

Title: Enhancing the removal of pollutants from coke wastewater by bioaugmentation: A scoping study

Short Title: Scoping bioaugmentation for coke wastewater treatment

Authors: Eleanor Raper^a, Tom Stephenson^a, Francisco Simões^a, Raymond Fisher^b, David, R. Anderson^b, Ana Soares^{a*}.

Affiliation:

^a Cranfield University Water Sciences Institute, Cranfield University, Cranfield, MK43 0AL, UK. e.raper@cranfield.ac.uk (+44(0)1709 843500), T.Stephenson@cranfield.ac.uk (+44(0)1234 754054), f.a.simoes@cranfield.ac.uk, ^{*}a.soares@cranfield.ac.uk, (Corresponding Author: +44(0)1234 758121),

^b Tata Steel, Group Environment, Swinden Technology Centre, Rotherham, S60 3AR, UK. ray.fisher@tatasteel.com (+44(0)1709 843500), david.anderson@tatasteel.com (+44(0)1709 820166).

ABSTRACT

BACKGROUND

Bioaugmentation and biostimulation were investigated for their ability to improve the removal of thiocyanate (SCN⁻), polycyclic aromatic hydrocarbons (PAHs), phenol and trace metals in coke wastewater. Additionally, the ability of the microorganisms supplemented with the bioaugmentation product to survive in a simulated river water discharge was evaluated.

RESULTS

A commercially available bioaugmentation product composed mainly of *Bacillus* sp. was mixed with activated sludge biomass. A dose of 0.5 g/L increased the removal of Σ6PAHs (sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene) by 51% and reduced SCN⁻ below 4 mg/L enabling compliance with the EU Industrial Emissions Directive (IED). Biostimulation

(supplementing micronutrients and alkalinity) allowed compliance for both SCN^- and phenol (<0.5 mg/L).

Bacillus sp. accounted for 4.4% of the microbial population after 25 hours (1.5 g/L dose) which declined to 0.06% after exposure to river water (24 hours). Exposure of the activated sludge biomass to river water resulted in a 98.6% decline in viable cell counts.

CONCLUSION

To comply with the IED, bioaugmentation and biostimulation are recommended for the treatment of coke wastewater to enable an effluent $\Sigma 6\text{PAHs}$ of 6.6 $\mu\text{g/L}$, 0.3 mg/L phenol and 1.2 mg/L SCN^- . Such techniques are not anticipated to impact on downstream river water quality.

KEYWORDS

Bioaugmentation Biostimulation Thiocyanate Phenol Trace metals
Polycyclic aromatic hydrocarbons

INTRODUCTION

Bioaugmentation involves the addition of microorganisms, selected for their specialised characteristics, to a treatment process in order to enhance removal of target pollutants whilst biostimulation involves the addition of supplements such as nutrients and micronutrients to improve microbial metabolism and consequently improve treatment efficiencies^{1,2}. Bioaugmentation and biostimulation, therefore, offer different routes by which an activated sludge process (ASP) can be upgraded to treat persistent pollutants. Bioaugmentation has

been demonstrated to successfully improve the treatment of many industrial wastewaters whilst biostimulation has been reported to be important in nutrient limited industrial wastewaters ³⁻⁸.

Coke wastewaters are formed in the production of coke, used in steel manufacturing, and originate from the quenching of hot coke masses, washing of ammonia stills and cooling and washing of coke oven gases ⁹. Coke wastewaters contain a mixture of nitrogenous compounds and organic compounds, the concentrations of which are highly variable in response to the composition of the coals used in the coke ovens and the operational conditions ¹⁰. Coke wastewaters are typically characterised by ammonia concentrations of 50 - 500 mg/L, thiocyanate (SCN^-) concentrations of 100 - 400 mg/L and phenol concentrations of 60 – 400 mg/L ^{4,10,11}. The sum of 6 polycyclic aromatic hydrocarbons ($\Sigma 6\text{PAHs}$: sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene) was previously reported at $179 \pm 35 \mu\text{g/L}$ ¹². Total trace metals (sum of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd and Pb) were also reported at a concentration of $4216 \mu\text{g/L}$ with individual trace metal concentrations ranging from $0.13 \mu\text{g/L}$ (Cd) to $3612 \mu\text{g/L}$ (Fe) ¹². These wastewaters are regulated under the Industrial Emissions Directive (IED) and emission limits introduced in 2016 require that effluents are characterised by $<4 \text{ mg/L } \text{SCN}^-$, $<50 \mu\text{g/L } \Sigma 6\text{PAHs}$, $<0.5 \text{ mg/L}$ phenols and $<1000 \mu\text{g/L}$ trace metals (sum of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn) and mercury (Hg)) ¹³.

Coke wastewaters are typically treated through an ASP. Bioaugmentation has been shown to be successful in increasing the removal of phenol from industrial wastewaters. Duque et al. ⁷

added a 2-fluorophenol degrading strain to a rotating biological contactor and demonstrated the ability of the strain to enable 2-fluorophenol degradation up to 50 mg/L. The strain was also able to cope with periods of substrate absence. Furthermore, the addition of phenol degrading bacteria to a biological contact oxidation reactor improved total phenol removal from 66% to 80%¹⁴. Removal of phenol from coke wastewater was considered by Zhu, Tian and Chen¹⁵ who isolated two different strains of *Pseudomonas* (sp.PCT01 and PTS02). In synthetic wastewaters both strains performed similarly at phenol concentrations of ca. 235 and 460 mg/L, completely degrading the phenol within ca. 9 and 18 h respectively. In actual coke wastewater, with maximum phenol concentrations of 450 mg/L, degradation rates were reduced for both species. Although the investigation successfully isolated phenol degrading bacteria, the investigation used pure cells and did not investigate the ability of the added strains to survive in a mixed culture.

Biostimulation has been shown to have great potential for improved removal of PAHs. Sun et al.¹⁶ researched the impact of both bioaugmentation and biostimulation on the removal of PAHs from soil at a former coke work sites. Over 3 months total PAH concentrations dropped by 24% under control conditions, 35.9% with bioaugmentation and by 59% with biostimulation. Combined bioaugmentation and biostimulation resulted in only small improvements in total PAH removal compared to biostimulation alone, however, the combined action of biostimulation and bioaugmentation led to increased removal of heavy molecular weight PAHs. Bioaugmentation therefore had an important role in the removal of heavy molecular weight PAHs. Nutrient addition was also demonstrated to be important in the establishment of a cyanide degrading consortium treating effluent from a coke wastewater pre-denitrification ASP¹⁷.

Whilst many studies have considered the ability of exogenous microorganisms to survive within a wastewater treatment process to be an important aspect of bioaugmentation, no study has considered the survivability in the receiving waterbody after treated effluent is discharged from an ASP. Survival of exogenous microorganisms in the receiving waterbody may result in ecological impacts which could potentially be detrimental to the balance of the ecosystem. Domestic wastewater treatment plants, for example, have been confirmed as sources of nitrifying bacteria in freshwater bodies¹⁸. Whilst the seeding of nitrifying bacteria was typically beneficial in this study, introduction of other species could potentially have detrimental impacts to the ecosystem as a result of changes in the composition of the bacterial population. The question therefore exists whether microorganisms supplemented through bioaugmentation are able to survive in ASP and subsequently receiving water bodies, opening up the possibility for negative impacts in the natural environment.

