.-R., Pieper, D. H. and Höfle, M. G. (2003)
898 'Bacterial community dynamics during biostimulation and bioaugmentation experiments

899 aiming at chlorobenzene degradation in groundwater', *Microbial Ecology*, 46(2), pp. 161–
900 176. doi: 10.1007/s00248-003-2005-8.

901 Yu, K. S. H., Wong, a H. Y., Yau, K. W. Y., Wong, Y. S. and Tam, N. F. Y. (2005) 'Natural
902 attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic
903 hydrocarbons (PAHs) in mangrove sediments.', *Marine pollution bulletin*, 51(8–12), pp.
904 1071–7. doi: 10.1016/j.marpolbul.2005.06.006.

905 Yu, L., Peng, D. and Pan, R. (2012) 'Shifts in nitrification kinetics and microbial community
906 during bioaugmentation of activated sludge with nitrifiers enriched on sludge reject water.',
907 *Journal of biomedicine & biotechnology*, 2012, p. 691894. doi: 10.1155/2012/691894.

908 Yu, L., Peng, D. and Ren, Y. (2011) 'Protozoan predation on nitrification performance and
909 microbial community during bioaugmentation.', *Bioresource technology*. Elsevier Ltd,
910 102(23), pp. 10855–60. doi: 10.1016/j.biortech.2011.09.060.

911 Zhang, J., Wen, D., Zhao, C. and Tang, X. (2014b) 'Bioaugmentation accelerates the shift of
912 bacterial community structure against shock load: a case study of coking wastewater
913 treatment by zeolite-sequencing batch reactor.', *Applied microbiology and biotechnology*,
914 98(2), pp. 863–73. doi: 10.1007/s00253-013-4848-3.

915 Zhang, X., Li, X., Zhang, Q., Peng, Q., Zhang, W. and Gao, F. (2014a) 'New insight into the
916 biological treatment by activated sludge: The role of adsorption process.', *Bioresource*
917 *technology*. Elsevier Ltd, 153, pp. 160–4. doi: 10.1016/j.biortech.2013.11.084.

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