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Prediction of Performance of the Moving-Bed Biofilm Pilot Reactor Using Back-Propagation Artificial Neural Network (BP-ANN)

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Coal gasification stripped gas liquor (CGSGL) wastewater contains large quantities of complex organic and inorganic pollutants which include phenols, ammonia, hydantoins, furans, indoles, pyridines, phthalates and other monocyclic and polycyclic nitrogen containing aromatics, oxygen- and sulphur containing heterocyclic compounds. Most conventional aerobic systems for coal gasification wastewater treatment are not sufficient in reducing pollutants such as chemical oxygen demand (COD), phenols and ammonia due to the presence of toxic and inhibitory organic compounds. The current paper reports on the degradation of aromatic compounds and the reduction of hard COD in CGSGL using a Moving-Bed Biofilm Reactor (MBBR) system. The inoculum contained a genetically enhanced mixed culture of *Pseudomonas putida*, *Pseudomonas plecoglossicida*, *Rhodococcus erythropolis*, *Rhodococcus qingshengii*, *Enterobacter cloacae*, *Enterobacter asburiae* strains of bacteria, seaweed and diatoma. Consistently high hard COD removal (>88 %) and degradation of targeted phenolic compounds (>93 %) was achieved in the reactor with no loss of biodiversity in the consortium culture. The performance of the reactor outside the observable range was projected using a Back-Propagation Artificial Neural Network (BP-ANN) developed in this study.

1. Introduction

South Africa has no known petroleum reserves, but it has large quantities of coal reserves projected to last another 200 years under the current use. Coal can be converted to liquid fuel through a gasification liquification process. Sasol uses the patented Sasol-Lurgi process to convert low ranking coal to carbon monoxide as the first step towards the creation of the liquid fuel (Ginster and Matjie, 2005). Unfortunately, the low ranking coal used in the Sasol-Lurgi process contains large amounts of compounds with polar and hydrophilic functional groups such as –OH, –COOH, –O, $–NH_2$, and –SH (Molva, 2004). A number of coal gasification plants in China also use lignite coals as a raw material to produce gas via the Lurgi process. Jin et al. (2013) reported the presence of phenols, cyanides, thiocyanates, polycyclic aromatic hydrocarbons (PAHs), nitrogen-, oxygen-, and sulphur-containing heterocyclic compounds in the coking wastewater. In other related studies, coal gasification wastewater was reported to contained significant amounts of hydrogen sulphide (H_2S) (Gai et al., 2008), ammonia (NH_3) and carboxylic acids (Liu et al., 2013), and long chain alkanes (Ji et al., 2015).

Due to the mixed nature and complexity of the compounds in the coal gasification wastewater, treatment of this wastewater using physical chemical processes tends to be expensive and most of the time ineffective. Currently, the non-biodegradable component of coal gasification wastewater is mostly removed by solvent extraction and ammonia stripping. The latter process produces a secondary stream of highly toxic effluent (Yang et al., 2006).

Microorganisms containing the monooxygenase and dehydrogenase enzymes such as *Pseudomonas putida* (Shen and Wang, 1995), *Pseudomonas aerigunosa* (Oboirien and Chirwa, 2007), and *Alcaligenes eutropha* (Ornston, 1966) are known to facilitate ring cleavage of aromatic compounds leading to degradation of a range of aromatic compounds. Selection and packaging of such compounds into optimised culture soups could

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provide opportunities for treating highly toxic wastewater at a low cost.
In this study, the removal of chemical oxygen demand (COD), phenols and ammonia-nitrogen in a hybrid fixed-film bioreactor (H-FFBR) inoculated with a mixed culture of bacteria containing aromatic compound organisms was investigated. To achieve the above objective, the compounds in the wastewater were characterised. Furthermore, the genetic makeup of organisms in the reactor was determined using PCR followed by genetic sequencing and 16S rRNA genotype fingerprinting. The performance data collected over a period of 6 months was used to calibrate a self-learning Back-Propagation Artificial Neural Network (BP-ANN) to be used for control purposes. The BP-ANN used in this study was originally adopted from Hajmeer and Basheer (2002). A version of the trainable neural network model was tested by Jacobs and Chirwa (2015) for the evaluation of parameters in a phenol-degrading/Cr(VI)-reducing culture.

