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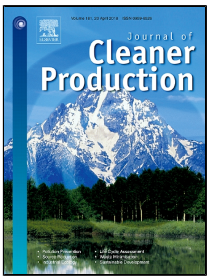

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Influences of pH and organic carbon on oxalate removal by alkaliphilic biofilms acclimatized to nitrogen-deficient and supplemented conditions

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Abstract

Accumulation of organic impurities (specifically oxalate) in Bayer liquor is a significant constrain to refine alumina. Microbial oxidation is a low-cost approach to remove organics, but hostile conditions of Bayer liquor (pH > 14 and nitrogen (N) deficiency) makes it challenging. The solution remains with selection of an appropriate haloalkaliphilic culture and alumina refineries currently have access to two types of bacterial cultures (N-supplemented and N-deficient cultures). To date there is no comparative assessment of the two cultures to examine which one is more suitable to reduce operational risks (i.e. with higher removal efficiencies over a broader range of pH) and costs.

Hence, this study compared oxalate removal efficiencies of two packed bed biofilm reactors (N-supplemented and N-deficient) on exposure to a range of influent pH and simple organic compounds. Both reactors were operated (>265 days) at pH 9 and pH influence was compared in batch experiments. Results suggested that both biofilms could tolerate a broad pH range (7-10). The optimal specific oxalate removal rate of N-supplemented reactor was restricted to pH 9, whereas the maximal rate was maintained over a wider pH range (7 - 8) in N-deficient reactor. In this range, the N-deficient system outperformed the N-supplemented system (105 vs. 130 mg-oxalate /h.g-biomass). Although acclimatized primarily with oxalate, both biofilms simultaneously oxidized other organics (acetate, formate, malonate and succinate) without a noticeable influence on oxalate removal. This study suggests that N-deficient systems are more versatile and better suited to remove organic impurities in Bayer liquor.

Key words: bioreactor, pH, nitrogen fixation, oxalate, organics

1. Introduction

Bayer process is used to refine alumina (Al_2O_3) from aluminium (Al) bearing bauxite minerals. In the Bayer process, crushed bauxite is digested in a hot concentrated solution of sodium hydroxide (NaOH) to produce sodium aluminate ($NaAlO_2$). To recover aluminium trihydroxide ($Al(OH)_3$), the process liquor is cooled down and an $Al(OH)_3$ crystallisation seed is introduced to trigger crystallisation of $Al(OH)_3$. On separation, the spent NaOH is recycled to the process (Meyers, 2004; Whelan et al., 2003). In addition to Al, the bauxite minerals also contain organic impurities and due to continuous re-cycling, these impurities accumulate in process liquor to concentrations that hinder Al recovery. The organics present in bauxite range from very complex high molecular weight humic substances to simple organic acids (Power et al., 2012; Whelan et al., 2003). Of these organic compounds, sodium oxalate ($Na_2C_2O_4$) is the most harmful impurity as it reduces process efficiency and product quality due to its co-precipitation with $Al(OH)_3$.

Some alumina refineries have used biological processes to remove oxalate from Bayer liquor (McKinnon and Baker, 2012). However, high pH (>14) and nitrogen (N) deficiencies of Bayer liquor, makes it a stream to treat biologically, and hence, uptake of the technology is limited possibly due to fears of operational failures. Initial studies that examined biological removal of oxalate, neutralised Bayer liquor to create a suitable environment for oxalate degrading microorganisms (Brassinga et al., 1989). To avoid pH neutralisation, Worsley Alumina Pty Ltd for the first time examined the use of alkaliphilic bacteria to remove oxalate (Morton et al., 1991). A typical alkaliphilic bacterium, has a pH tolerance range of 8.5 – 11 and the use of an alkaliphilic culture (Horikoshi, 1999), therefore helped better manage operational risks and costs of biological oxalate removal.

Although alkaliphilic bacteria have a broader tolerance of pH, their optimum biological activities remain in a much narrow pH range (Banciu et al., 2008; Sorokin et al., 2001). This partially may be

a result of ammonia toxicity arising with a higher prevalence of unionised NH_3 at higher pH values (Sousa et al., 2015). Compared to NH_4^+ ions, free NH_3, readily diffuses through cell membranes and disrupts the proton balance inside cells, interrupting cellular metabolic processes (toxic) (Sousa et al., 2015). At a given alkaline pH, the free NH_3 concentration is depended on the total ammonia nitrogen (TAN) concentration and a low TAN concentration results in a low free NH_3 concentration (pK_a = 9.25), which is less toxic to microorganisms. However, free NH_3 toxicity and tolerance vary between different bacterial inocula (Wang et al., 2016), and our knowledge around pH and removal rates of oxalate by different cultures is incomplete and this has prevented alumina refineries from having better control of their biological treatment processes.

