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Research paper

Performance of a continuously stirred tank bioreactor system connected in series for the biodegradation of thiocyanate and free cyanide

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ABSTRACT

A microbial consortium which was largely dominated by *Thiobacillus* sp. and *Serratia* sp. was evaluated for the biodegradation of thiocyanate (SCN^-) and free cyanide (CN^-) under neutral to alkaline conditions, in a two-staged stirred tank bioreactor system operated in series. The bioreactors were operated across a range of residence times (7 d to 24 h), SCN^- (100–1000 mg SCN^-/L) and CN^- (200–450 mg CN^-/L) concentrations at room temperature (21–25 °C). The bioreactors were characterised by high SCN^- degradation efficiencies (>99.9%) throughout the experimental run except when the microorganisms were temporarily shocked by a pH increase and the introduction of CN^- within the system. Similarly, high CN^- biodegradation efficiencies (>99.9%) were observed subsequent to its introduction to the system. Planktonic microbial activity tests by organisms within the bioreactor system revealed high SCN^- and CN^- degradation efficiencies (>80%); a direct indication of high planktonic microbial activity within the bioreactor system. Furthermore, there was an observed total nitrogen removal by the organisms within the system, which demonstrated the nitrification and denitrification capacity of the organisms while the sulphate concentration increased as a result of SCN^- biodegradation, over a period of approximately 300 days. This is the first report on the simultaneous biodegradation of high CN^- and SCN^- concentrations, coupled with nitrogen removal under alkaline conditions. The results demonstrated the potential of the process to treat CN^- and SCN^- laden wastewaters.

1. Introduction

Cyanide is produced naturally by a variety of micro- and/or macrobiotic species in minute concentrations as compared to the cyanide wastes which are largely generated from the mining industry [1]. In the mining industry, free cyanide is used as a preferred lixiviant for the recovery of base (e.g Ni, Cu, Zn, etc) and precious metals (Au, Pt, Ag) from free milling, complex and refractory sulphide ores, in a process known as the cyanidation process [2,3]. In this process, cyanide reacts with a variety of chemical constituents within the ore, forming chemical complexes which vary widely in their stability and solubility [4]. The major chemical constituents resulting from the cyanidation process are free cyanide, thiocyanate and metal-complexed cyanides, as weak acid (Cu, Cd, Ni, Zn, etc.) and strong acid dissociable cyanides (Au, Ag, Fe, Co, etc.) [5,6]. The prevalence and co-existence of these chemical compounds is harmful to both the environment and living organisms. This effect has been observed elsewhere, where artisanal small scale gold mining (ASGM) operations in West African countries such as Burkina Faso, whereby the effluent from such mining activities directly affected the lives of living organisms, including humans, that reside in close proximity to these ASGMs'. Such activities have contributed immensely to environmental destruction [7] [8].

The co-existence of SCN^- and CN^-, as the major pollutants, can result in the contamination of the receiving water bodies, which in-turn reduces the availability of potable and usable water reserves [9]. Therefore, this necessitates the development of robust and environmentally benign processes that would reduce the CN^- and SCN^- concentrations to acceptable levels which are suitable for discharge, without hampering ecological settings. The development of such methods has been enforced by the International Cyanide

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Management Code (ICMC), a code which was established by the International Cyanide Management Institute (ICMI) (www.cyanide-code.org) [10]. These processes are differentiated into recovery and destruction based techniques. Recovery based techniques are mainly utilised to recover CN^- for reuse [11,12], while the destruction based techniques are mainly utilised to decompose cyanide and its complexes, including its degradation by-products, such that the wastewater can meet regulatory discharge standards [13,14]. The latter technique has been widely utilised in the mining industry. However, the currently utilised destruction techniques have contributed to environmental deterioration due to the production of hazardous by- or end-products. Biological destruction of CN^- and SCN^- in wastewaters has gained popularity due to the robustness, environmentally friendliness and economic viability of the process. In this process, microorganisms are employed to degrade cyanide compounds via a series of enzymatic reactions which have been widely documented [15] [16,17]. Industrial processes such as the ASTER^{TM} process and the operations at the Homestake and LaRonde gold mines in Canada [50], have demonstrated the feasibility and robustness of a biological process for treating cyanide-laden wastewaters. In most cases, the biodegradation process produces bicarbonate alkalinity which neutralises the acidity that is produced from the biodegradation process. These reactions are summarised from Eqs. (1)–(3) [18,19] .

$$CN^- + O_2 + 2H_2O \rightarrow HCO_3^- + NH_3 \quad (1)$$

$$SCN^- + 2O_2 + 3H_2O \rightarrow HCO_3^- + NH_4^+ + SO_4^{2-} + H^+ \quad (2)$$

$$HCO_3^- + H^+ \rightarrow H_2O + CO_2 \quad (3)$$

However, recent research has mainly focused on the utilisation of pure microbial cultures that are mainly operated in batch systems [20–22]. Such studies provide fundamental research as they seek to understand the fundamental roles that pure cultures play in the biodegradation process, with a purpose of constructing a high-strength microbial community that would effectively degrade these compounds. Numerous studies have focused on the biodegradation of CN^- and SCN^- separately and in batch cultures, with limited reports on the co-metabolism of these contaminants in continuous systems. Hence, the aim of this study was to evaluate the biodegradation of CN^- and SCN^- in a dual-stage continuously stirred tank bioreactor system operated in series, using microbial communities containing cyanide and thiocyanate degrading organisms.