Although the full-scale coke wastewater treatment plant is capable of achieving high removals for many pollutants, the high variability of the coke wastewater composition means that the ASP is not able to consistently meet the new IED emission limits, particularly for $\Sigma 6$ PAHs. Removal of $\Sigma 6$ PAHs to the emission limit of 50 $\mu\text{g/L}$ is challenging, as coke making wastewaters are characterised by an abundance of heavy molecular weight PAHs which are characterised by high n-octanol/water partition coefficients ($\log K_{ow}$)^{19,20}. Of the PAHs investigated, $\log K_{ow}$ values range from 5.12 - 7.66^{21,22}. Additionally, although SCN^- removals are high, typically 99%, the treatment of SCN^- has been recognised for its sensitivity²³. When instability occurs in the treatment process thiocyanate removals are the first to decline after nitrification. Bioaugmentation and biostimulation may provide the answer. Despite this, bioaugmentation for the removal of PAHs has focussed on the treatment of contaminated soils and groundwater rather ASP applications^{16,24}.

This study is an initial investigation to understand whether bioaugmentation or biostimulation could enhance the removal of PAHs and SCN^- from coke wastewater to enable consistent compliance with the IED. The potential of bioaugmentation to remove SCN^- from coke wastewater was investigated for the first time. Furthermore, the survivability of microorganisms supplemented through a bioaugmentation product in a simulated river water discharge was investigated, targeting a gap in knowledge within the field of bioaugmentation.

EXPERIMENTAL

Coke wastewater and activated sludge seed

Coke wastewater was collected from a full-scale, wastewater treatment plant treating coke wastewater from a steel producing works. The wastewater had been subjected to tar separation and ammonia stripping and then combined with site drainage wastewater. Biomass used in batch tests was taken from the sites ASP operating under aerobic conditions and at a hydraulic retention time (HRT) of ca. 25 hours. Aeration was provided via a Vitox oxygen injection system. Temperature was maintained between 20 and 25°C. Biomass was characterised by a sludge age of approximately 38 days.

Batch tests to assess effectiveness of bioaugmentation towards pollutant removal and viability

Batch tests were conducted to assess the impact of bioaugmentation and biostimulation (micronutrient/alkalinity addition) on the treatment of SCN^- , $\Sigma 6\text{PAHs}$, phenol and trace metals in the coke making wastewater. Coke wastewater and activated sludge biomass were

combined to produce a mixed liquor suspended solids (MLSS) of 5400 mg/L replicating the full-scale ASP MLSS concentrations. Samples were placed in Erlenmeyer flasks on an incubated shaker plate (Grant-bio Orbital Shaker - Incubator ES-80) at 190 rpm (previously optimised to ensure a dissolve oxygen of 3 mg/L, comparable to the full-scale vitox system) and a temperature of 25°C, for 25 hours to simulate the full-scale ASP. Samples were replicated in at least duplicate. The impact of alkalinity addition, micronutrient addition and bioaugmentation was investigated by spiking the coke wastewater. Alkalinity was added at the previously optimised dose of 300 mg/L (as CaCO₃) through the addition of sodium carbonate (Na₂CO₃)²⁵. A micronutrient solution was designed taking account of the coke wastewater characterisation and activated sludge nutrient requirements reported by Burgess, Quarmby and Stephenson⁶ (Table I). A commercially available bioaugmentation product was added at doses of 0.1, 0.5 and 1.5 g/L. Reported bioaugmentation doses vary significantly from 0.007 to 0.75 g/L, and do not appear to correlate to pollutant concentration. Therefore, doses tested in this study were selected to cover a broad range which would be feasible for full-scale applications^{4,26}. Samples were taken regularly. Thiocyanate was analysed at 0, 10, 13, 17, and 25 h. Phenol, sum nitrogen (sum of nitrite-nitrogen (NO₂⁻-N), nitrate-nitrogen (NO₃⁻-N), ammonia-nitrogen (NH₄⁺-N) and thiocyanate-nitrogen (SCN⁻-N)), soluble chemical oxygen demand (sCOD), PAHs and trace metals were analysed at 0 and 25 h.

Table I: Composition of micronutrient solution used in batch tests.

Batch tests to investigate viability of the bioaugmentation product

Samples of mixed liquor were taken from the batch tests at 0 h and 25 h to assess the ability of the microorganisms supplemented through the bioaugmentation product to survive. Additionally, further batch tests were completed to mimic the effluent discharge into a receiving river water taking into consideration an existing real scenario. Mixed liquor from the bioaugmentation batch tests was diluted with river water at a ratio of 1:1,690 to account for the dilution effect upon discharge according to typical river water volumes of the river receiving discharge from the full-scale treatment plant. The mixed liquor and river water batch tests were placed on an incubated shaker plate at 190 rpm and a temperature of 25°C. Furthermore, a worst-case scenario approach was used and it was assumed that removal of activated sludge microorganisms from the effluent was low, due to poor settling in the secondary sedimentation tank in the wastewater treatment plant.

Chemical analysis

Samples were immediately filtered through 0.45 µm filters (VacuCap 90, Pall Corporation). Nitrite-nitrogen, NO_3^- -N, NH_4^+ -N and sCOD were analysed using Merck cell test kits according to the manufacturer's instructions. Thiocyanate was analysed colourmetrically by complex reaction with iron (III) at a wavelength of 465 nm (based on The Institute of Gas Engineers analytical method for thiocyanate)²⁷. Phenol (mono) was analysed by complex reaction with 4-aminoantipyrene at a wavelength of 510 nm (based on ISO 6439:1990)²⁸. Both were analysed using a UNICAM spectrophotometer. pH was recorded using a Jenway 3540 pH meter (UK). Total nitrogen was calculated through the sum of NO_2^- -N, NO_3^- -N, NH_4^+ -N and SCN^- -N. Although TN is defined as containing organic nitrogen, coke wastewaters contain very little organic nitrogen and therefore the method used is a good

approximation ²⁹. Total suspended solids were analysed according to standard methods ³⁰. Trace metals were analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) according to BS EN 14385:2004 ³¹. Polycyclic aromatic hydrocarbons were extracted using dichloromethane and then analysed by Gas Chromatography Mass Spectrometry according to US EPA Method 8270 ³². Data was analysed using a paired T-Test with 95% confidence level.

Bacterial speciation and viability

Flow cytometry was used to count the number of viable cells in the batch test samples based on the method described by Lipphaus et al. ³³. Samples were diluted with Evian water (Evian, Évian-les-Bains, France), filter-sterilized through a 0.2 µm filter and stained using SYBR® Green I (Life Technologies Ltd., Paisley, UK) and propidium iodide (Life Technologies Ltd., Paisley, UK) as staining agents. A vortex mixer was used to ensure cells were evenly distributed and to ensure effective staining. Samples were then incubated for 13 minutes at 37°C before being analysed on the flow cytometer, BD Accuri C6 with a 488nm solid-state laser (Becton Dickinson U.K. Ltd., Oxford, UK), using the standard gate method ³⁴. In cells with membrane damage the propidium iodide partially replaces the SYBR® Green I, a change which is detected primarily in the FL3 detector (emission filter 670 LP). Viable cell counts can therefore be obtained by excluding propidium iodide fluorescent cells from the remaining SYBR® Green I stained cell count.