N is a major essential element that is required for bacterial growth. The issue related to N deficiencies of Bayer liquor is typically circumvented by external supplementation of N (e.g. NH_3). Loss of N from Bayer liquor due to volatilisation of NH_3 at high pH is an operational challenge for refineries and the current strategy is to maintain an excess concentration, with an overdose of N. As previously mentioned, higher TAN concentrations result in higher NH_3 toxicities, which in turn affects oxalate removal rates of bacteria. On the other hand, a bacterial inoculum able to fix atmospheric nitrogen would negate the need to externally supplement N and thus could minimise operational risks and costs. Haloalkaliphilic diazotrophs and their ability to facilitate removal of oxalate from Bayer liquor was demonstrated for the first time by (Weerasinghe Mohottige et al., 2017) and this has provided the alumina industry a choice of bacterial cultures to facilitate a costs effective, low risk biological process to remove oxalate.

A typical alumina refinery produces >1000 m^3 Bayer liquor /h (Chen et al., 2007). If a biological process is to maintain a low concentration of oxalate in Bayer liquor, an efficient side stream biological process is needed. In order to facilitate an efficient side stream process, a well-characterised (i.e. physicochemical and kinetically) bacterial culture is needed. Although current

alumina refineries have access to two main types of cultures (i.e. an N-supplemented and an N-deficient inoculum) to date no effort has gone towards a comparative assessment to understand which culture would be best suited to reduce operational risks and costs. Considering the very high pH of Bayer liquor (> 14) there is an operational need to determine pH range where optimum removal of oxalate takes place. Further, in addition to oxalate, there is accumulation of other simple organic impurities such as sodium acetate, sodium formate, sodium succinate and sodium malonate in Bayer liquor (McKinnon and Baker, 2012; Tilbury, 2003) and implications of these organic compounds on the removal of oxalate is also currently unexplored.

Therefore, in this study we compared oxalate removal efficiencies of two packed bed biofilm reactors (one supplemented and the other not supplemented with N) by exposing the reactors to a range of influent pH values (7 to 12) and simple organic compounds. The two reactors were operated aerobically at pH 9 for a period of 275 days using a synthetic medium that mimicked (i.e. in terms of salinity and alkalinity) the Bayer process liquor. This study offers new insight into pros and cons of using each of the bacterial cultures to remove oxalate in Bayer liquor.

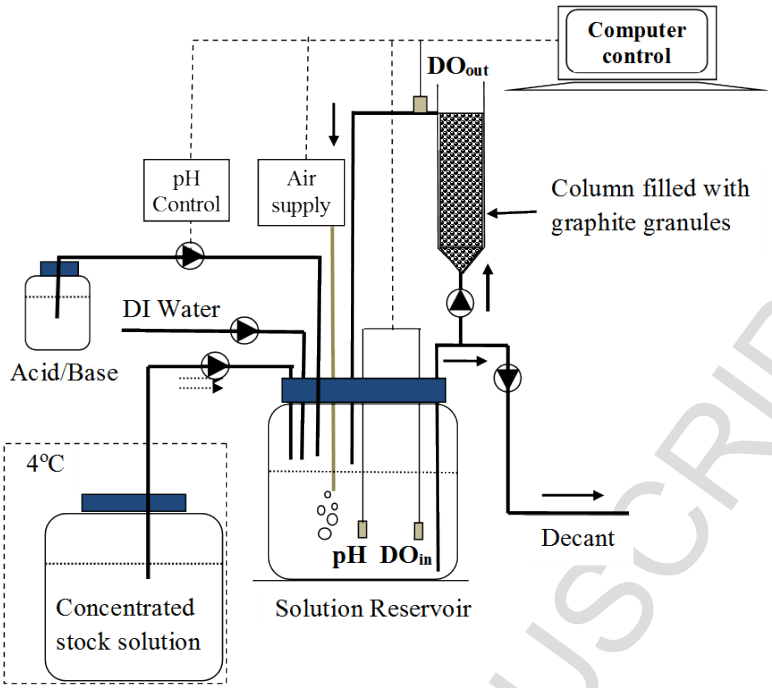
2. Materials and methods

2.1 Aerobic bioreactor system

Experiments were carried out using two identical laboratory-scale aerobic bioreactors as described in Weerasinghe Mohottige et al. (2017). Each reactor was composed of a packed bed glass column and a recirculation bottle (Fig. 1). The internal diameter and height of the glass column was 55 mm and 400 mm, respectively and total available bed volume of the reactor was 650 ml. Each column was packed with 480 g of air dried graphite granules (3-5 mm diameter, KAIYU Industrial (HK) Ltd) that made up a bed volume of 600 ml. Once packed in the column, there was a 210 ml void volume in the granular column. The 2 L glass recirculation bottle served as a solution reservoir to enable

129 recirculation of liquid through the granular column. The two reactors were operated in sequencing-
130 batch mode with a cycle length of 4 h. During the first 2 mins of the cycle, 10 times concentrated
131 solutions of carbon and nutrient was pumped into the recirculation bottle together with deionised
132 water to make up a 1.3 L volume of fresh influent. The solution in the recirculation bottle was
133 continuously aerated with a sparger over the entire period of reactor cycle to maintain a near
134 saturation level of dissolved oxygen (DO) in the liquid. The aerated liquor (i.e. a total working
135 volume of approximately 1.5 L) was continuously recirculated through the packed bed column in an
136 upward-flow direction at a flow rate of 9.6 L/h. At the end of each cycle, the liquid (~1.3 L) in the 2
137 L bottle was decanted to replenish with new solution, while the packed bed remained submerged
138 (~210 ml).