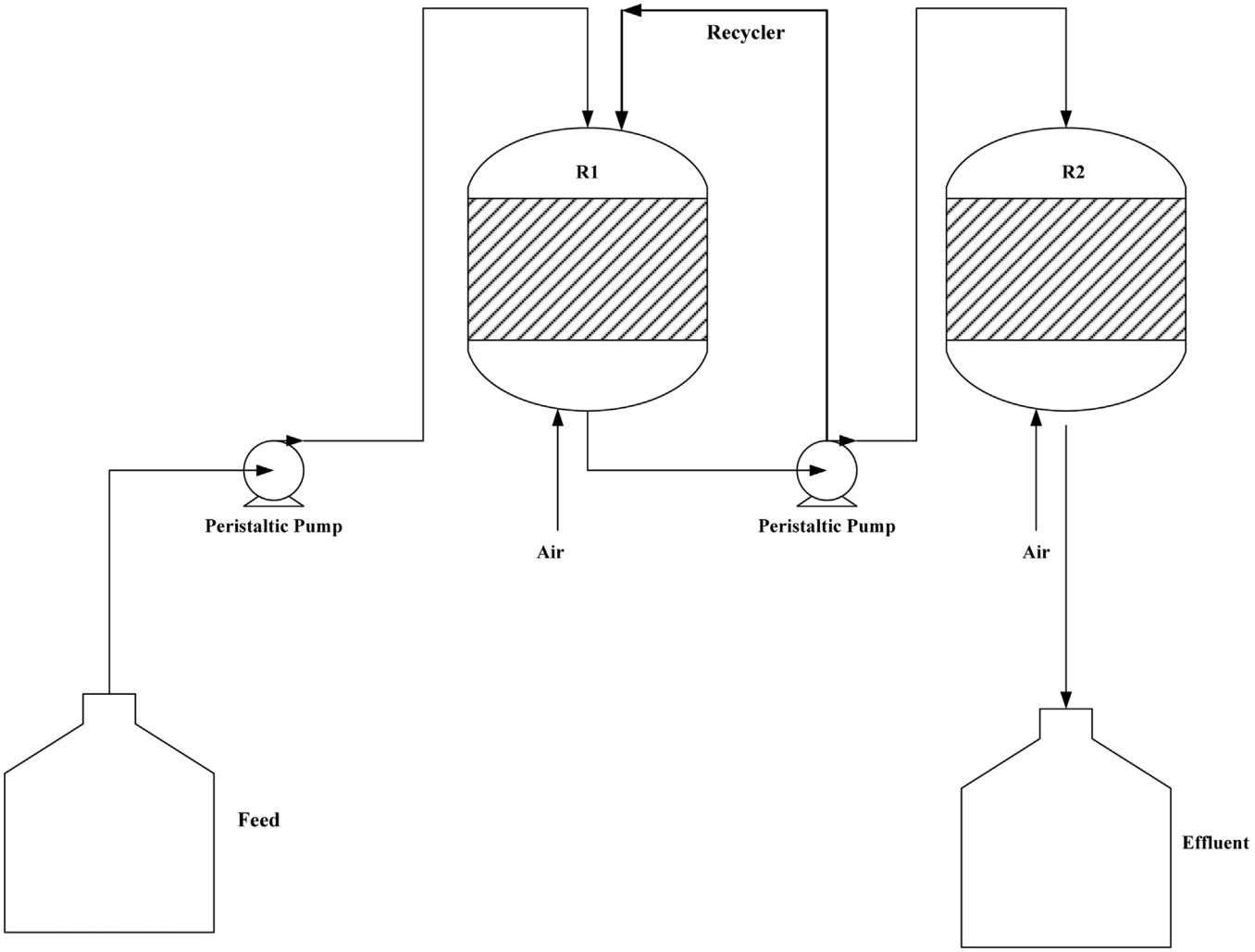

Fig. 1. A schematic demonstration of the dual stage continuous stirred tank reactor system.

2. Materials and methods

2.1. Microbial culture

Microorganisms were isolated by gravimetric sampling utilising synthetic wastewater solution in an Erlenmeyer flask (500 mL) containing (g/L); K_2HPO_4 (3.4), KH_2PO_4 (4.3), Glucose (0.01), SCN^- (0.2) and CN^- (0.2), at a pH of 10 (±0.05). The solution was exposed for two months to allow airborne microorganisms to settle in the media, outside the laboratory. The pH of the media was adjusted to alkaline conditions as most cyanide containing wastewaters are around the alkaline range [19]. After a two month period, the culture was transferred and maintained in a 1L stirred tank bioreactor, operated at a 48 h hydraulic retention time using the aforementioned media. Aeration was set at 400 mL/min and the reactor was operated at room temperature (21 to 25 °C). This enrichment culture served as an inoculum for the continuous biodegradation study. A volume (50 mL) of this culture was withdrawn from the bioreactor and the cells were harvested by centrifugation at 10 000*g* for 10 min at 4 °C followed by re-suspension in a phosphate buffer (pH 7.0). The total DNA was extracted directly from the recovered cells using a commercial DNA extraction kit (Promega, Madison,Wisconsin, USA), as per manufacturer's instructions. The presence of the genomic DNA was assessed using a 1% (w/v) molecular grade agarose gel containing 0.5 μg/mL ethidium bromide (EtBr), using 1X Tris-acetate-ethylenediamine tetraacetic acid (TAE) electrophoresis buffer at 100 V for 1 h.

The purified DNA was PCR amplified using the 16S rRNA forward bacterial primers 27F-16S-5′-AGAGTTTGATCMTGGCTCAG-'3 and reverse primers 518R-16S-5′-ATTACCGCGGCTGCTGG-'3 [23] that targeted the V1 and V3 regions of the 16S rRNA. The PCR amplicons were sent for sequencing at Inqaba Biotechnical Industries (Pretoria, South Africa), a commercial NGS service provider. Briefly, the PCR amplicons were gel purified, end repaired and illumina® specific adapter sequence were ligated to each amplicon. Following quantification, the samples were individually indexed, followed by a purification step. Amplicons were then sequenced using the illumina® MiSeq-2000, using a MiSeq V3 (600 cycle) kit. 20 Mb of the data (2 × 300 bp long paired end reads) were produced for each sample. The Basic Local Alignment Search Tool (BLAST)-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

2.2. Microbial community structure analysis using scanning electron microscopy (SEM)

Microbial samples were concentrated by centrifugation at 10 000*g* for 5 min at 4 °C and were fixed in 2.5% glutaraldehyde overnight at 4 °C. The glutaraldehyde solution was discarded and the fixed organisms were washed twice with phosphate buffer (pH 7.0), followed by microbial dehydration in an alcohol series, i.e. 50%, 70% and 100% (v/v) ethanol, for a period 12 h at 4 °C. The samples were dried using a hexamethyldisilazane (HMDS) solution and were visualised using a scanning electron microscope (Nova NanoSEM 230) after the samples (0.05–0.1 g wet sample) were mounted on a stub that was later coated with carbon.