Polymerase chain reaction (PCR) was used to identify different microbial genera and species present in the bioaugmentation and river simulated batch tests. River simulation samples which were aqueous in nature were filtered through a sterile 0.2 µm membrane polycarbonate filter (GE Life Sciences, UK) and placed in a lysing matrix tube, whilst mixed liquor from the batch tests was placed directly in the lysing matrix tube. Deoxyribonucleic acid (DNA) was extracted using the MPBIO FastDNA Spin Kit for soil (Santa Ana, USA). The V4 and V5 regions of the 16S ribosomal RNA gene were targeted with the universal primers 515F and 926R³⁵. Error correcting golay barcodes enabled sample multiplexing³⁶. HighPrep magnetic beads (Magbio, Gaithersburg USA) were used to purify the PCR products which were subsequently purified using QuantiFluor ONE (Promega, Madison USA). An equimolar pool of amplicons was sequenced using Illumina MiSeq with 2x300 v2 chemistry (Illumina, San Diego USA). The number of reads per sample varied from 338000 to 1.6M after quality filtering. The sequences were analysed using QIIME 1.9³⁷ and were grouped at 97% similarity to create operational taxonomic units (OTUs). Representative sequences from each OTU were then taxonomically assigned using the SILVA 16S rRNA gene database v123.1³⁸. The identification of a sequence which matched unambiguously to a sequence in the database at a 97% similarity gave an exact species identification. An 16S sequence unambiguously matched to a database sequence but for which the taxonomy was unavailable resulted in a species being defined as “uncultured”. A 16S sequence which was identical to more than one sequence of the genus (at a 97% similarity) was defined as “ambiguous”. A 16S sequence for which the species data did not exist but was identified at genus level was defined as “other”. Data was analysed using a paired T-Test with 95% confidence level.

RESULTS AND DISCUSSION

Coke wastewater characterisation

On average the full-scale coke making wastewater contained 221 mg/L of phenol and 195 mg/L of thiocyanate (SCN^-) (Table II). Trace metal concentrations ranged from 0.13 $\mu\text{g/L}$ Cd to 3612 $\mu\text{g/L}$ Fe. Therefore, Fe constituted the majority of the total trace metals measured at 4216 $\mu\text{g/L}$ (Table II). The $\Sigma 6\text{PAHs}$ presented typical total concentrations of 179 $\mu\text{g/L}$. Individual PAHs varied from 13.6 $\mu\text{g/L}$ (benzo[g,h,i]perylene) to 64.4 $\mu\text{g/L}$ (fluoranthene). The new emission limit for the $\Sigma 6\text{PAHs}$ to achieve following treatment is 50 $\mu\text{g/L}$, hence effective treatment of PAHs in the treatment facility is crucial to achieve the required limit¹³.

Table II: Coke wastewater characterisation.

Impact of bioaugmentation and biostimulation on SCN^- removal

Thiocyanate degradation took place in 3 phases: acclimatisation, rapid degradation and reduced degradation. During the 3 phases the concentration of SCN^- was significantly different, according to T-test statistical analysis. Under control conditions SCN^- declined by 58 mg/L over the first 13 hours as the biomass became acclimatised to the wastewater. After this acclimatisation, the SCN^- declined rapidly by 58 mg/L in 4 hours. The degradation then continued at a reduced rate with a further decline of 38 mg/L between 17 hours and 25 hours (Figure I). All test conditions demonstrated this 3-phase removal trend.

Figure I: Impact of bioaugmentation and biostimulation on SCN⁻ degradation ○ - Control, □ -micronutrients, Δ - alkalinity and bioaugmentation doses of ● - 0.1 g/L ■ - 0.5 g/L ▲- 1.5 g/L.

The addition of micronutrients led to a 9% increase in degradation, in the first 10 hours, compared to control conditions with SCN⁻ concentrations decreasing to 108 ± 11 mg/L. This increased degradation continued with a 12% improvement at 13 hours and a more substantial 39% improvement being observed at 17 hours. After 25 hours, SCN⁻ concentrations fell to 0.7 ± 1.2 mg/L compared to 2.7 ± 4.6 mg/L under control conditions allowing consistent compliance with the <4 mg/L SCN⁻ emission limit. The addition of micronutrients therefore had a marked benefit on SCN⁻ degradation kinetics and enabled a small but important improvement in SCN⁻ degradation after 25 hours, ensuring compliance with the IED. This suggests that coke wastewater does not contain the required micronutrients for the indigenous SCN⁻ degraders, which is a common occurrence in industrial wastewater⁶. Similar to micronutrient addition, the provision of alkalinity improved SCN⁻ removal. After 13 hours of incubation in batch tests, there was a 16% difference in SCN⁻ removal between the control and tests with added alkalinity. Whilst this difference declined to 1.7% at 25 hours, degradation of SCN⁻ was complete, therefore alkalinity addition could also ensure compliance with the 4 mg/L SCN⁻ emission limit. The activated sludge used in these tests had a high abundance of *Thiobacillus* (26%) (Figure II), which are species known to be involved in SCN⁻ degradation. As SCN⁻ degraders are autotrophic in nature, the improved SCN⁻ removal through biostimulation may be associated with the increased concentrations of the required micronutrients or inorganic carbon associated with alkalinity addition which may be utilised by autotrophic thiocyanate degraders present in the biomass^{23,39}.

Figure II: Operational taxonomic unit (OTU) abundance □ **Bioaugmentation product** ■
Indigenous activated sludge biomass ≡ **Bioaugmentation batch test effluent (1.5 g/L)** // **Bioaugmentation batch test effluent (1.5 g/L) after 25 hours contact with river water.**

A bioaugmentation dose of 0.1 g/L resulted in a similar degradation trend to control conditions in the first 13 hours. However, final SCN^- concentrations at 25 hours were higher than under control conditions at 4.3 ± 2 mg/L. The addition of bioaugmentation product at 0.1 g/L therefore offered no benefit to SCN^- degradation over a 25 hour period. At an increased dose of 0.5 g/L there was a notable delay in the time required for degradation to proceed. This delay may be associated with the acclimatisation required for the bioaugmented bacteria to adapt to the wastewater conditions or increased competition between species⁴⁰. Despite the initial delay in degradation, by 13 hours the average SCN^- concentration was comparable to control conditions at 95 ± 17 mg/L. At 25 hours the final SCN^- concentration was 0.7 ± 1.2 . This therefore offered a small improvement compared with control tests and ensured compliance with the emission limit. A dose of 1.5 g/L resulted in complete degradation of SCN^- and after time 17h of incubation, the SCN^- concentration was significantly different from the control. The bioaugmentation product was dominated by *Bacillus* species including *Bacillus cereus* (54%) and *Bacillus* other (37%). *Mycobacterium* (other) were present at an abundance of 9%. Improved degradation may therefore be associated with the bioaugmentation product as some *Bacillus* sp. are associated with SCN^- removal⁴¹.