139 Data acquisition and control hardware (CompactRio National Instruments, USA) and software
140 (Labview, National Instrument, USA) were used for continuous monitoring and control of the
141 reactors. Online monitoring of DO and pH were carried out using a luminescent DO probe (PDO2,
142 Barben Analyzer Technology, USA) and an intermediate junction pH probe (Ionode IJ44, Ionode Pty
143 Ltd, Australia), respectively. Two DO probes were used to measure the DO concentrations at the inlet
144 and outlet of the packed column, with one immersed in the synthetic solution in the 2 L bottle and
145 the other placed at the outlet of the column reactor (Fig. 1). All experiments were carried out at room
146 temperature (~ 23˚C).



147

148 **Fig. 1.** A schematic diagram of the aerobic packed bed bioreactor set-up. Adapted by Weerasinghe
149 Mohottige et al. (2017).

150 *2.2 Influent for N-supplemented and N-deficient reactors*

151 A highly saline and alkaline synthetic medium was used as the influent to simulate the salinity and
152 alkalinity of Bayer process liquor of the alumina industry. The working solution contained 2.0 g/L
153 $Na_2C_2O_4$ and 25 g/L NaCl. The pH of the working solution was 9.0 -9.5and was adjusted using 2 M
154 NaOH. Additionally, the working solution also contained a nutrient medium.

155 The nutrient medium was consisted of (per L): 50 mg NH_4Cl, 125 mg $NaHCO_3$, 51 mg $MgSO_4 \cdot 7H_2O$,
156 15 mg $CaCl_2 \cdot 2H_2O$, 20.5 mg $K_2HPO_4 \cdot 3H_2O$, and 1.25 ml of trace element solution. The trace element
157 solution contained (Per L): 0.43 g $ZnSO_4 \cdot 7H_2O$, 5 g $FeSO_4 \cdot 7H_2O$, 0.24 g $CoCl_2 \cdot 6H_2O$, 0.99 g
158 $MnCl_2 \cdot 4H_2O$, 0.25 g $CuSO_4 \cdot 5H_2O$, 0.22 g $NaMoO_4 \cdot 2H_2O$, 0.19 g $NiCl_2 \cdot 6H_2O$, 0.21 g
159 $NaSeO_4 \cdot 10H_2O$, 15 g ethylenediaminetetraacetic acid (EDTA), 0.014 g H_3BO_3, and 0.05 g
160 $NaWO_4 \cdot 2H_2O$.

Compared to the N-supplemented reactor, the working solution of the N-deficient reactor contained a lower concentration of $Na_2C_2O_4$ (0.8 g/L). NH_4Cl was removed from the nutrient medium of N-deficient reactor and replaced with 10 mg/L yeast extract. Yeast extract is commonly used to supplement essential nutrients when culturing nitrogen fixing bacteria (Gauthier et al., 2000; Sorokin et al., 2008).

2.3 Reactor start up

The inoculum for the reactors was sourced from two locations. Since the Bayer liquor is a saline solution (typical NaCl concentration of 0.43 M NaCl) (Hind et al., 1997) and coastal sediments are also known to contain oxalic acid, a sediment sample was collected from a local beach (Floreat beach, Western Australia) for microbial enrichment. Another two samples were collected from a local wetland reserve (Perry Lake reserve, Western Australia). Rhizosphere microorganisms are likely exposed to oxalic acid due to the accumulation of oxalic acid by most plant cells as a metabolic end product. Also, Western Australian soils are highly deficient in N and as such diazotrophs are also likely to dominate the rhizosphere (Bahadur and Tripathi, 1976). Hence, Perry Lake samples were obtained from the vicinity of the rhizosphere of native plants. Equal weights from all three different inocula samples (total weight of 225 g each) were combined and suspended in two separate 2 L glass bottles, which contained 1.5 L of N-supplemented and N-deficient feed solutions, respectively. Initially, the $Na_2C_2O_4$ concentrations in the working solutions were maintained low (100 mg/L) to prevent substrate inhibition of microorganisms. The two vessels were aerated at room temperature for 3 weeks to enrich aerobic oxalate degrading microorganisms. During enrichment, aeration was regularly interrupted for a period of 1 h once per week to allow the contents of both bottles to settle. Subsequently, the supernatant liquid (1 L) was discarded and replenished with respective fresh feed solutions.

After 3 weeks of enrichment, the sediments in both vessels were vigorously disturbed by shaking to dislodge microbial cells from sediment material. Subsequently, the two packed bed column reactors

were connected to the vessels as illustrated in Fig. 1 and the reactors were operated in batch mode. The liquid containing the dislodged biomass was re-circulated through the respective reactors to facilitate colonisation and growth of a N-supplemented and a N-deficient biofilm on the carriers. The reactors were operated for another 2-3 d with the sediments in the recirculation bottle. Thereafter, all sediment was completely removed from each of the recirculation bottles. Biofilm enrichment in the column reactors was allowed to continue with weekly replenishment of feed solutions (1.3 L) in the 2 L recirculation bottles.