2.3. Experimental set-up and procedures

The bioreactors, with a 1L working volume, were arranged in a dual-stage mode, using the New Brunswick BioFlo 110 reactors (New Brunswick Scientific Co., INC, New York, USA), which were operated in series (denoted as R1 and R2) (see Fig. 1). The effluent from R1 was recycled back to R1 relative to the set hydraulic retention time (HRT). Hence, R1 operated at half of the set HRT. Mixing was achieved using overhead stirrers driving Rushton impellers, for continuous mixing at 250 rpm. Air was introduced from the bottom of the reactors at a flow rate of 400 mL/min and the reactors were operated at room temperature (21–25 °C) with no temperature control, in an air-conditioned laboratory The feed solution contained CN^- (200–450 mg CN^-/L), SCN^- (100–1000 mg SCN^-/L), phosphate sources (as 3.4 g/L KH_2PO_4 and 4.3 g/L K_2HPO_4), glucose (0.1 g/L) and magnesium (as 0.6 g/L $MgCl_2.6H_2O$). The growth media was pumped into the reactor system using a peristaltic pump at different feed flow rates, depending on the desired hydraulic retention time. In this system, nutrient recycling to R1 was evident as demonstrated in Fig. 1.

During the start-up period, the initial pH of the feed solution was not controlled and it ranged from 6.9 to 7.1. The reactors were inoculated with a seed culture at a concentration of 10% (v/v) (equivalent to 100 mL of culture in 900 mL of media), in media containing 150 mg SCN^-/L only. The reactors were initially ran in batch mode until complete degradation of SCN^- (after 10 days) and thereafter, the system was allowed to stabilise for an additional 5 days to allow the consortium to utilise the produced total nitrogen prior to the bioreactors being operated in a continuous mode where the hydraulic residence time (HRT) (from 7 to 1 day), SCN^- concentration (100–1000 mg SCN^-/L) and CN^- concentrations (200–450 mg CN^-/L) were varied throughout the experimental run.

The system was switched to continuous mode on day 15 and operated at a 7 day HRT at a feed SCN^- concentration of 100 mg SCN^-/L. On day 82, the HRT was changed to 5 days while retaining a constant feed SCN^- concentration of 100 mg SCN^-/L. On day 119, the HRT was changed to 2.5 days and on day 137, the HRT was adjusted to 1 day; thereafter, this HRT was maintained throughout the rest of the experimental run. On day 156, the SCN^- concentration was adjusted to 150 mg SCN^-/L subsequent to SCN^- feed increment to 250 mg SCN^-/L on day 178. Prior to SCN^- increment to 500 mg SCN^-/L on day 189, the reactors were inoculated with *Pseudomonas aeruginosa* STK 03, *Exiguobacterium acetylicum* and *Bacillus marisflavi* at a concentration of 1%(v/v) from a previously grown culture at 30 °C in nitrogen-free MM described in [24]. These organisms were previously determined to be CN^- and SCN^- degraders in separate studies [24,25]. The reactors were temporarily operated in a batch mode over a 24 h period, to allow the organisms to acclimatize to the system and thereafter, the SCN^- concentration was increased to 500 mg SCN^-/L on day 189. These organisms were introduced to the system to counter the TN concentration build-up within the system and to aid in the removal of SCN^-, CN^- and TN. On day 204, the SCN^- feed concentration was further increased to 1000 mg SCN^-/L, which was maintained until the end of the experiment. On day 224, the pH of the feed solution was adjusted to 9.9 and CN^- was introduced at a concentration of 200 mg CN^-/L; a CN^- threshold tolerance for most microorganisms [26]. The CN^- concentration was further increased to 450 mg CN^-/L on day 261 and the reactors were operated under these conditions (450 mg CN^-/L, 1000 mg SCN^-/L and pH 9.9) until the end of the experiment (day 308). On day 280, microbial samples were withdrawn from R1 and R2 for SEM analysis as described in Section 2.2.

2.4. Microbial activity tests

Microbial activity tests were conducted on planktonic microbial species, when the operational parameters were changed. This was done to assess the activity of the planktonic organisms within the system when the operational parameters were changed. The media contained 3.4 g/L KH_2PO_4, 4.3 g/L K_2HPO_4, glucose (0.1 g/L), 0.6 g/L $MgCl_2{\cdot}6H_2O$ and 100 mg SCN-/L (for SCN^- degradation) and 100 mg CN^-/L (for CN^- degradation). For SCN^- activity tests, the

pH was adjusted to 7.0 (±0.05) while for CN^- activity tests, the pH was adjusted to 9.9 (±0.05).

2.5. Analytical methods

CN^- was determined using the barbituric acid-pyridine method [27], while ammonium was detected using the Berthelot reaction [28]. Sulphates were determined turbidimetrically using barium sulphate [29]. The nitrites were detected using the method developed by Rider and Mello [30] and SCN^- was detected using the ferric method [31]. Nitrate was determined using a Merck nitrate test kit (14773) (Merck, Germany). The combination of ammonium, nitrate and nitrite was referred to as total nitrogen (TN). The solution pH was adjusted using 1 M sodium hydroxide (NaOH) or 1 M sulphuric acid (H_2SO_4) and the pH was measured using a Crison Basic20 pH meter (Barcelona, Spain) which was calibrated daily using a three-point calibration method (pH 4.00, 7.00 and 10.00).