Impact of bioaugmentation and biostimulation on $\Sigma 6\text{PAH}$ removal

The IED applies to total PAHs and compliance with the 50 µg/L emission limit is challenging. As the PAHs need to be solubilised in order for microbial degradation to occur a focus was therefore given on the ability of bioaugmentation and biostimulation to improve the removal of dissolved PAHs (Table III). The concentration of Σ6PAHs, under control conditions, increased from 3.5 µg/L to 13.4 µg/L after 25 hours. This increase is believed to result from desorption of PAHs from the suspended biomass solids. After 25 hours, under control conditions, benzo[a]pyrene and benzo[b/k]fluoranthene, both characterised by a molecular weight of 252 g/mol accounted for 66% of the Σ6PAHs at a concentration of 5.2 and 3.6 µg/L respectively. The higher the number of fused rings in a PAH compound the higher the molecular weight and greater the persistence of the compound. Indeno[1,2,3-cd]perylene and benzo[g,h,i]perylene, both with a molecular weight of 276 g/mol, had lower concentrations of 1.7 and 1.9 µg/L respectively. As they are characterised by higher molecular weights their lower concentration may be explained by lower rates of desorption. Fluoranthene, characterised by the lowest molecular weight of 202 g/mol and therefore most easily degraded was present at 1.0 µg/L.

The addition of micronutrients led to no improvement in Σ6PAHs removal. At 12.7 µg/L Σ6PAHs were comparable to control conditions (13.4 µg/L). Micronutrient limitations have previously noted as a possible cause behind the failure of benzo[a]pyrene degradation⁴². Despite this, the current investigation suggests that the availability of micronutrients is not a limiting factor for the removal of PAHs in coke wastewater. Bioaugmentation, on the other hand, was beneficial to the removal of Σ6PAHs at a dose of 0.5 g/L. A notable improvement in PAH removal was observed with Σ6PAHs declining to 6.6 µg/L, representing a 51% decrease in Σ6PAH concentrations. Lower molecular weight PAHs are more prone to microbial attack and degradation which explains the high removal of fluoranthene which

declined by 60% to 0.4 µg/L. A 50% reduction was observed for both benzo[b/k]fluoranthene and benzo[a]pyrene. For the highest molecular weight compounds, indeno[1,2,3-cd]perylene and benzo[g,h,i]perylene, a 53% reduction was also observed with concentrations falling to 0.8 and 0.9 µg/L respectively. The improved removals were noteworthy due to the abundance of heavy molecular weight PAHs. At a lower dose (0.1 g/L) there was no improvement to Σ6PAH removal (16.4 µg/L). Additionally, tripling the bioaugmentation dose only led to a further 10% improvement in Σ6PAH removal.

The bioaugmentation product was dominated by *Bacillus cereus* (54%), *Bacillus* (other) (37%) and *Mycobacterium* (other) (9%) (Figure III) which have been associated with the degradation of a range of PAHs^{43,44}. For instance, *Mycobacterium* are known for their good catabolic capabilities for PAHs with 5-benzene rings as they have mycolic acids which aid in the uptake of hydrophobic PAHs⁴⁴⁻⁴⁷. The failure of bioaugmentation at 0.1 g/L suggests that the exogenous microorganisms were unable to establish themselves within the activated sludge mixed liquor. As higher doses had a positive impact on Σ6PAH removal, it is likely that the inoculum size at the 0.1 g/L dose was simply insufficient to overcome pressures such as grazing by protozoa and or insufficient numbers to be able to compete with the indigenous population⁴⁸. The increased removal of Σ6PAHs at a dose of 0.5 g/L suggests that the exogenous microorganisms quickly acclimatised to the wastewater and laboratory conditions and enhanced the indigenous population of PAH degrading bacteria. Increased doses did not result in substantial improvements in the removal of Σ6PAHs suggesting that another factor became limiting in the system such as nutrients and carbon. Furthermore, the microbial degradation of PAHs can also be limited by the rate at which PAHs can be transferred to the microbial cells⁴⁷.

Table III: Impact of bioaugmentation and biostimulation on effluent PAH concentrations.

Removals in relation to the control are reported in brackets e.g. (21%).

Impact of bioaugmentation and biostimulation on trace metal removal

The impact of bioaugmentation and biostimulation was subsequently investigated for trace metal removal (Table IV). Under control conditions the sum trace metals increased from 47.2 to 56.4 µg/L. This increase was believed to be the result of desorption from the suspended solids which can be impacted by changes to the pH and mass flux balances⁴⁹. The addition of micronutrients and bioaugmentation led to little impact to the sum trace metal concentration (Table IV). Although small improvements were observed through bioaugmentation the percentage improvements were within the method uncertainty range and therefore no clear conclusions could be drawn about the significance of the data. The main improvement seen was for Zn which was reduced from 13 µg/L (control conditions) to 1 µg/L at a dose of 0.1 g/L and 1.5 g/L. Despite this, at 0.5 g/L Zn removal was lower (9 µg/L) giving an unclear correlation between bioaugmentation dose and removal potential.

Table IV: Impact of bioaugmentation and biostimulation on trace metal concentration.

Removals in relation to the control are reported in brackets e.g. (25%).

Impact of bioaugmentation and biostimulation on sCOD and nitrogen removal

The removal for sCOD under control conditions was 97%. Similarly, sCOD remained at 97% in all the biostimulation tests and bioaugmentation tests with doses of 0.1 and 0.5 g/L. However, at the higher dose of 1.5 g/L, the sCOD removal declined to 88%, with 53 mg/L sCOD remaining. This may have resulted from bacterial degradation through endogenous metabolism due increased competition and reduced survival ⁴⁰.

Phenol removals were unaffected by bioaugmentation with all tests showing a removal of >98%. Under all conditions phenol was reduced from 74 mg/L to 1 mg/L and below. Alkalinity addition led to a reduction in phenol concentration to 0.3 mg/L allowing compliance with the <0.5 mg/L emission limit. Ammonia-nitrogen concentrations were expected to increase as a result of SCN⁻ degradation ^{9,39}. Alkalinity addition, however, led to ammonia-nitrogen removal (Table V) which was consistent with the stimulation of autotrophic nitrifying bacteria.

Table V: Impact of bioaugmentation and biostimulation on sCOD, phenol and ammonia-nitrogen in batch tests after 25 h of incubation.

Bacterial speciation, abundance and viable cell counts

It was important to understand whether the addition of exogenous microorganisms through bioaugmentation would impact microorganism speciation in the receiving river waterbody and or the viable cell counts present after exposure to river water. A Special Area of

Protection (SPA) exists downstream of the discharge point for effluent from the full-scale coke wastewater treatment ASP which then subsequently flows into an estuary which is designated as a Special Area of Conservation (SAC), under the EU Habitats Directive ^{50,51}. As such it is important to understand whether the exogenous microorganisms hold the potential to have negative consequences within the receiving waterbody as a result of interactions with the native microorganisms such as competition and predation which may have further impacts higher up the food chain resulting in further impacts to the ecosystem.

Figure II shows the OTU abundance for the bioaugmentation product, indigenous activated sludge biomass, batch test effluent combined with river water and batch test effluent combined with river water after 24 hours. It can be observed that the indigenous activated sludge biomass was characterised by a high abundance of an uncultured species of *Thiobacillus* (26%), an uncultured species of *Mizugakiibacter* (13%), an ambiguous species of *Rhodanobacter* (11%) and an ambiguous species of *Comamonas* (12%). *Thiobacillus* is associated with the degradation of SCN^- whilst *Mizugakiibacter* and *Rhodanobacter* have been associated with their iron-oxidising and nitrate reducing abilities ^{39,52}. *Comamonas* bacteria have been associated with a wide range of abilities including the degradation of phenol ⁵³.