During enrichment, oxalate removal in the reactors was monitored every two weeks. Depending on the removal of oxalate, the cycle length of the reactors was decreased gradually to 4 h. After the 4 h cycle length was achieved, the oxalate concentration in the feed was increased to coincide with increasing oxalate removal rates. The increase of oxalate loading rate was terminated when no further increase in the rate of oxalate removal was observed. During stable operation, the final $Na_2C_2O_4$ concentrations in the feed of N-deficient and N-supplemented reactors were 0.8 g/l and 2.0 g/l, respectively.

2.4 Cyclic studies

After 21 days of microbial inoculation, cyclic studies were carried out once every two weeks during the enrichment period to determine the oxalate removal rate of the bioreactors. During steady state of operation, the frequency of sampling was varied based on specific experiments. During routine cyclic studies, hourly sampling was carried out (over the 4 h cycle). During sampling, 3 ml volumes were withdrawn from the 2 L re-cycle bottles and immediately filtered through 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia) and the filtrates were collected in a 2 ml Eppendorf tube. The samples were immediately stored at 4°C until analyzed.

2.5 Effect of in-reactor pH on oxalate degradation rate

Once the routine cyclic studies revealed stable reactor performance, the impact of in-reactor pH fluctuations on oxalate removal rate was examined. The normal operation of the reactors was at a pH range of 9 – 9.5. The influence of different in-reactor pH values on oxalate removal rate was examined in a pH range of 7.0 to 12. pH values below 7 were not tested due to practical relevance. During the experiment, in-reactor pH set point was feedback-maintained using the programmable logic controller to dose either acid (1 M HCl) or base (1M NaOH). The biofilm was exposed to each of the in-reactor pH values over a single reactor cycle of 4 h. In the subsequent 5 cycles, the biofilm was exposed to normal operational conditions (i.e. exposed to a pH of 9 – 9.5) in order to prevent acclimatisation of biofilm to a pH value other than pH 9 – 9.5. The influent DO during this experiment was maintained at 8 mg/L. The $Na_2C_2O_4$ concentration introduced at the beginning of the cycle was 2 g/L and 0.8 g/L for N-supplemented and N-deficient reactors, respectively. During the experiment, hourly liquid samples were collected and immediately filtered through 0.22 µm syringe filters (Cat No SLGNO33NK, Merck Millipore, USA) for oxalate measurement.

2.6 Removal of other organics and their influence on oxalate removal

Bayer liquor contains other simple organic compounds such as sodium acetate, sodium formate, sodium succinate and sodium malonate (McKinnon and Baker, 2012). In this experiment, the influence of these organics on the removal of oxalate and their individual removal efficiencies were examined in both the N-supplemented and the N-deficient reactors. The effects of sodium acetate, sodium formate, sodium succinate and sodium malonate for each reactor were independently examined in 4 separate batch experiments. In each of the batch experiments, the additional carbon source was included to create an initial in-reactor chemical oxygen demand (COD) molar ratio of approximately 1:1 between the additional carbon source and the oxalate (Table 1). During these experiments, the influent pH (9.5) and DO (8 mg/L) were maintained at a constant. The removal of carbon sources was monitored over a 4 h cycle length by collecting hourly liquid samples (3 ml). On collection, the samples were immediately filtered through 0.22 µm syringe filters (Cat No

234 SLGNO33NK, Merck Millipore, USA) to enable quantitation of residual carbon. In between
235 exposure to each carbon source, the biofilm was allowed to recover under normal operational
236 conditions (i.e. exposed to oxalate only) over five consecutive cycles.

237 Table 1. Concentrations of organic mixtures used in batch experiments

	Influent Concentration (mM)				
	Oxalate	Acetate	Formate	Malonate	Succinate
Control	12	0	0	0	0
C-Mix-1	6	1.5	0	0	0
C-Mix-2	5.5	0	3.5	0	0
C-Mix-3	6	0	0	1.5	0
C-Mix-4	6	0	0	0	1

238

239 *2.7 Assessment of N-deficient conditions*

240 To confirm the N-deficient and N-supplemented conditions in the two reactors, the following
241 assessments were carried out.

242 *2.7.1 In-reactor ammonia-N, nitrite-N and nitrate-N concentrations*

243 Ammonia-N, nitrite-N and nitrate-N concentrations in both N-deficient and N-supplemented reactors
244 were monitored with frequent analysis of in-reactor liquid samples. Approximately 2 ml volumes of
245 liquid samples were withdrawn and immediately filtered through 0.22 µm syringe filters (Cat No
246 SLGNO33NK, Merck Millipore, USA) and the filtrates were analyzed for residual ammonia, nitrite
247 and nitrate using ion chromatography.