3. Results and discussion

3.1. Microbial culture characterisation and identification

Prior to the inoculation of the reactors, the inoculum was identified and characterised using the 16S rDNA amplicon gene sequencing approach. This culture-independent technique provides a direct and meaningful insights on the microbial composition of a consortium without prior culturing. This is advantageous as researchers are able to fully elucidate the microbial composition of a particular ecological habitat, as compared to the biased culture-dependent techniques which only focuses on the culturable organisms. This is disadvantageous since 1% of the total microbial composition that exists can be cultured on suitable growth media [32]; hence, the detected organisms would not truly represent the actual consortium composition within the reactor. The inoculum was mainly dominated by bacteria (67%) (Fig. 2a) which was majorly constituted by bacteria belonging to the Pretobacteria (23.95%) and unknown/non assigned (57.52%) phylum (Fig. 2b). In this study, non-assigned and/or unknown organisms constituted the majority of the organisms within the inoculum and this was also confirmed by the family profile (see Fig. 2c), and this was later attributed to the prevalence of uncultured bacteria (Fig. 2d). The dominant bacterial species which were detected in the inoculum was majorly constituted with *Thiobacillus* sp. and *Serratia* sp. *Thiobacillus* species have been largely documented as autotrophic SCN^- degrading organisms [31] while *Serratia* sp. have been observed to possess CN^- degradation capabilities [22,23], ensuring maximum degradation of both SCN^- and CN^-.

Amongst the 47.97% of the unclassified organisms, referred to as "other" in Fig. 2d, are organisms which belong to the *Microbacterium*, *Sphingomonas*, *Methylobacterium*, *Burkholderia*, *Pseudomonas*, *Raoultella*, *Klebsiella*, *Serratia*, *Acinetobacter*, *Cyanobacter*, *Bacillus* and *Corynebacterium* genus (see Table S1). The organisms belonging to these genus have been observed to possess CN^- and SCN^- biodegradation capacity [34]. *Microbacterium* sp. and *Sphingomonas* sp. were previously identified in the ASTER™ process as the dominant organisms for the biodegradation of SCN^- [35,36], while in separate studies, *Burkholderia* sp. have been determined to be effective SCN^- and CN^- degraders [37,38]. Furthermore, *Klebsiella* sp. were identified and characterised as having high SCN^- and CN^- biodegradation rates [39–41], including nitriles [42]. These organisms are of economic importance as they are able to produce methane from the biodegradation process [21]. Similarly, *Serratia* sp., *Bacillus* sp., *Pseudomonas* sp. and *Methylobacterium* sp. have been observed to be highly effective in the degradation of CN^- and SCN^- [22,43–45] and are also able to utilise the produced biodegradation end-products. Recently, Mpongwana

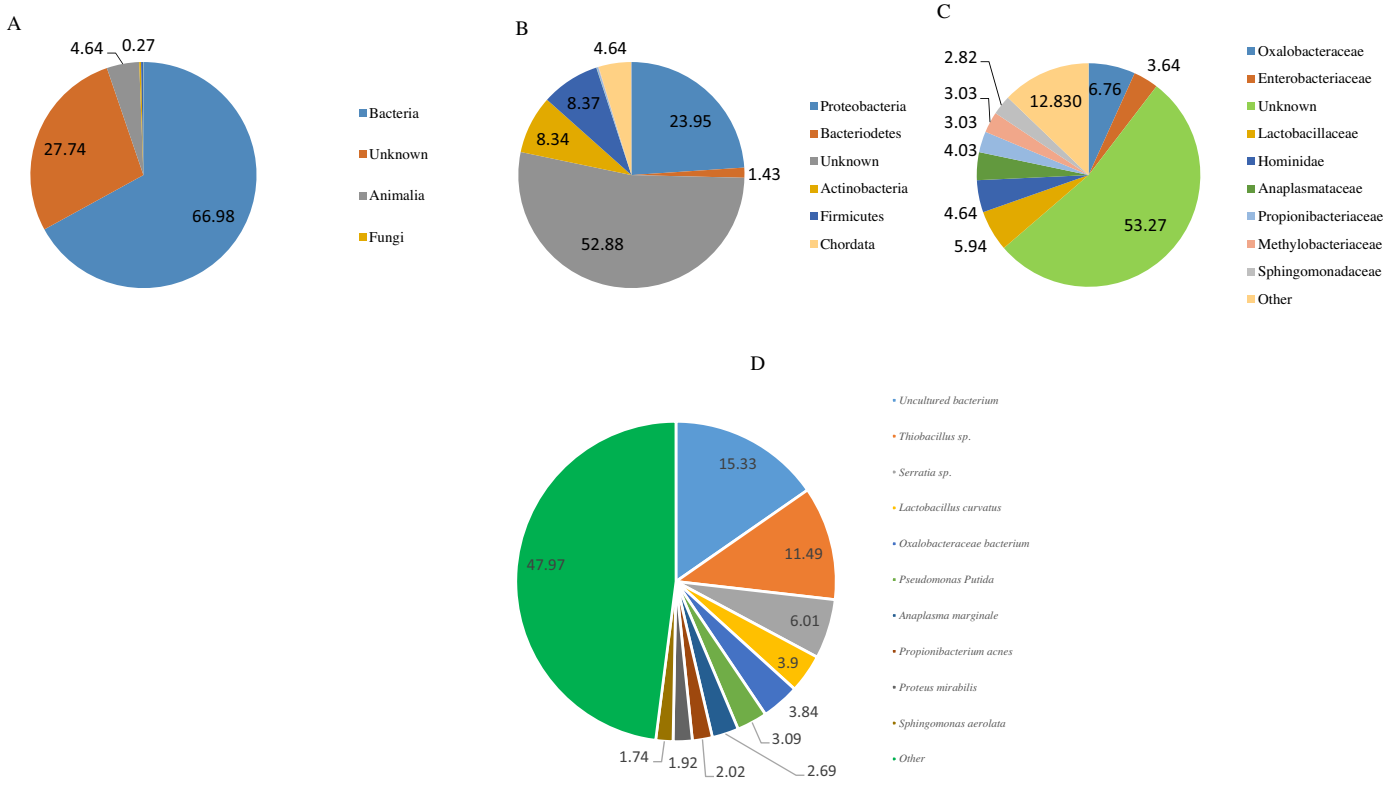

Fig. 2. A microbial characterization profile demonstrating the (a) taxonomy, (b) phylum, (c) family and (d) Genus of the inoculum.