2. Materials and methods

2.1 Moving-bed biofilm reactor setup

The moved-bed hybrid fixed-film bioreactor (H-FFBR) consisted of 3 aeration compartments in series designated as Zone 1, Zone 2 and Zone 3 (Figure 1). The specific respective volumes for the Zones 1, 2 and 3 were 250 L, 150 L and 600, respectively, resulting in a total bioreactor volume 1,000 L. The bioreactor was acclimatised to the feed over a period of time such that the reactor was subjected to at least three complete bacterial sludge ages.

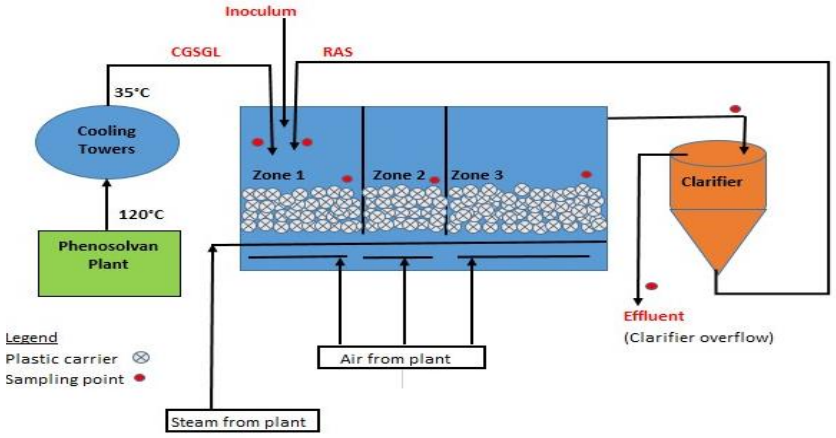

Figure 1: Configuration of the moving-bed, hybrid fixed-film bioreactor pilot plant (H-FFBR) used in the study

2.2 Inoculum culture characterisation

DNA primers 27F (5'-AGAGTTTGATCMTGGCTCAG-'3) and 518R (5'-ATTACCGCGGCTGCTGG-'3) were used in the amplification of the 16S rRNA gene using the Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, UK) PCR system following the manufacturer's instructions. The amplicon libraries were purified using the Agencourt® Ampure® XP bead protocol (Beckman Coulter, USA). The library concentration was measured using the NEBNext® Library Quant assay kit for Illumina® (New England Biolabs, UK). The library pool was sequenced on a MiSeq™ (Illumina, USA) using the MiSeq™ Reagent kit v3, 600 cycles PE (Illumina, USA). The final pooled library was at 10 pM with 20 % PhiX as control. The DNA sequence for each pure colony was then uploaded to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). A phylogenetic tree was constructed from the identified 16S rRNA sequences using the neighbor-joining method in the MEGA Version 6 software (Tamura et al., 2013).

2.3 Analytical methods

2.3.1 COD measurement

Chemical oxygen demand (COD) was determined by oxidation of oxidizable COD using a sodium dichromate solution. Using readily oxizable substance, sodium dichromate is readily reduced to the trivalent (III) state while oxidising organic compounds (APHA, 2005).

2.3.2 Measurement of metals

Samples for soluble metal analyses (sodium, calcium, iron, silica, potassium, copper, zinc, lead, vanadium, manganese, chromium, cobalt, nickel, aluminium, molybdenum and magnesium) were preserved with 10 mL

concentrated nitric acid per litre sample and stored at 6±2 ºC. Samples were filtered through 0.45 µm membrane filters (Merck Millipore, South Africa) and analysed by inductively-coupled plasma-optical emission spectrometry (ICP-OES) (Agilent Technologies, USA) following the U.S. EPA Method 200.7 (1979) (U.S. EPA, 1979). The ICP-OES was operated and controlled using the installed Agilent ICP Expert II software (Agilent Technologies, USA).

2.3.3 Semi-volatile compounds

Aromatic and semi-volatile compounds in water samples were characterised using the Perkin-Elmer Clarus 500 GC-MS equipped with Clarus 600T equipped with headspace (Perkin Elmer, Connecticut, USA). Separation of compounds in the GC was performed in a Perkin–Elmer Elite—5MS capillary column (30 m×0.25 mm ID ×0.5 µm fixed phase) with helium as a carrier gas with the GC operating in a split-less mode. The oven temperature was kept initially at 60 °C for 5 min, followed by an increase to 300 °C at a rate of 15 ºC/min.