248 *2.7.2 Acetylene reduction assay*

249 The acetylene reduction assay was based on a method proposed by Sprent (1990). After 250 days of
250 operation, two media samples were withdrawn from each N-deficient (16 ml) and N-supplemented
251 (20 ml) reactors and were placed in four 150 ml bottles. Thereafter, 50 ml of N-deficient and N-
252 supplemented growth media (pH 9) containing 2 g/L of oxalate were introduced into the respective

bottles. The bottles were subsequently capped (using butyl rubber stoppers), crimped (with an aluminium seal) and flushed with helium for 3 min to remove any N_2 from the samples. Subsequently 2 % of the headspace helium was removed and replaced with pure oxygen in one N-deficient and one N-supplemented bottle. In the remaining two bottles, the headspace was completely flushed with pure oxygen. Subsequently, 2 % of the headspace volume of all four bottles was replaced with acetylene (produced by reacting DI water with calcium carbide ~ 1 g in a 1 L conical flask and the acetylene captured with displacement of water in a column). The sample was then incubated in an environmental shaker at 28°C. Liquid and headspace gas sampling were carried out on all 4 bottles at time intervals of 3, 16, 24, 40 and 48 h. Once collected, the liquid samples were immediately filtered through 0.22 µm syringe filters (Cat No SLGNO33NK, Merck Millipore, USA) and the filtrates were analyzed for residual oxalate.

2.8 Analysis of samples

2.8.1 Analysis of organic carbon and nitrogen species

Organic carbon and nitrogen species in samples were analyzed using a Dionex ICS-3000 reagent free ion chromatography (RFIC) system equipped with an IonPac® AS18 4 x 250 mm column. The eluent for the system was potassium hydroxide (KOH) and the eluent flow rate was maintained at 1 ml/min. The eluent KOH concentration was 12-45 mM from 0-5 min, 45 mM from 5-8 min, 45-60 mM from 8-10 min and 60-12 mM from 10-13 min. Ammonium (NH_4^+-N) was measured with the same RFIC but with a IonPac® CG16, CS16, 5 mm column. Methansulfonic acid (30 mM) was used as an eluent with a flow rate of 1 ml/min. The temperature of the two columns was maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppressioin® recycle mode) for concentration determination. COD measurements of the filtered liquid samples were measured using a closed reflux dichromate method (HACH Method 8000, HACH Ltd).

2.8.2 Gas analysis

Acetylene and ethylene gases in the headspace were analyzed using a Trace 1300 gas chromatograph (ThermoFisher Scientific, USA) fitted with a flame ionisation detector (FID). Rt®-U-BOND, (30 m, 0.32 mm ID, 10 µm, Cat.# 19752, Restek, USA) capillary column was used to measure the gases. A 100 µl volume of gas was manually injected using a gas tight syringe into a split injector (split flow 40 ml/min) maintained at 200 ˚C. Helium was used as the carrier gas and the flow rate through the capillary column was maintained at a constant pressure of 53.1 kPa. The initial oven temperature was set at 100 °C for 1 min. Subsequently the temperature was raised to 150 °C at a rate of 25 °C/min and was finally held for 4 min. FID temperature was maintained at 230 ˚C and analysis was carried out using Chromeleon software (Version 7.1.2.1478).

2.8.3 Estimation of dry biomass weight in reactors

Known volumes of graphite biomass carriers were removed from the reactors and immersed in a known volume of deionized water in 50 mL Falcon tubes. Subsequently, the tubes were subjected to ultra-sonication (Sanophon ultrasonic cleaner - 90 watts and 50 Hz) for 3 min to dislodge the attached biofilm from the carriers. The suspensions containing the dislodged cells were then decanted into new 50 ml Falcon tubes and the carriers were sonicated once more for 3 min in deionized water. This final suspension was combined with the previous one and suspended solids (SS) and the volatile suspended solids (VSS) of the suspension were measured using methods detailed in the Standard Methods for Water and Wastewater Analysis (American Public Health Association. et al., 1995).

3. Results and Discussion

3.1 Highly alkaline conditions impacted N-deficient more than N-supplemented reactor

The performance of the two aerobic reactors from start-up until steady state of operation is detailed in Weerasinghe Mohottige et al. (2017). Except when specific batch experiments were carried out, the in-reactor pH of both reactors was maintained at approximately 9–9.5 throughout the entire period

of the study. The oxalate removal rate of both reactors was low at the start of the reactors. Since the inoculum used in both reactors was sourced from neutral pH environments, the immediate exposure to a highly alkaline environment may have negatively impacted the indigenous microorganisms present in the inoculum. While the negative impact appears to be less with the N-supplemented reactor, the impact on the N-deficient reactor appears to be profound (Weerasinghe Mohottige et al., 2017). The oxalate removal rate of the N-supplemented reactor steadily increased without any notable lag phase. In contrast, a long lag period of 75 days was detected for the N-deficient reactor where oxalate removal remained negligible (Weerasinghe Mohottige et al., 2017). Often it is assumed that a lag phase enables adaptation of microorganisms to a new environmental condition (Madigan et al., 2000). This may include the repair of macromolecular damage (Dukan and Nystrom, 1998) and the synthesis of necessary cellular components required for growth.