The abundance of OTUs was tracked in the batch test with the addition of 1.5 g/L of the bioaugmentation product. After 25 hours under batch test conditions *Bacillus cereus* was detected, at an abundance of 3.4% whilst *Bacillus* (other) was detected at an abundance of 0.96% (Figure II). This suggests that some of the inoculated *Bacillus* bacteria were maintained in the activated sludge. A total abundance of 4.4% *Bacillus* species suggests that

the population was potentially still able to play a role in the activated sludge like other species present in relatively low abundances such as nitrifying bacterial populations which account for 3-10% of the bacterial population in an ASP⁵⁴. Long-term studies would be required, however, to assess whether the population was sustained or whether maintenance dosing would be required^{48,55}. *Mycobacterium* on the other hand decreased to an abundance of just 0.03%. It is possible that this species was outcompeted by indigenous bacteria or was unable to survive in the coke wastewater as a result of toxic compounds such as phenol and SCN⁻^{8,56}. The OTU abundance data can give an indication of the causes behind the failure of a dose of 0.1 g/L to impact PAHs removal. At this lower dose, the species abundance may have been too low within the activated sludge to play an important degradative role.

Of particular interest was the ability of the exogenous bacteria to survive simulated river water discharge. When effluent from the batch test was exposed to river water for 24 hours *Bacillus* sp. were detected at just 0.06% relative abundance. Although a reduction was expected due to the dilution associated with the combination of activated sludge effluent and the river discharge the continued low abundance after 24 hours would suggest that the *Bacillus* sp. were unable to thrive in the river ecosystem. *Mycobacterium* (found in the bioaugmentation product) was detected, however, this was identified as an indigenous species to the river water (abundance of 4%) and as the abundance from the activated sludge was very low the increased abundance is believed to be associated with their presence in the river water (Figure II). Operational taxonomic units associated with the bioaugmentation product did not therefore become abundant in the river water suggesting that they may have succumbed to predatory pressures.

Viable cell counts increased in line with the bioaugmentation dose from 2.07×10^8 (control) to 2.17×10^8 (dose of 0.1 g/L), 2.30×10^8 (dose of 0.5 g/L) and 2.52×10^8 (dose of 1.5 g/L) (Figure III). It is possible that this was attributed to the synergistic activities of the exogenous and indigenous bacteria. The number of viable cells declined firstly as a result of dilution. The dilution applied (1:1,690) corresponded to a theoretical decrease of 99.94% cell counts in the effluent of the batch tests. Nevertheless, when accounting for the cell count within the river water, the dilution represented an average viable cell count decline of 93%.

Figure III: Impact of bioaugmentation on viable cell counts ■ - Bioaugmentation batch test effluent, ■ - Bioaugmentation batch test effluent after 0 hours contact with river water and □ - Bioaugmentation batch test effluent after 25 hours contact with river water.

After 25 hours exposure to the river water under typical treatment conditions (control) the viable cell count declined to 2.9×10^6 representing a further reduction in the viable cell count of 89%. The reduction in the number of viable cells may have resulted from competition between for the limited resources available in the receiving waterbody or due to the inability of the activated sludge bacteria to survive under the environmental conditions associated with the river water. The number of viable cells was therefore 98.6% lower than in the original activated sludge biomass. There was a significant difference between the cell counts of the bioaugmentation effluent at time 0 (0 hours, that accounted for dilution in the river water) and after 25h of incubation in the river water. As a worst-case scenario was modelled, assuming no settling in the clarifier, it would be expected that viable cell count reductions would in fact be higher under normal operational conditions. Similar reductions were observed when bioaugmentation was applied.

CONCLUSION

Bioaugmentation using a commercially available product rich in *Bacillus* and *Mycobacterium* sp. at a dose of 0.5 g/L resulted in a 51% improvement in the removal of Σ 6PAHs and enabled compliance with the SCN^- emission limit of <4 mg/L. Thiocyanate removal was also improved by both micronutrient and alkalinity addition ensuring compliance with the emission limit. Phenol removal was improved by alkalinity addition typically enabling compliance with the 0.5 mg/L emission limit. Biostimulation should be optimised for the removal of SCN^- and phenol. Operational taxonomic unit abundance data showed that the exogenous bacteria added through bioaugmentation at a dose of 1.5 g/L, accounted for 4.4% of the activated sludge biomass after 25 hours. After the activated sludge biomass was exposed to river water for 24 hours *Bacillus* sp. associated with the bioaugmentation product were detected at 0.06% suggesting that they were unable to thrive in the river ecosystem. The viable cell count for the activated sludge biomass declined by 93% as a result of dilution with the river water and a further 89% after 25 hours exposure to river water suggesting low survival of bacterial cells in the river water. Bioaugmentation and biostimulation are recommended for their application to coke wastewater having been demonstrated to be capable of producing an effluent characterised by an effluent Σ 6PAHs of 6.6 $\mu\text{g/L}$, 0.3 mg/L phenol and 1.2 mg/L SCN^- which complies with the IED emission limit. Bioaugmentation is not anticipated to impact on downstream river water quality.

ACKNOWLEDGEMENTS

This work was supported by Tata Steel UK and the Natural Environment Research Council
[grant number NE/K007424/1].