et al. [33] observed that *Serratia* sp. were able to degrade CN^- and utilise the end products under cyanogenic conditions while Mekuto et al. [25] observed nitrification and aerobic denitrification proficiency of *Pseudomonas aeruginosa* STK 03. The presence of

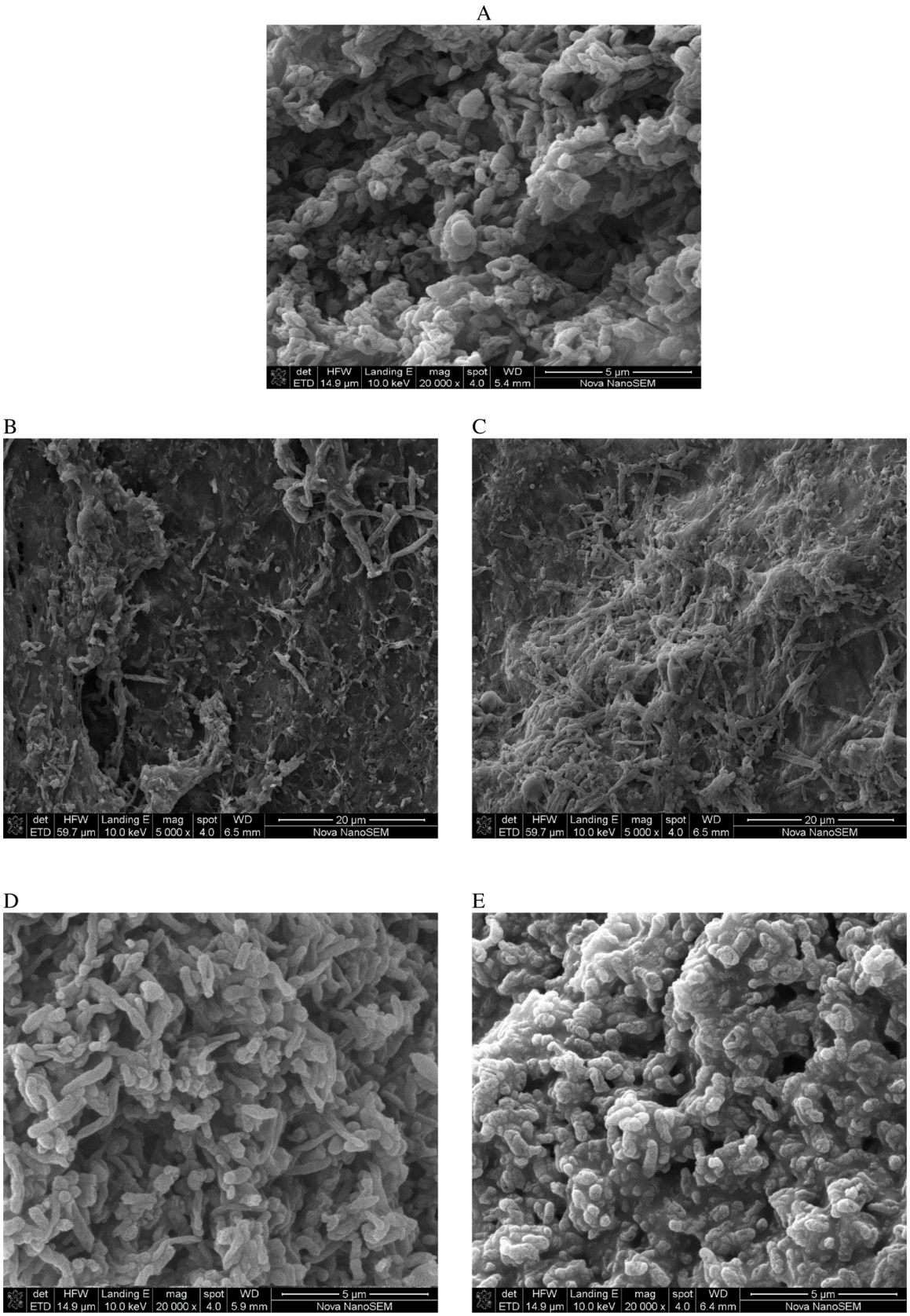

Fig. 3. Scanning electron microscopy images demonstrating microbial structure of the (a) inoculum, (b,c) microorganisms in reactor 1 at 52 and 273 days respectively, (c,d) microorganisms in reactor 2 at 52 and 273 days respectively.

these complex microorganisms reflect the high-strength nature of the consortium for effective biodegradation of CN^- and SCN^-.

3.2. Microbial community structure using SEM

The inoculum was majorly constituted of rod shaped organisms in combination with yeast-like organisms (Fig. 3a). On day 52, the community structure changed in R1 as this reactor was dominated by filamentous organisms and minimal rod shaped organisms (Fig. 3b), and on day 273, biofilm formation was evident which comprised of filamentous, rod-shaped and yeast-like organisms (Fig. 3c). This demonstrated microbial growth within the system as a result of nutrient uptake by the organisms. Similarly, the community structure in R2 on day 52 was mainly colonised by rod-shaped organisms (see Fig. 3d) which later transformed into a slimy-like structure (Fig. 3e), with observed rod-shaped organisms that were embedded within the slimy biofilm structure. Furthermore, the image also demonstrated the minute apertures within the structure of the biofilm, which provided channels for solute transport and uptake within the inner structure of the biofilm.