2.3.4 Polar aromatic compounds

Hydrophilic aromatic compounds were measured using the Waters 2695 high performance liquid chromatograph (HPLC) (Waters Corporation, Massachusetts, USA) equipped with the Waters Photo Diode Array (PDA) detector operated at λ = 254 nm, and a Waters PAH C_{18} column (4.6 mm × 25 cm with 5 µm packing) operated at a column temperature of 25 ºC. Extraction was done in a 1:1 ratio of sample and acetonitrile.

3. Results and discussion

3.1 Microbial culture composition

The dominant kingdom classification for the biofilm was bacteria (99.8 %) with 0.2 % consisting of archaea, fungi and protozoa. The dominant phyla were Proteobacteria (75.0 %), Firmicutes (3.66 %), Bacteriodetes (5.16 %) and Actinobacteria (1.65 %). The dominant classes were Beta-proteobacteria (32.9 %), Alpha-proteobacteria (34.8 %), Gamma-proteobacteria (13.6 %), Bacteriodetes (4.14 %) and Actinobacteria (1.65 %). Uncultured bacteria comprised 14.53 % and 12.91 % of the uncultured phylum and class classifications respectively (Figure 2). The bacterial BLAST nucleotides were 99 % homologous to three strains of *Pseudomonas putida*, two strains of *Enterobacter cloacae*, one strain of *Enterobacter asburiae* and one strain of *Rhodococcus erythropolis*. These strains are tolerant and effective in high conductivity wastewaters and the hypothesis is that these strains will improve the biodegradation of petroleum hydrocarbons (oils and grease), alkanes and aromatic compounds typically found in CGSGL.The core genera and specific species in the actual bioengineered inoculum contained several Pseudomanads which are intended for the biodegradation of aromatic compounds. Several species from the Rhizobial sources were also identified (Table 1).

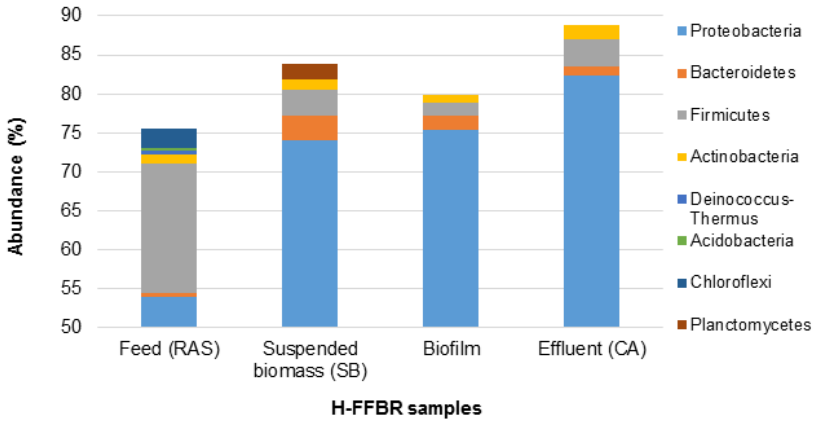

Figure 2: Relative abundance of bacterial phyla in the H-FFBR (excluding unidentified phyla)

3.2 Moving-bed bioreactor performance

Phenol (C_6H_6O); isoquinolinones (C_9H_7N) and saturated carboxylic acids ($C_4H_8O_2$; $C_6H_{12}O_2$; $C_7H_{14}O_2$; $C_8H_{15}O_2$; $C_{16}H_{32}O_2$) were detected in the effluent when phenol (C_6H_6O), isoquinolinones (C_9H_7N), substituted cyclopentanones (C_6H_6O), substituted benzoic acids ($C_8H_8O_2$) and phenylbutenones ($C_{10}H_{10}O$) were detected in the feed. Thus, these compounds were degraded slower when phenylbutenones (substituted aromatic alkenone) were present in the feed. Hydantoins ($C_5H_8N_2O_2$; $C_6H_{10}N_2O_2$) were only detected in the effluent

when phenol (C_6H_6O), aniline (C_6H_5NH), substituted cyclopentanones (C_6H_6O), furans (C_6H_6O) and pyridines ($C_6H_7N_9$) were detected in the feed. In this phase of the project, phenol was used as a surrogate for the majority of aromatic compounds confirmed to be present using GC-MS. The removal of total phenols ranged between 62 % and 93 % with an average of 78 %, thus 22 % of the total phenols were soluble, but non-biodegradable (Figure 3). The removal of phenols was affected by factors such as substrate inhibition effect, pH, temperature, biomass concentration, microbial community and their metabolic potential, and nutrient concentration. Biodegradation was also affected by the positions of the methyl groups of methyl phenols. The p-substituted phenols were more readily biodegradable than the m- or the o-substituted phenols since they are weaker electron donors (Rava et al., 2015).

