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.

<table>
<thead>
<tr>
<th>Wastewater origin</th>
<th>BAT emission limit (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Produced Water (Oil and gas wastewater)</td>
<td>Hydrocarbon oil index: 0.1 – 2.5</td>
<td>(European Commission, 2014)</td>
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<tr>
<td></td>
<td>COD: 30 – 125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TN: 1 – 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(European Commission, 2014)</td>
</tr>
<tr>
<td>Food and Milk:</td>
<td>Oil and grease: &lt; 10</td>
<td>(European Commission, 2006)</td>
</tr>
<tr>
<td>e.g. Raw dairy, Cheese, Mixed dairy, palm oil mill effluent</td>
<td>COD: &lt;125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOD₅: &lt; 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TN: &lt; 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP: 0.4-5</td>
<td></td>
</tr>
<tr>
<td>Glass manufacturing</td>
<td>COD: &lt; 5-130</td>
<td>(European Commission, 2012)</td>
</tr>
<tr>
<td></td>
<td>Total hydrocarbons: &lt; 15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ammonia (as NH₄): &lt; 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenol: &lt; 1</td>
<td></td>
</tr>
<tr>
<td>Coke making wastewater:</td>
<td>COD: &lt; 220</td>
<td>(European Commission, 2013a)</td>
</tr>
<tr>
<td></td>
<td>BOD₅: &lt; 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCN: &lt; 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PAHs*: 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenols: 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TN: &lt; 15-50</td>
<td></td>
</tr>
</tbody>
</table>

*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,
Microorganisms can be genetically engineered to over-express degradation genes or to exhibit increased survivability (McClure, Fry and Weightman, 1991; Nüβlein 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 Chaudhryt, 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 Nüβlein 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;
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. *Burkholderia pickettii*, a quinoline degrading species, was shown to have a beneficial role at any location; nevertheless, its positive impact was higher when *Burkholderia 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 ($4.3 \times 10^4$ 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 Pillay, 2006; Tyagi,
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 rate 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.
Table 2: Reasons for bioaugmentation failures and possible improvement techniques.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Technique to overcome problem</th>
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<tbody>
<tr>
<td>Predation (Overgrowth of protozoa)</td>
<td>High initial doses (Ramadan, El-Tayeb and Alexander, 1990)</td>
</tr>
<tr>
<td>(Goldstein, Mallory and Alexander, 1985; Ramadan, El-Tayeb and Alexander, 1990; Songzhe et al., 2009; Martín-Hernández, Suárez-Ojeda and Carrera, 2012)</td>
<td>Protection from grazing (Songzhe et al., 2009)</td>
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<td></td>
<td>Heat treatment (Ikeda-Ohtsubo, Miyahara, Yamada, et al., 2013)</td>
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<tr>
<td>Competition for nutrients between indigenous and inoculated bacteria</td>
<td>Supplementation of nutrients (biostimulation) (Ramadan, El-Tayeb and Alexander, 1990)</td>
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<tr>
<td>(Ramadan, El-Tayeb and Alexander, 1990; Yu et al., 2005; Martín-Hernández, Suárez-Ojeda and Carrera, 2012)</td>
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<tr>
<td>Insufficient inoculations</td>
<td>Repeated inoculations (Boon et al., 2003)</td>
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<td>(Ramadan, El-Tayeb and Alexander, 1990)</td>
<td>Continual inoculations (Abeyesinghe et al., 2002)</td>
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<td>Poor biofilm formation</td>
<td>Immobilisation/encapsulation (Stormo and Crawford, 1992; Quan, Shi, Liu, Lv, et al., 2004)</td>
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<td>(Park et al., 2008; Songzhe et al., 2009)</td>
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<tr>
<td>Wash-out</td>
<td>High initial doses (Ramadan, El-Tayeb and Alexander, 1990)</td>
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<tr>
<td>(Chong, Pai and Chen, 1997; Park et al., 2008)</td>
<td>Immobilisation/encapsulation (Stormo and Crawford, 1992; Quan, Shi, Liu, Lv, et al., 2004)</td>
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<tr>
<td>Decline of inoculated bacteria due to toxins</td>
<td>Protection from adverse environmental conditions (Songzhe et al., 2009)</td>
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<tr>
<td>(Goldstein, Mallory and Alexander, 1985)</td>
<td>Allow acclimatisation period (Stephenson and Stephenson, 1992)</td>
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<tr>
<td></td>
<td>Use autochthonous bioaugmentation (Ueno et al., 2007)</td>
</tr>
<tr>
<td>Alternative substrates available</td>
<td>Detailed understanding of ecological background (Songzhe et al., 2009)</td>
</tr>
<tr>
<td>(Goldstein, Mallory and Alexander, 1985; Chitra et al., 1995; Quan, Shi, Liu, Lv, et al., 2004; Mahin et al., 2011)</td>
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<tr>
<td>Large inoculations disturbing balance of ecosystem</td>
<td>Careful consideration of dose rate</td>
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<td>(Bouchez et al., 2000)</td>
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<tr>
<td>Periods of starvation</td>
<td>Higher dose rate to allow survival in the system for longer time periods (Martin-Hernández, Suárez-Ojeda and Carrera, 2012)</td>
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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).
Table 3: Examples of bioaugmentation applied to compounds present in industrial wastewaters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Scale</th>
<th>Application</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(Onyia et al., 2001)</td>
<td>Laboratory</td>
<td>Palm oil effluent</td>
<td>15 mg/L of mixed cultures led to 100% increase in nitrification. Reduced HRTs led to 20% reduction in land requirement.</td>
</tr>
<tr>
<td>(Obbard and Shan, 2001)</td>
<td>Laboratory</td>
<td>Prawn aquaculture wastewaters</td>
<td>Immobilised bacteria allowed total ammonical nitrogen reduced from 3 mg/L to 0.5 mg/L allowing ponds to remain at optimal conditions.</td>
</tr>
<tr>
<td>(Ma et al., 2009)</td>
<td>Full-scale</td>
<td>Petrochemical wastewaters</td>
<td>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.</td>
</tr>
<tr>
<td>Aromatic compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Qu et al., 2011)</td>
<td>Laboratory</td>
<td>Synthetic alkaline wastewaters</td>
<td>Addition of Pseudomonas 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%).</td>
</tr>
<tr>
<td>(Fang et al., 2013)</td>
<td>Laboratory</td>
<td>Coal gasification wastewater</td>
<td>Bioaugmentation increased removal efficiencies from 66% to 80% despite high variation in levels of phenol (500-3000 mg/L).</td>
</tr>
<tr>
<td>(Duque et al., 2011)</td>
<td>Laboratory</td>
<td>2-fluorophenol wastewaters</td>
<td>2-fluorophenol degrading species FP1 allowed treatment of waters subjected to shock loads of up to 50 mg/L of 2-fluorophenol.</td>
</tr>
<tr>
<td>(Martín-Hernández, Suárez-Ojeda and Carrera, 2012)</td>
<td>Laboratory</td>
<td>p-nitrophenol (PNP) wastewaters</td>
<td>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.</td>
</tr>
<tr>
<td>(Straube et al., 2003)</td>
<td>Laboratory and pilot-</td>
<td>PAH contaminated soil</td>
<td>Bio-surfactant producer <em>Pseudomonas aeruginosa</em> strain 64 increased PAH</td>
</tr>
</tbody>
</table>
scale degradation from 23% to 34%. Bioaugmentation and biostimulation increased degradation to 87%. Biostimulation alone increased degradation to 86%.

(Sun et al., 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.

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

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