3.3. Performance of the bioreactor system

A dual staged bioreactor system was established and operated at room temperature (22 to 27 °C) for a period of approximately 300 days. During the start-up period, the concentration of SCN^- was set a 150 mg SCN^-/L and complete biodegradation of SCN^- was observed after 22 days with an initial pH range of 6.9–7.1 (Fig. 4). After the start-up period, it was evident that the organisms had acclimatized and this was verified by high degradation efficiencies (>90%), in both R1 and R2, when the overall HRT was set at 7 days. The residual SCN^- concentration was below 10 mg SCN^-/L. Similarly, the biodegradation efficiencies increased drastically when the HRT was decreased from 7 days to 2.5 days, with an observed degradation efficiency of >99.9% from day 82 to day 135. This was followed by a further decrease in residence time (to 1 day), where the high degradation efficiencies were maintained. Due to this, the concentration of SCN^- was increased to 150 mg SCN^-/L (day 156) and subsequently to 250 mg SCN^-/L (day 178) which was accompanied by high degradation efficiencies of >99.9%.

On day 189, prior to the SCN^- increment to 500 mg SCN^-/L, the reactors were inoculated with *Pseudomonas aeruginosa* STK 03 [25], *Exiguobacterium acetylicum* and *Bacillus marisflavi* [24]. These organisms were previously observed to biodegrade CN^- and SCN^- under alkaline conditions, and would hence, assist in accelerated biodetoxification of these contaminants. During this period, biofilm development was noticed on the walls, impellers and stainless steel piping system of the reactors. Furthermore, the growth of these organisms resulted in the blockage of the air sparging system, thus resulting in microaerobic conditions. The introduction of these organisms to the bioreactor system further bolstered and ensured satisfactory degradation efficiencies, resulting in the degradation efficiencies exceeding 99%. When the SCN^- concentration was increased to 1000 mg SCN^-/L on day 204, there was an observed inhibition on microbial activity. This was confirmed by the microbial activity tests where the biodegradation efficiency was below 90% (Fig. 5). The activity was later regained after 18 days and this was followed by a deliberate pH increase to 9.9 and the introduction of CN^- at a concentration of 200 mg CN^-/L on day 224. This proved to be detrimental to the performance of the organisms as the residual SCN^- concentration averaged 280 (R1), 155 (R2) and 50 mg SCN^-/L in the effluent respectively, from day 224 to day 240. However, after this period, the complete degradation of SCN^- was observed, achieving an overall SCN^- degradation rate >180 mg SCN^- L^{-1} h^{-1} which was above the 80 mgL^{-1} h^{-1} reported by Van Zyl et al. [46]. During the stress period, complete degradation of CN^- was observed (Fig. 6), and this demonstrated the adequacy of the biofilm for effective degradation of both CN^- and SCN^-. This was denoted from the regained high degradation efficiencies observed (>99.9%), which were maintained until the end of the experimental run. The maximum CN^- degradation rate was achieved at 450 mg CN^-/L, where an overall biodegradation rate exceeded 17 mg CN^- L^{-1} h^{-1} whereas >7 mg CN^- L^{-1} h^{-1} was achieved at 200 mg CN^-/L, exceeding the 0.5 mg CN^- L^{-1} h^{-1} achieved by White et al. [47].

Although high degradation efficiencies were achieved on this study, with the wastewater meeting the regulatory discharge limits, there were days where the wastewater did not meet the regulatory discharge limits. Following the introduction of CN^- to the system, effluent CN^- concentration ranged from 0.34 to 45 mg CN^-/L from day 228 to day 237. Similarly, the CN^- concentration ranged from 0.30 to 12.00 mg CN^-/L between day 261 to day 189, which did not meet the regulatory discharge limits of 0.007 and 0.01 mg CN^-/L as enforced by the World Health Organization and the South African Water Affairs Department [48,49]. A pre-denitrification stage, as suggested later on, would allow for the complete degradation of CN^- to ensure removal of residual CN^- in the wastewater.

The biodegradation of CN^- and SCN^- results in the production of nitrogenous compounds in the form of ammonium (from CN^-

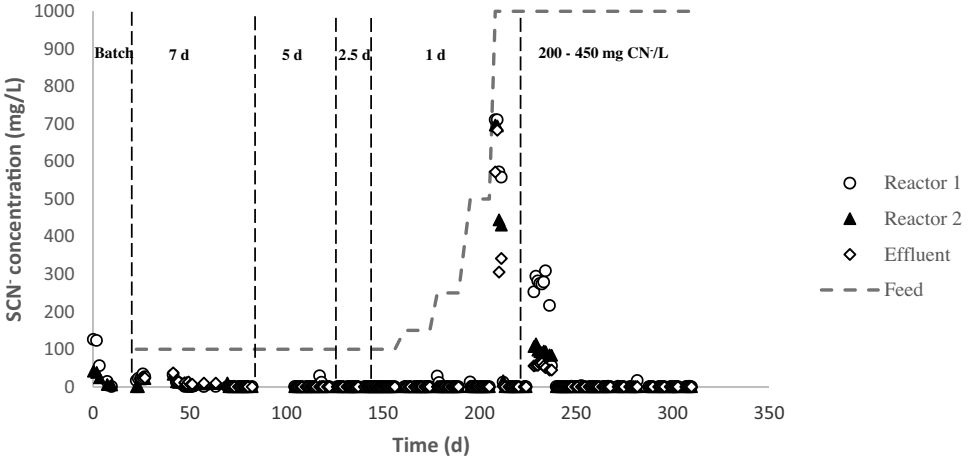

Fig. 4. Residual thiocyanate and free cyanide concentrations as a function of feed concentration and time.