Table 1: Core genera and species across the H-FFBR

Feed (RAS)	Biofilm	Suspended biomass (SB)	Effluent (CA)
Pseudomonas aeruginosa (AF440523.1) (6-8 %)	*Thauera butanivorans* (NR_040797.1) (5-10 %)	*Ochrobactrum anthropi* (AB120120.1) (2-4 %)	*Rhodoplanes cryptolactis* (AB087718.1) (1-8 %)
Rhodoplanes cryptolactis (AB087718.11) (2-5 %)	*Pseudaminobacter salicyclatoxidans* (NR_028710.1) (3-5 %)	*Thauera butanivorans* (NR_040797.1) (1-4 %)	*Pseudomonas putida* (AE015451.1) (0.3-5 %)
Xanthobacter polyaromaticivorans (AB106864.1) (1-3 %)	*Pseudomonas aeruginosa* (AF440523.1) (1-3 %)	*Pseudomonas aeruginosa* (AF440523.1) (1-3 %)	*Pseudomonas aeruginosa* (AF440523.1) (0-4 %)
Diaphorobacter nitroreducens (AB076856.1) (0.6-2 %)	*Diaphorobacter nitroreducens* (AB076856.1) (1-2 %)	*Ancylobacter polymorphus* (NR_04279.1) (0.6-2 %)	*Diaphorobacter nitroreducens* (AB076856.1) (0-3 %)

[(a)]Pooled data from sample set 1 and sample set 2

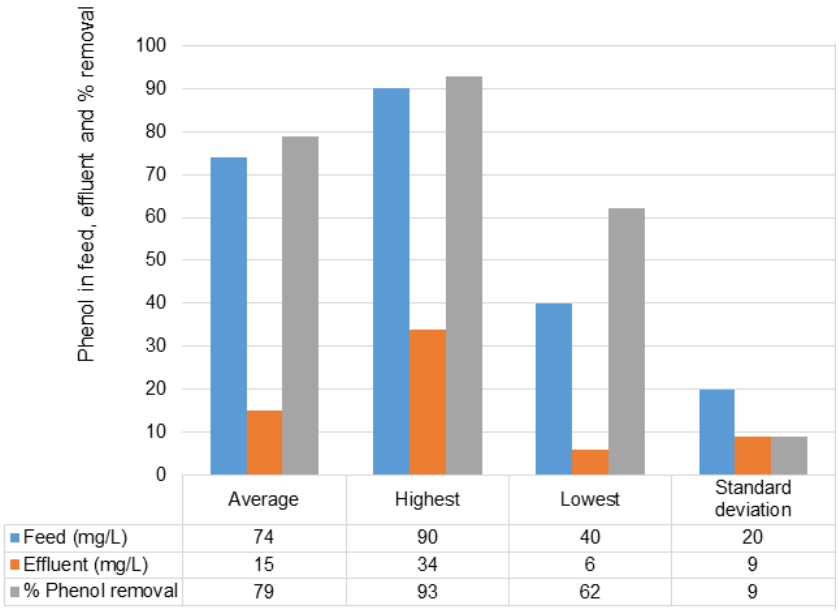

Figure 3: Figure showing removal rate of phenol across the H-FFBR system

3.3 Calibration and Application of BP-ANN (TANN)

The neural network was trained using a back-propagation algorithm (Figure 4) with the goal of achieving a minimum net error on the validation data set while preventing overtraining or memorisation (Sewsynker and Kana, 2016). The experimental data set were randomly divided into two sets (i) 75 % of the data were used for training and (ii) 25 % of the data were used for the validation and testing process. A net error value on the validation data set of 0.018 was achieved after 3700 training epochs. The accuracy of the developed model

was assessed on fourteen novel process conditions (validation data set). With this data set, the regression analyses on predicted and observed process outputs, and, the coefficients of determination (R^2) were calculated for each model output.