The specific oxalate removal rate in N-supplemented reactor (110 mg/h.g-biomass) was slightly higher than in N-deficient (87 mg/h. g-biomass) reactor after 215 d of reactor operation (Weerasinghe Mohottige et al., 2017) and this difference in specific rates indicated that microorganisms in the N-supplemented reactor were more efficient at oxidizing oxalate than those in the N-deficient reactor. The low specific oxalate removal rate on the other hand, could be a result of (1) an inhibition of activity due to exposure to high alkaline conditions, (2) a lower oxalate oxidizing activity of the microorganisms under N-deficient conditions and /or (3) a lower initial oxalate concentration in N-deficient reactor. In addition to having differences in specific oxidation rates, the overall biomass concentrations in the two reactors were also notably different. The VSS of the N-supplemented reactor (10 mg/ml of graphite media) was 1.25 times higher than that of the N-deficient reactor (8 mg/ml of graphite media) (Weerasinghe Mohottige et al., 2017). The low biomass concentration in the N-deficient reactor is another reason for the observed low rate of oxalate removal in this reactor.

Influence of pH on microbial activity and growth is well demonstrated in literature. For example, a pH value of 8 was noted desirable for ammonia oxidizing bacteria, while a pH value greater than 7.5

was completely inhibitory towards nitrite oxidizing bacteria (Villaverde et al., May 1997). In this instance, the activity of both organisms was found to be dependent on specific free NH_3 concentrations. Accordingly, free NH_3 inhibition and the limitation of ammonia (due to volatilisation) are challenges associated with an N-supplemented reactor when exposed to high pH. Both of the above factors however, may not be causing any impact on the N-supplemented reactor due to an oversupply of ammonia. However, further studies are required to validate this.

In the absence of an inorganic source of N, the oxalate oxidizing activity recorded in the N-deficient reactor suggested that the microorganisms there would have to fulfil their N requirements plausibly via biological fixation of atmospheric N. It is well known that the enzyme responsible for catalysing the fixation of N is nitrogenase (Hernandez et al., 2009). This enzyme is known to be inhibited when exposed to highly alkaline conditions (Yang et al., 2014). According to Hadfield and Bulen (1969) and Igarashi et al. (2005), the pH dependence of nitrogenase activity exhibits a bell-shaped relationship, whereby an optimal activity is at approximately pH 7-8. Therefore, an increase of pH beyond 8 significantly reduces activity, not due to high pH inactivation of MoFe-proteins in nitrogenase, but rather as a consequence of a complex, mechanism-based reaction (Yang et al., 2014). Accordingly, at pH 9, the N-deficient reactor was likely to have suffered a severe shortage of N (both as a result of limited fixation of atmospheric N and volatilisation of the produced ammonia), specifically impacting the growth of microorganisms. The low biomass concentration that prevailed in the reactor provides an indirect indication towards an inefficient fixation of N in this reactor. Overall, the high pH condition appears to have negatively impacted on the performance of the N-deficient reactor, specifically in terms of maintaining a suitable concentration of biomass required for higher loading rates desirable for practical application.

3.2 The acetylene reduction assay on N-deficient biomass confirmed low nitrogenase activity

No inorganic source of nitrogen was included in the feed of the N-deficient reactor. Frequent measurement of NH_4-N and NO_x-N showed no measureable concentration of ammonia and NO_x-N in the N-deficient reactor. Although an inorganic source of nitrogen was not included, some organic nitrogen was introduced into the reactor in the form of yeast extract. The 10 mg/L concentration of yeast extract in the feed is unlikely to fulfil all N requirements of the biomass. An insignificant amount of inorganic nitrogen also may have occurred in N-deficient reactor through hydrolysis of organic nitrogen sources such as dead cells. Overall, nitrogen requirements of the N-deficient reactor, however, were likely fulfilled via biological nitrogen fixation.

Biological nitrogen fixation can be indirectly quantified using acetylene reduction assay (Hardy et al., 1968). Instead of N_2, in this assay, acetylene is reduced by nitrogenase enzyme to form ethylene and by monitoring ethylene, the activity of nitrogenase can be assessed. When acetylene reduction assay was carried out using biomass from the N-deficient reactor, no ethylene was detected with both 2 and 100 % of oxygen over a 48 h incubation period. Exposure to higher concentration of oxygen is known to inactivate nitrogenase (Compaore and Stal, 2010a; Staal et al., 2007) and this could be one reason for the observed negative result with 100 % oxygen. However, the N-deficient reactor was at all times operated near saturation (8 mg/L) levels of dissolved oxygen. Hence the biofilm in the reactor can be assumed to be tolerant towards oxygen when fixing nitrogen. Specifically, the 2 % oxygen used in the assay was unlikely to cause any inhibitory effect as similar concentrations have been widely used in other acetylene reduction assays reported in the literature (Compaore and Stal, 2010b).

Overall the failure to obtain a measureable quantity of ethylene, suggested very low nitrogenase activity in the biomass of the N-deficient reactor. This supported the hypothesis that a severe nitrogen limitation (due to a low nitrogenase activity) in the N-deficient reactor failed to facilitate a high biomass concentration in N-deficient reactor.

375 *3.3 The performance of the N-deficient reactor improved at close to neutral pH*

376 It is well known that proteins (e.g. enzyme) can be denatured at pH values outside the optimal range
377 and this optimal range is largely species dependent. When considering the need to dilute the Bayer
378 process liquor to reduce pH, having an understanding about the optimal pH range of the biological
379 oxalate removal process becomes invaluable, as this would help reduce the treatment costs.
380 Accordingly, the influence of pH on biological oxidation of oxalate under both N-supplemented and
381 N-deficient conditions was examined (Fig. 2).