References

1. Gerardi, M. *Wastewater Bioaugmentation and Biostimulation*. (DEStech Publications, Inc., 2015).
2. Tyagi, M., da Fonseca, M. M. R. & de Carvalho, C. C. C. R. Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation* **22**, 231–41 (2011).
3. Zhang, J., Wen, D., Zhao, C. & Tang, X. Bioaugmentation accelerates the shift of bacterial community structure against shock load: a case study of coking wastewater treatment by zeolite-sequencing batch reactor. *Appl. Microbiol. Biotechnol.* **98**, 863–73 (2014).
4. Bai, Y., Sun, Q., Zhao, C., Wen, D. & Tang, X. Bioaugmentation treatment for coking wastewater containing pyridine and quinoline in a sequencing batch reactor. *Appl. Microbiol. Biotechnol.* **87**, 1943–1951 (2010).
5. Kim, Y. M., Park, D., Lee, D. S. & Park, J. M. Inhibitory effects of toxic compounds on nitrification process for cokes wastewater treatment. *J. Hazard. Mater.* **152**, 915–921 (2008).
6. Burgess, J. E., Quarmby, J. & Stephenson, T. Role of micronutrients in activated sludge-based biotreatment of industrial effluents. *Biotechnol. Adv.* **17**, 49–70 (1999).
7. Duque, A. F., Bessa, V. S., Carvalho, M. F. & Castro, P. M. L. Bioaugmentation of a rotating biological contactor for degradation of 2-fluorophenol. *Bioresour. Technol.* **102**, 9300–3 (2011).
8. Martín-Hernández, M., Suárez-Ojeda, M. E. & Carrera, J. Bioaugmentation for treating transient or continuous p-nitrophenol shock loads in an aerobic sequencing batch reactor. *Bioresour. Technol.* **123**, 150–6 (2012).
9. Kim, Y. M., Park, D., Jeon, C. O., Lee, D. S. & Park, J. M. Effect of HRT on the biological pre-denitrification process for the simultaneous removal of toxic pollutants from cokes wastewater. *Bioresour. Technol.* **99**, 8824–32 (2008).
10. Marañón, E. *et al.* Treatment of coke wastewater in a sequential batch reactor (SBR) at pilot plant scale. *Bioresour. Technol.* **99**, 4192–8 (2008).
11. Vázquez, I., Rodríguez, J., Marañón, E., Castrillón, L. & Fernández, Y. Simultaneous removal of phenol, ammonium and thiocyanate from coke wastewater by aerobic biodegradation. *J. Hazard. Mater.* **137**, 1773–80 (2006).
12. Raper, E. *et al.* Enhancing the removal of hazardous pollutants from coke making wastewater by dosing activated carbon to a pilot-scale activated sludge process. *J. Chem. Technol. Biotechnol.* (2017). doi:10.1002/jctb.5231
13. European Commission. *Best Available Techniques (BAT) Reference Document for Iron and Steel Production. Industrial Emissions Directive 2010/75/EU BREF-IS*, (2013).
14. Fang, F., Han, H., Zhao, Q., Xu, C. & Zhang, L. Bioaugmentation of biological contact oxidation reactor (BCOR) with phenol-degrading bacteria for coal gasification wastewater (CGW) treatment. *Bioresour. Technol.* **150**, 314–20 (2013).
15. Zhu, X., Tian, J. & Chen, L. Phenol degradation by isolated bacterial strains: Kinetics study and application in coking wastewater treatment. *J. Chem. Technol. Biotechnol.*

- 87, 123–129 (2012).
16. Sun, G. D. *et al.* Isolation of a high molecular weight polycyclic aromatic hydrocarbon-degrading strain and its enhancing the removal of HMW-PAHs from heavily contaminated soil. *Int. Biodeterior. Biodegrad.* **90**, 23–28 (2014).
 17. Park, D., Lee, D. S., Kim, Y. M. & Park, J. M. Bioaugmentation of cyanide-degrading microorganisms in a full-scale cokes wastewater treatment facility. *Bioresour. Technol.* **99**, 2092–6 (2008).
 18. Brion, N. & Billen, G. Wastewater as a source of nitrifying bacteria in river systems: The case of the River Seine downstream from Paris. *Water Res.* **34**, 3213–3221 (2000).
 19. Abu-salah, K., Shelef, G., Levanon, D., Armon, R. & Dosoretz, C. G. Microbial degradation of aromatic and polyaromatic toxic compounds adsorbed on powdered activated carbon. *J. Biotechnol.* **51**, 265–272 (1996).
 20. Jeon, C. O. & Madsen, E. L. In situ microbial metabolism of aromatic-hydrocarbon environmental pollutants. *Curr. Opin. Biotechnol.* **24**, 474–81 (2013).
 21. Popp, P., Bauer, C. & Wennrich, L. Application of stir bar sorptive extraction in combination with column liquid chromatography for the determination of polycyclic aromatic hydrocarbons in water samples. *Anal. Chim. Acta* **436**, 1–9 (2001).
 22. de Maagd, P. G.-J., ten Hulscher, D. T. E. M., van den Heuvel, H., Opperhuizen, A. & Sijm, D. T. H. M. Physicochemical properties of polycyclic aromatic hydrocarbons: Aqueous solubilities, n-octanol/water partition coefficients, and Henry's law constants. *Environ. Toxicol. Chem.* **17**, 251–257 (1998).
 23. Staib, C. & Lant, P. Thiocyanate degradation during activated sludge treatment of coke-ovens wastewater. *Biochem. Eng. J.* **34**, 122–130 (2007).
 24. Straube, W. L. *et al.* Remediation of Polyaromatic Hydrocarbons (PAHs) through Landfarming with Biostimulation and Bioaugmentation. *Acta Biotechnol.* **23**, 179–196 (2003).
 25. Raper, E., Fisher, R., Anderson, D. R., Stephenson, T. & Soares, A. Optimisation of carbon supply for the nitrification and denitrification of coke wastewaters. *Submitt. Pap.* (2017).
 26. Jianlong, W., Xiangchun, Q., Libo, W., Yi, Q. & Hegemann, W. Bioaugmentation as a tool to enhance the removal of refractory compound in coke plant wastewater. *Process Biochem.* **38**, 777–781 (2002).
 27. The Institution of Gas Engineers. *Recommended analytical methods for gas works and coke oven effluents: Booklet 3: Thiocyanate.* (1971).
 28. ISO. *ISO 6439:1990: Water quality — Determination of phenol index — 4-Aminoantipyrine spectrometric methods after distillation.* (1990).
 29. Vázquez, I., Rodríguez-Iglesias, J., Marañón, E., Castrillón, L. & Álvarez, M. Removal of residual phenols from coke wastewater by adsorption. *J. Hazard. Mater.* **147**, 395–400 (2007).
 30. Eaton, A. *Standard methods for the examination of water and wastewater.* (American Public Health Association, 2005).
 31. British Standards Institution. BS EN 14385: 2004 Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). (2004).
 32. US EPA. US EPA Method 8270, Revision C, Semivolatile Organic Compounds by

- Gas Chromatography-Mass Spectrometry (GC/MS). (1996).
33. Lipphaus, P. *et al.* Microbiological tap water profile of a medium-sized building and effect of water stagnation. *Environ. Technol.* **35**, 620–628 (2014).
 34. Gatza, E., Hammes, F. & Prest, E. Assessing Water Quality with the BD Accuri™ C6 Flow Cytometer. (2013).
 35. Quince, C., Lanzen, A., Davenport, R. J. & Turnbaugh, P. J. Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* **12**, 38 (2011).
 36. Hamady, M. *et al.* Microbial community profiling for human microbiome projects : Tools, techniques, and challenges. *Next-Generation DNA Seq.* 1141–1152 (2012). doi:10.1101/gr.085464.108
 37. Caporaso, J. G. *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods Corresp.* **7**, 335–336 (2010).
 38. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **41**, 590–596 (2013).
 39. Raper, E., Stephenson, T., Fisher, R., Anderson, D. R. & Soares, A. Characterisation of thiocyanate degradation in a mixed culture activated sludge process treating coke wastewater. *Submitt. Pap.* (2017).
 40. Bouchez, T. *et al.* Ecological study of a bioaugmentation failure. *Environ. Microbiol.* **2**, 179–90 (2000).
 41. Lee, C., Kim, J., Chang, J. & Hwang, S. Isolation and identification of thiocyanate utilizing chemolithotrophs from gold mine soils. *Biodegradation* **14**, 183–188 (2003).
 42. Juhasz, A. L. & Naidu, R. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. *Int. Biodeterior. Biodegradation* **45**, 57–88 (2000).
 43. Samanta, S. K., Singh, O. V. & Jain, R. K. Polycyclic aromatic hydrocarbons: Environmental pollution and bioremediation. *Trends Biotechnol.* **20**, 243–248 (2002).
 44. Haritash, A. K. & Kaushik, C. P. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *J. Hazard. Mater.* **169**, 1–15 (2009).
 45. Mueller-Spitz, S. R. & Crawford, K. D. Silver nanoparticle inhibition of polycyclic aromatic hydrocarbons degradation by Mycobacterium species RJGII-135. *Lett. Appl. Microbiol.* **58**, 330–337 (2013).
 46. García-Díaz, C., Ponce-Noyola, M. T., Esparza-García, F., Rivera-Orduña, F. & Barrera-Cortés, J. PAH removal of high molecular weight by characterized bacterial strains from different organic sources. *Int. Biodeterior. Biodegradation* **85**, 311–322 (2013).
 47. Johnsen, A. R., Wick, L. Y. & Harms, H. Principles of microbial PAH-degradation in soil. *Environ. Pollut.* **133**, 71–84 (2005).
 48. Ramadan, M. A., El-Tayeb, O. M. & Alexander, M. Inoculum size as a factor limiting success of inoculation for biodegradation. *Appl. Environ. Microbiol.* **56**, 1392–6 (1990).
 49. Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E. & Tack, F. M. G. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review. *Sci. Total Environ.* **407**, 3972–3985 (2009).
 50. Joint Nature Conservation Committee. Humber estuary : Annex I habitats that are a