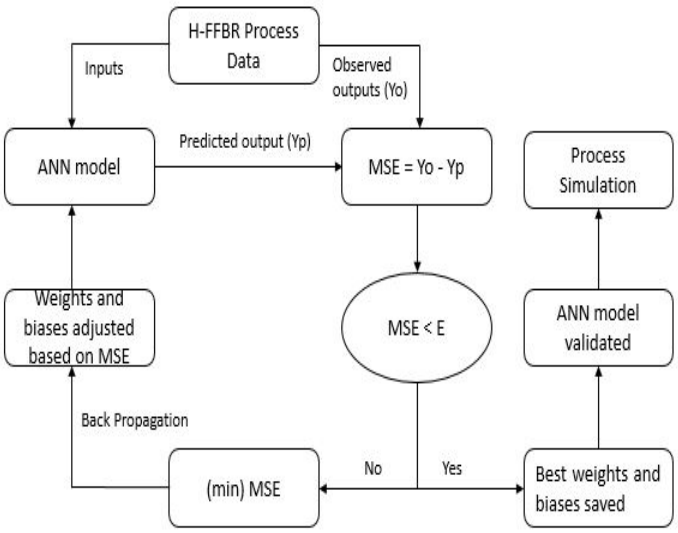

Figure 4: Back-propagation training algorithm used for the model ANN training, where MSE = mean square of differences and E = target minimum error between model data and measured values

The validation was achieved by using the Artificial Neural Network (ANN) model to predict the process output values of Zone 1 Biofilm thickness, Zone 2 Biofilm thickness, Zone 3 Biofilm thickness, Zone 1 OUR, Zone 2 OUR, Zone 3 OUR, Suspended Biomass (MLSS), COD removal (%) and Phenol removal (%) based on fourteen process conditions not previously exposed to the model (Figure 5). The output values gave varied coefficients of determination (R^2) up to 0.96 (average 0.85, excluding outliers). High coefficient of determination (R^2>0.7) suggests a higher reproducibility and accuracy in the model when subjected to the novel H-FFBR operational conditions. Thus, the higher predicted outputs (R^2>0.7) accounted for more than 70 % variation in the observed data. The only coefficients below 0.7 were for o-Phosphates removal (R^2 = 0.06) and for phenol removal (R^2 = 0.57 %). The removal of o-Phosphates was not considered significant enough to be estimated using the developed ANN model since R^2 outliers have a negative effect on ANN based model development as was earlier observed by Rorke et al. (2017).

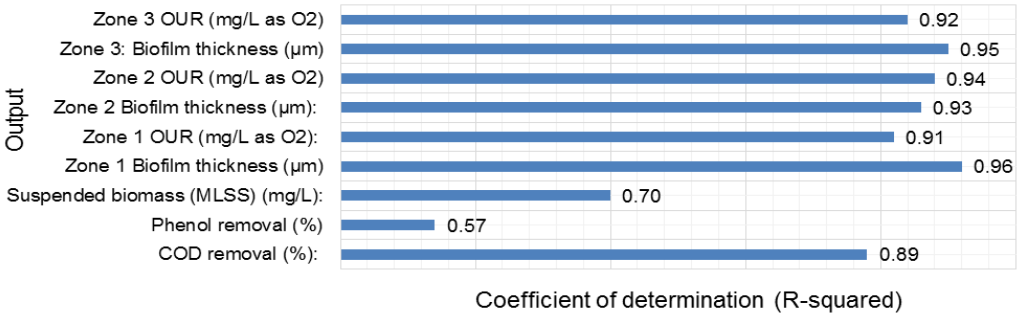

Figure 5: Comparative regression (R^2) values for each output using the Trained Artificial Neural Network (TANN).

4. Conclusions

Diluted CGSGL was successfully treated in a pilot H-FFBR reactor using a bioengineered inoculum of phenol degrading organisms. A diverse microbial community in the inoculum removed most of the hard COD including phenolic compounds. However, nitrogen compounds were resistant to degradation due to the

absence of autotrophic ammonia-oxidising bacteria in the inoculum. The microbial community diversities in the reactor were significantly dissimilar thus indicating that microbial communities were affected by the different growth environments in the H-FFBR. Part of the observed data was used to train a predictive Artificial Neural Network developed in this study which successfully predicted biofilm growth rate in phases not used during the training phase of the model.

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