382 Considering that the biomass in both reactors was acclimatized at pH 9, it was hypothesised that both
383 reactors would demonstrate optimal oxalate removal rates at pH 9. As hypothesised, the N-
384 supplemented reactor showed an optimal oxidation rate (105 mg/h.g biomass) (Figs. 2A and 2B) at
385 pH 9. Interestingly, for the N-deficient reactor, the highest oxalate removal rate (130 mg/h.g biomass)
386 was detected at a slightly lower pH range of 7-8 (Figs. 2C and 2D). Compared to the oxalate removal
387 rate at pH 9 (63 mg/h.g biomass), the removal rate at pH 7 was approximately 2 times higher in the
388 N-deficient reactor. At this pH range, the specific oxalate removal rate was even higher than that of
389 the N-supplemented reactor. These results suggested that the microorganisms in both reactors were
390 equally active in oxidizing oxalate given that the respective pHs were maintained at an optimal
391 condition. Hence, for the N-deficient reactor to perform similarly to the N-supplement reactor (in
392 terms of treatment load), the biomass needs to be exposed to a lower pH of approximately 7–8.
393 However, a lower pH condition would translate into an increased cost of operation (i.e. increased
394 chemical cost for pH reduction) which is undesirable for practical application.

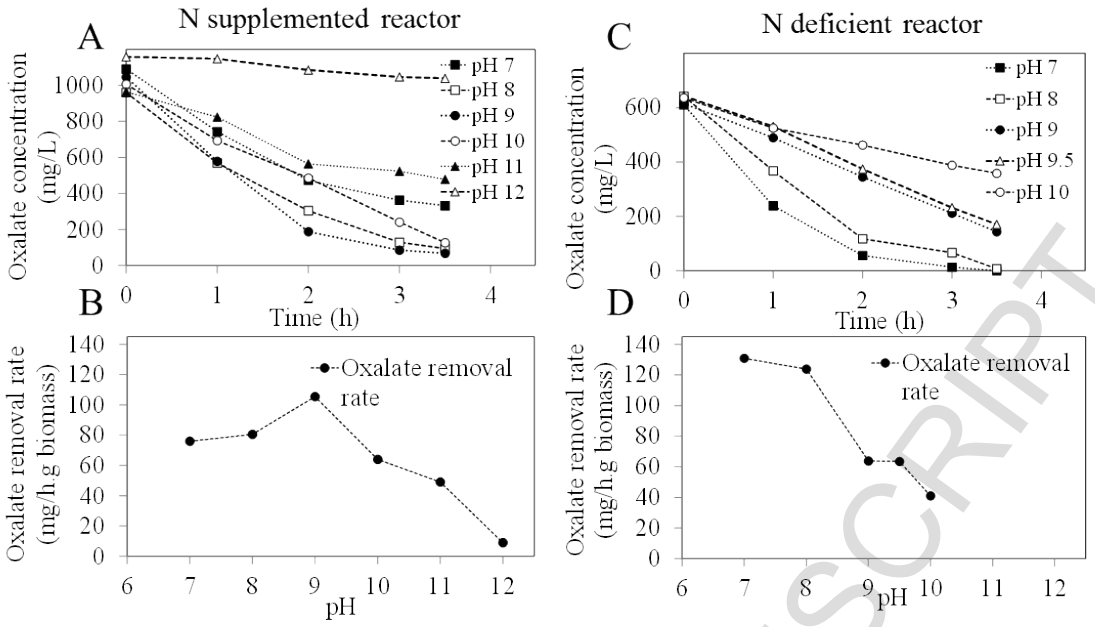
395 *3.4 A continuous influent flow can facilitate the maintenance of optimum pH*

396 Biological oxidation of oxalate generates carbonate resulting in a decrease of pH in the reactor.
397 During a reactor cycle, both N-supplemented and N-deficient reactors showed a gradual decrease of
398 in-reactor pH from approximately 9 down to 8.5. Hence, in general, if an optimum pH of 9 and 8 is

399 to be maintained in N-supplemented and N-deficient systems respectively, alkaline liquor needs to
400 be continuously fed into both systems to control pH (also facilitates a steady supply of oxalate). As
401 previously mentioned, at respective optimum pH values, the specific oxalate removal rates of both
402 processes were similar. Hence, at optimum pH, both N-supplemented and N-deficient systems are
403 able to produce a similar quantity of carbonate. As a consequence (i.e. with similar biomass
404 concentrations), both systems will demand a similar loading rate of influent to maintain its respective
405 optimum pH levels. Accordingly, by adopting a continuous mode of operation, the N-deficient
406 system has the potential to even outperform the N-supplemented system in terms of the influent load
407 that could be handled, the oxalate removal efficiency that could be achieved and low treatment cost
408 that could be maintained due to no requirements for an external nitrogen source.