- primary reason for selection of this site. Available at:
<http://jncc.defra.gov.uk/protectedsites/sacselection/sac.asp?EUCode=UK0030170>.
(Accessed: 7th September 2016)
51. Joint Nature Conservation Committee. SPA description: Humber Flats, Marshes and Coast. (2005). Available at: <http://jncc.defra.gov.uk/default.aspx?page=1996>.
(Accessed: 7th September 2016)
 52. Wang, R. *et al.* Chemoautotrophic denitrification based on ferrous iron oxidation: Reactor performance and sludge characteristics. *Chem. Eng. J.* **313**, 693–701 (2017).
 53. Zámocký, M., Godočíková, J., Koller, F. & Polek, B. Potential application of catalase-peroxidase from *Comamonas terrigena* N3H in the biodegradation of phenolic compounds. *Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol.* **79**, 109–117 (2001).
 54. Gerardi, M. H. *Nitrification and Denitrification in the Activated Sludge Process*. (John Wiley & Sons, 2002). doi:10.1002/0471216682
 55. Boon, N., Top, E. M., Verstraete, W., Steven, D. & Siciliano, S. D. Bioaugmentation as a Tool To Protect the Structure and Function of an Activated-Sludge Microbial Community against a 3-Chloroaniline Shock Load. *Appl. Environ. Microbiol.* **69**, 1511–19 (2003).
 56. Goldstein, R. M., Mallory, L. M. & Alexander, M. Reasons for possible failure of inoculation to enhance biodegradation. *Appl. Environ. Microbiol.* **50**, 977–83 (1985).
 57. RWE AG Pers.Comm. Activated lignite pricing. (2014).

1 *Table I: Composition of micronutrient solution used in batch tests.*

Micronutrient	Concentration (mg/L)
Riboflavin (B2)	1
Pyroxidine hydrochloride (B6)	0.2
Vitamin B12	0.5
Niacin	1
Biotin	0.5
Phosphate	3
Calcium	1
Magnesium	3
Copper	0.03
Zinc	0.5
Molybdenum	0.4

1 **Table II: Coke wastewater characterisation.**

	Concentration (mg/L) and standard deviation
sCOD	638 ± 8
Phenol (mono)	74 ± 1
SCN ⁻	128 ± 11
NH ₄ ⁺ -N	89 ± 1
NO ₃ ⁻ -N	9 ± 1
NO ₂ ⁻ -N	13 ± 1
PAHs (total) (µg/L)* †	170 ±30
PAHs (dissolved) (µg/L)*	3.5
Trace metals (total) (µg/L)** †	132 ±23
Total metals (dissolved) **	47.2
pH	8.2 ± 0

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

** Sum of As, Cd, Cr, Cu, Pb, Ni and Zn.

† Average data taken from full-scale site

- 1 *Table III: Impact of bioaugmentation and biostimulation on final effluent PAH concentrations. Removals in relation to the control are*
 2 *reported in brackets e.g. (21%).*

	μg/L					
	Fluoranthene	Benzo(b/k) fluoranthene	Benzo(a)pyrene	Indeno (1,2,3-cd) pyrene	Benzo(g,h,i)perylene	Σ6PAHs
Molecular weight (g/mol)	202	252	252	276	276	
Initial concentration:	0.6	1.3	0.8	0.4	0.4	3.5
Final concentrations:						
Control	1.0	5.2	3.6	1.7	1.9	13.4
Biostimulation:						
Micronutrient addition	0.8 (21%)	5.1 (1.9%)	3.5 (2.8%)	1.6 (5.9%)	1.7 (10.5%)	12.7 (5.3%)
Bioaugmentation dose:						
0.1 g/L	0.9 (13%)	6.4 (-)	4.6 (-)	2.2 (-)	2.3 (-)	16.4 (-)
0.5 g/L	0.4 (56%)	2.6 (50%)	1.8 (50%)	0.8 (50.6%)	0.9 (53.2%)	6.6 (51%)
1.5 g/L	0.3 (68%)	2.1 (59.6%)	1.5 (58.3%)	0.7 (60%)	0.7 (61.1%)	5.3 (60.1%)

Relative standard deviation of method: fluoranthene 14%, benzo(b/k)fluoranthene 14.9%, benzo(a)pyrene 8.1%, indeno(1,2,3-cd)pyrene 13.8%, benzo(g,h,i)perylene 14.2%.

- 1 *Table IV: Impact of bioaugmentation and biostimulation on final trace metal concentration. Removals in relation to the control are reported*
 2 *in brackets e.g. (25%).*

	µg/L							
	As	Cd	Cr	Cu	Pb	Ni	Zn	Sum
Initial concentration:	9.5	0.01	15	0.3	0.2	17	5	47.2
Final concentrations:								
Control	7.1	0.01	16	5.9	1.3	13	13	56.4
Biostimulation:								
Micronutrients	7.7 (-)	nd	15 (6.3%)	4.2 (29%)	0.9 (31%)	10 (23%)	16 (-)	54.5 (3.4%)
Bioaugmentation dose:								
0.1 g/L	6.7 (5.6%)	0.01 (-)	20 (-)	3.2 (46%)	1.4 (-)	12 (7.7%)	1 (92%)	44.4 (21%)
0.5 g/L	6.9 (2.8%)	0.01 (-)	17 (-)	3.8 (36%)	1.3 (-)	13 (-)	9 (31%)	51.1 (9.4%)
1.5 g/L	7.3 (-)	0.01 (-)	17 (-)	4.5(24%)	1.3 (-)	15 (-)	1 (92%)	46.2 (18%)

Relative standard deviation of method: As 11.9%, Cd 9.7%, Cr 15.5%, Cu 20.1%, Pb 16.9%, Ni 10.6%, Zn 26.2%