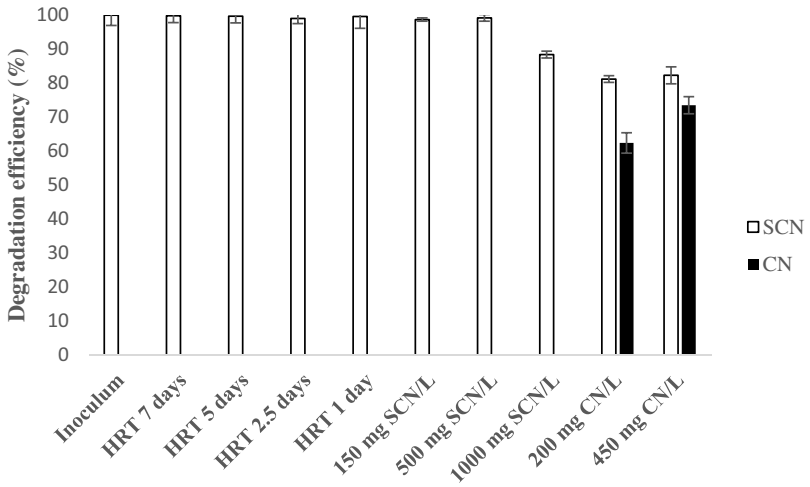

Fig. 5. Graphical profile of microbial activity tests.

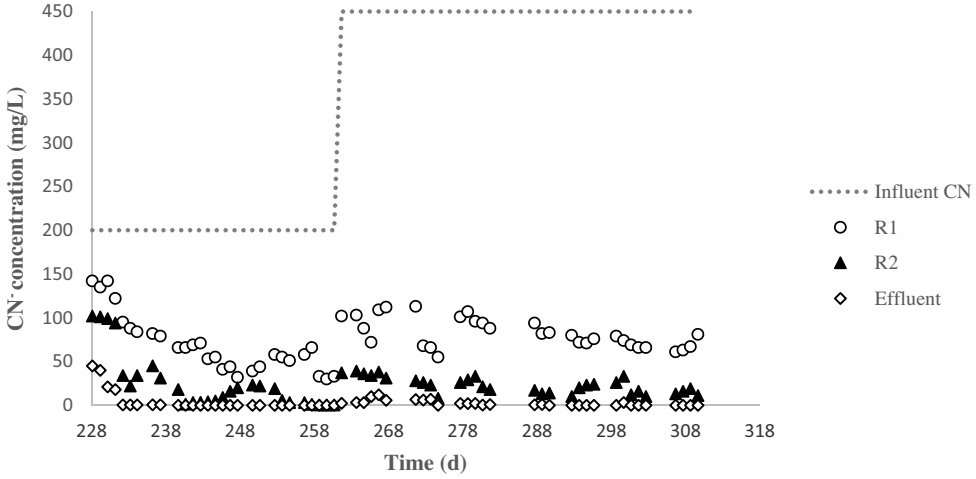

Fig. 6. Residual free cyanide concentration as a function of feed concentration and time.

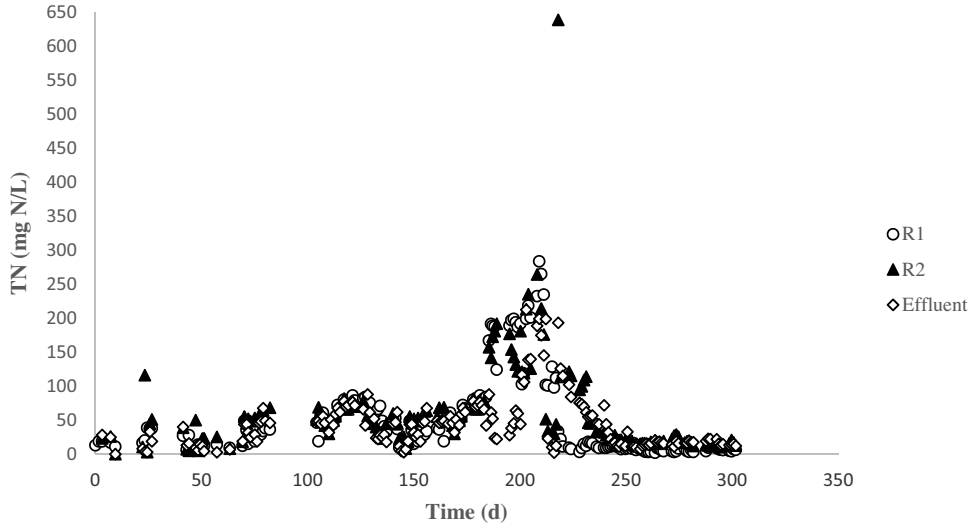

Fig. 7. Total nitrogen profile produced from thiocyanate and free cyanide biodegradation.

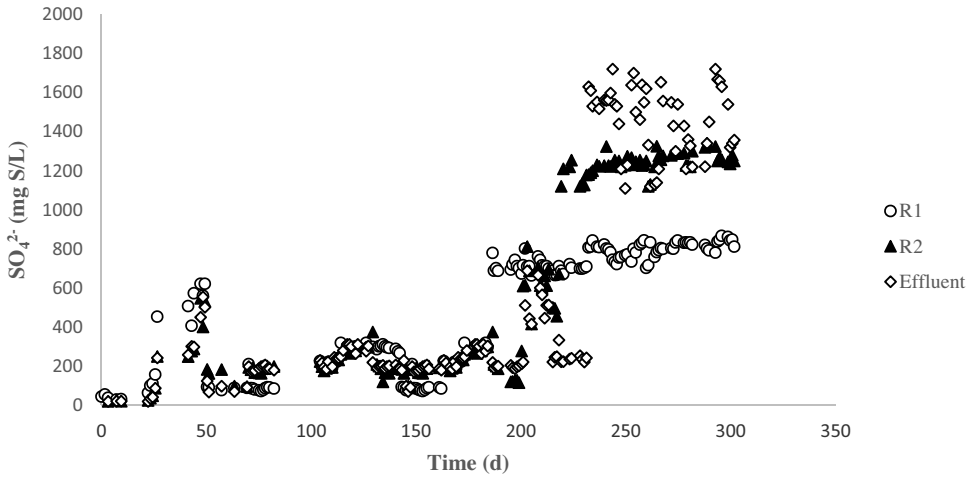

Fig. 8. Sulphate concentration profile as a function of time.

and SCN^- degradation), nitrites and nitrates (from ammonium metabolism), as demonstrated in Eqs. (1) and (2). The combination of these nitrogenous compounds was denoted as total nitrogen (TN). The biodegradation of SCN^- from day 0 to day 179 resulted in the fluctuation of TN which was below 100 mg N/L (Fig. 7). However, from day 188 to day 224, there was an observed increase and accumulation of TN within the reactor systems, suggesting reduced temporal microbial activity due to the increase in SCN^- concentration. In addition, the simultaneous increase in pH and the introduction of CN^- within the system stressed the organisms, thus leading to reduced microbial activity and reduced TN removal. Conversely, after the stress period, the organisms were able to remove TN, with an average effluent concentration of approximately 17 mg N/L. This suggested that the organisms were able to conduct heterotrophic nitrification and aerobic denitrification under cyanide and thiocyanate laden conditions. This phenomenon was observed by Mpongwana et al. [33], where the *Serratia* sp., *Yersinia* sp. and *Enterobacter* sp. were able to nitrify and aerobically denitrify under cyanogenic conditions. In addition, Razanamahandry et al. [7] observed nitrification potential of organisms that were able to degrade CN^-, while Mekuto et al. [43] observed simultaneous nitrification and aerobic denitrification in a continuous CN^- biodegradation system. These studies demonstrated the ability of SCN^- and CN^- degrading organisms to conduct heterotrophic nitrification and aerobic denitrification. However, complete TN removal is desired and can be achieved by incorporating an additional TN removal bioreactor and/or by introducing a pre-aerobic denitrification stage. In a separate study, the incorporation of a pre-denitrification stage resulted in the optimised TN removal from SCN^- biodegradation system; an operational strategy that can be taken into account for reactor configuration directed at industrial application [50].

As illustrated in Eq. (2), the biodegradation of SCN^- results in the production of sulphates. The concentrations of the produced sulphates, which were monitored throughout the experimental run (see Fig. 8), increased with an increase in SCN^- loading as a result of the biodegradation of SCN^-. The maximum sulphate concentration was observed when the SCN^- loading was increased to 1000 mg SCN^-/L which averaged approximately 1600 mg SO_4^{2-}-S/L. In a separate study, the production of sulphates was indirectly correlated with microbial growth and SCN^- biodegradation efficiencies [51] submitted). The authors suggested utilising the production of sulphates from SCN^- biodegradation systems as an indirect technique for microbial proliferation and colonisation since most SCN^- degraders are unable to utilise sulphates as a source of sulphur. Recent studies have demonstrated the efficacy of the sulphate-reducing ammonium oxidation (SRAO) processes on the reduction of sulphates in nitrogen removal processes [52,53],

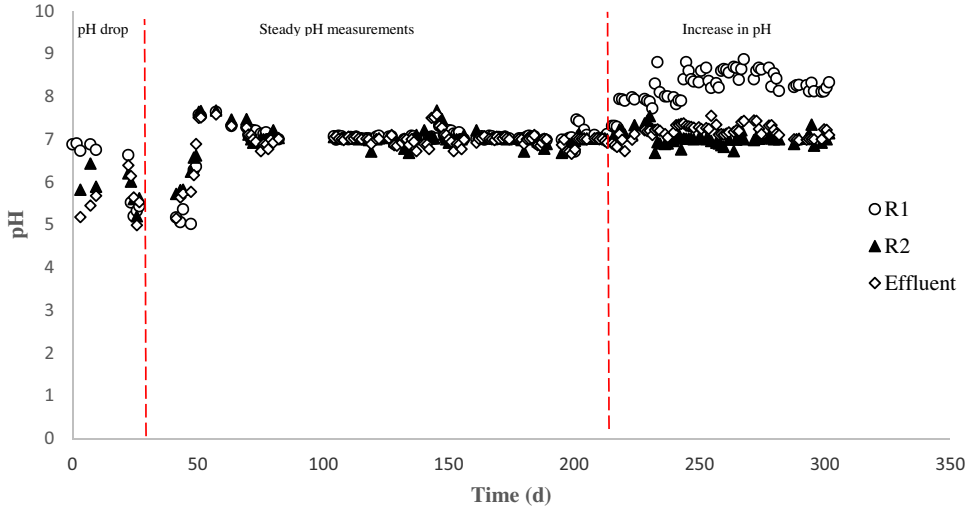

Fig. 9. Graphical representation of pH as a function of time.

and such a process should be considered as an alternative technique for the reduction of the produced sulphates such that the effluent is free of contaminants.

The differences in pH were assessed during the experimental run (Fig. 9). During the start-up period, the pH decreased sharply to an average of 5 across the bioreactors. The pH decrease was due to the acidity produced by the biodegradation process which might have superseded the bicarbonate alkalinity and/or the autotrophic organisms within the reactors rapidly utilised the bicarbonate as an alternative carbon source as seen in a study by Hung and Pavlostathis [54]. However, the pH increased when the system was switched into a continuous mode and the pH plateaued along neutrality after day 76. The pH was increased after day 224–9.9 but the measured pH in R1, R2 and the effluent averaged at 8.6, 7.1 and 7.0, respectively. Similarly, this observation was attributed to the acidity generated from the biodegradation of SCN^- which led to a pH decrease.

4. Conclusion

It was evident from this study that SCN^- and CN^- loading did not have a dire impact on the performance of the microbial communities that were present within the bioreactor system. Furthermore, changes in residence time had minimal impact on process performance as the organisms maintained an overall biodegradation efficiency of >99.9%, irrespective of the SCN^- and CN^- loading, a demonstration of microbial adaptability. However, the simultaneous changes in pH and the introduction of CN^- had a minimal effect on the performance of the system, with an observed recuperation of process performance thereafter. This was confirmed through planktonic microbial activity tests which revealed SCN^- and CN^- biodegradation efficiencies. Furthermore, the organisms were able to utilise the biodegradation by- and end-products, i.e. ammonium, nitrates and nitrites; an indication of nitrification and denitrification potential of the active microorganisms within the system. However, it is recommended that a pre-aerobic denitrification configuration be included as part of the reactor configuration for optimised TN removal using the consortium that is responsible for SCN^- and CN^- biodegradation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jece.2017.03.038.

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