1 *Table V: Impact of bioaugmentation and biostimulation on sCOD, phenol and ammonia-nitrogen in batch tests after 25 h of incubation.*

	pH		sCOD			Phenol			Ammonia-nitrogen		
	0 h	25 h	0 h (mg/L)	25 h (mg/L)	Removal (%)	0 h (mg/L)	25 h (mg/L)	Removal (%)	0 h (mg/L)	25 h (mg/L)	Removal (%)
Control	7.2	7.6 ± 0.3	422 ± 50	12	97	74 ± 7	0.9 ± 0.5	99 ± 0.5	69 ± 4	93 ± 2	-34
Biostimulation:											
Micronutrients	7.2	7.7 ± 0.1	"	"	"	"	1.2 ± 0.8	98 ± 1.1	"	89 ± 1	-28
Alkalinity	8.5	8.2 ± 0.1	"	"	"	"	0.3 ± 0.3	100 ± 0.3	"	68 ± 3	1.4
Bioaugmentation dose:											
0.1 g/L	7.2	7.6 ± 0	"	"	"	"	0.9 ± 0.3	99 ± 0.3	"	95 ± 1	-38
0.5 g/L	7.2	7.6 ± 0.1	"	"	"	"	1.0 ± 0.2	99 ± 0.2	"	95 ± 2	-38
1.5 g/L	7.2	7.5 ± 0.1	"	52 ± 2	88 ± 3	"	1.0 ± 0.1	99 ± 0.1	"	94 ± 2	-36

2

Figure 1.

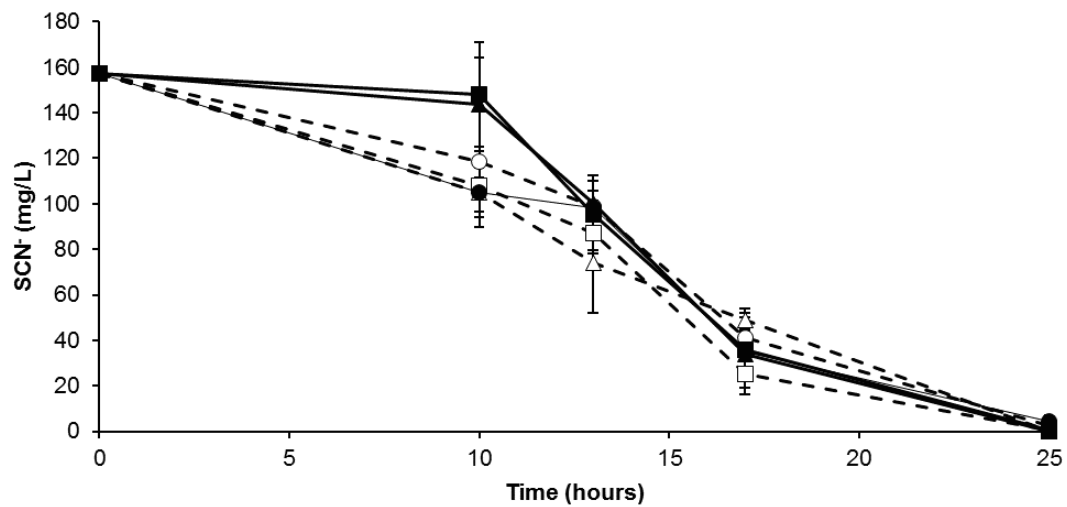


Figure I: Impact of bioaugmentation and biostimulation on SCN- degradation ○ - Control, □ - micronutrients, Δ - alkalinity and bioaugmentation doses of ● - 0.1 g/L ■ - 0.5 g/L ▲ - 1.5 g/L.

Figure 2

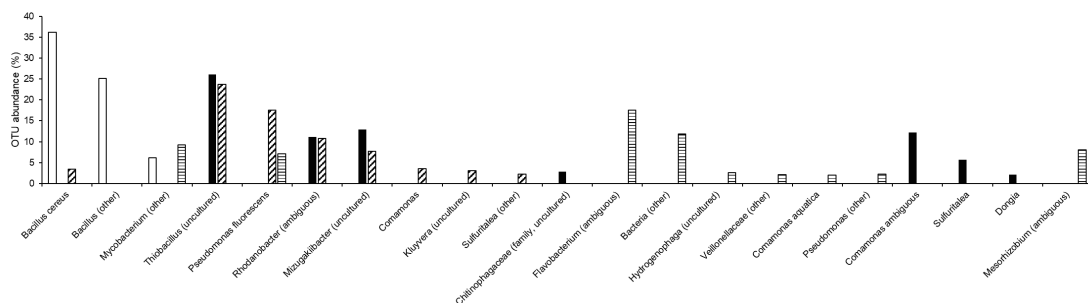


Figure II: Operational taxonomic unit (OTU) abundance □ Bioaugmentation product ■ Indigenous activated sludge biomass ▨ Bioaugmentation batch test effluent (1.5 g/L) ▩ Bioaugmentation batch test effluent (1.5 g/L) after 25 hours contact with river water.

Figure 3

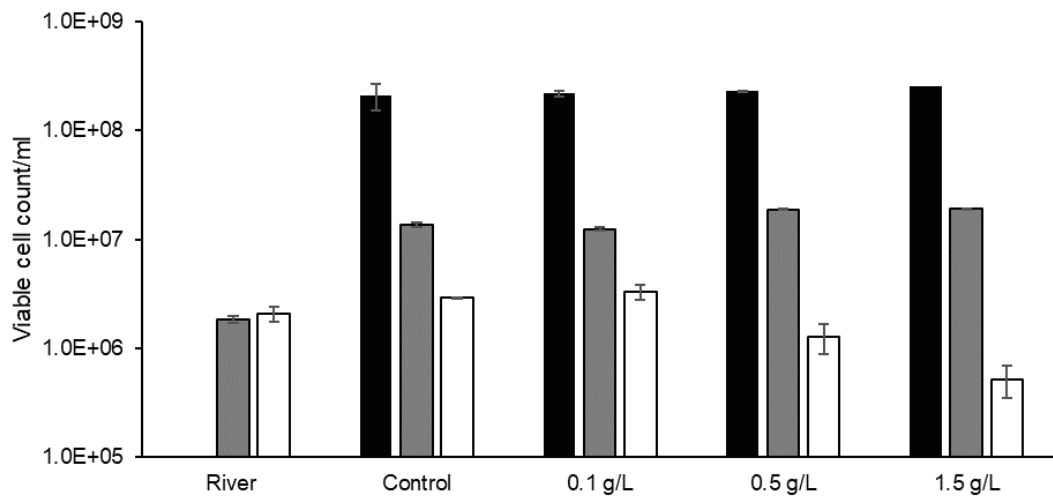


Figure III: Impact of bioaugmentation on viable cell counts ■ - Bioaugmentation batch test effluent, ■ - Bioaugmentation batch test effluent after 0 hours contact with river water and □ - Bioaugmentation batch test effluent after 25 hours contact with river water.

Enhancing the removal of pollutants from coke wastewater by bioaugmentation: a scoping study

Raper, Eleanor

2018-02-09

Attribution-NonCommercial 4.0 International

Eleanor Raper, Tom Stephenson, Francisco Simões et al. Enhancing the removal of pollutants from coke wastewater by bioaugmentation: a scoping study. *Journal of Chemical Technology and Biotechnology*, Volume 93, Issue 9, September 2018, pp. 2535-2543

<http://dx.doi.org/10.1002/jctb.5607>

Downloaded from CERES Research Repository, Cranfield University