409 The N-deficient process was able to maintain optimal performance even at pH 7 and accordingly, it
410 is even possible to achieve an effluent, which is near pH neutral with an N-deficient system using the
411 above approach. The performance of the N-deficient system increased (from 63 to 130 mg/h.g
412 biomass) when the pH was decreased from 9 to 7. The N-supplemented system on the other hand
413 underperformed at pH 7 as the specific oxalate removal rate decreased from 105 to 76 mg/h.g biomass
414 when the pH was changed from 9 to 7 (Fig. 2B). The poor performance of the N-supplemented reactor
415 at pH 7 could be a result of acclimatization of the microorganisms at pH 9. Future studies should
416 examine the specific oxalate removal rates of an N-supplemented and a N-deficient reactor
417 acclimatized at pH 7 in order to understand whether N-deficient reactors would continue to
418 outperform N-supplemented reactors at neutral pH.

419



420

421 **Fig. 2.** Oxalate concentrations during oxalate degradation and various initial pH values in the (A) N-
422 supplemented and (B) N-deficient reactors. The influence of pH on oxalate removal rate in (C) N-
423 supplemented and (D) N-deficient reactors as calculated based on initial 2 h of oxalate removal.

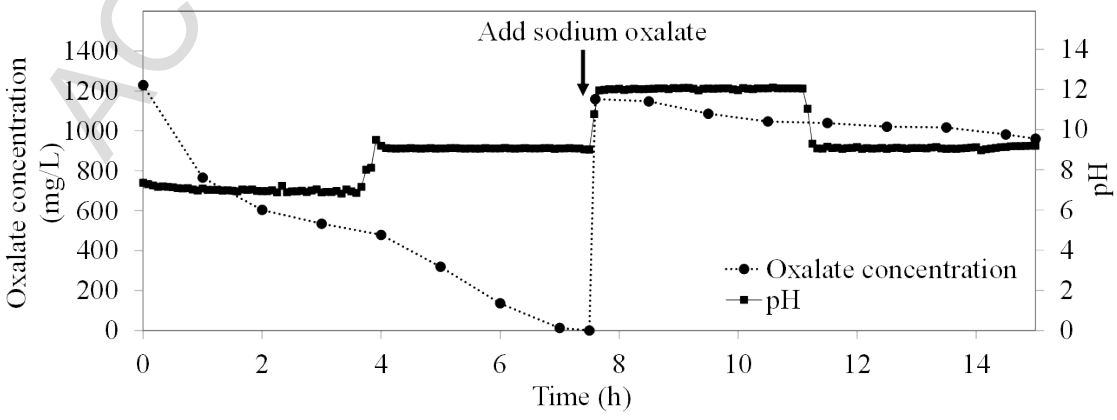
424 *3.5 The negative impact on N-supplemented reactor cause with exposure to in-reactor pH values of*
425 *> 11 are not readily reversible*

426 Microorganisms in the N-supplemented reactor demonstrated two rates of oxalate removal when
427 oxidizing oxalate at the optimal pH of 9 (Fig. 2A). A linear rate of oxalate removal (105.5 mg/h.g
428 biomass) continued until the residual oxalate reached approximately 185 mg/L. Subsequently another
429 lower linear rate (16.9 mg/h. g biomass) of removal was detected. At pH 8, a similar reduction in
430 oxalate removal rate was detected approximately at the same residual oxalate concentration. The
431 oxalate removal profile at pH 10 did not however show a distinct rate change and maintained a
432 consistent rate of removal (64 mg/h.g biomass) throughout the experiment. The residual oxalate
433 concentration at pH 10 did not reach the 185 mg/L threshold during the entire period of experiment
434 and this may be the reason for the non-occurrence of the second lower rate of oxalate removal. The

435 change of oxalate removal rate from high to low at the above residual oxalate concentration is likely
436 a response of microorganisms to substrate affinity.

437 The initial oxalate removal rates of both pHs 7 and 11 experiments were approximately 2 times lower
438 compared to the rate observed at pH 9. Due to the reduced removal rates, the residual oxalate
439 concentrations at pH 7 and 11 did not reach the 185 mg/L threshold to trigger a lower oxalate removal
440 rate similar to that observed with pHs 9 and 8 experiments. Nevertheless, pH 7 and 11 experiments
441 showed a reduction in removal rates after 2 h of oxidation and this reduction in oxalate removal rates
442 was likely an inhibitory response to pH. The very low oxalate removal rate observed with the pH 12
443 experiment confirmed that pH 12 was remarkably detrimental to the biofilm.

444 A separate experiment was conducted to evaluate whether the observed pH inhibition was reversible
445 (Fig. 3). The results suggested that the inhibition caused by a short-term (4 h) exposure of the biofilm
446 to pH 7 was reversible, as a subsequent correction of the pH to 9 showed an instantaneous increase
447 of oxalate removal rate (Fig. 3). However, exposing the biofilm to pH 12 for a similar period (4 h)
448 resulted in an inhibition of oxalate removal that could not be readily reversed, as a subsequent
449 exposure to pH 9 failed to revert the oxalate removal activity of the biofilm (Fig. 3). The inhibitory
450 effect on the biofilm, even with such a short-term exposure to pH 12, was noted to be severe, with
451 the biofilm requiring more than 18 reactor cycles (3 days) to regain original stable activity (data not
452 shown).



453

Fig. 3. Reversible and irreversible inhibition of oxalate removal by N-supplemented reactor at in-reactor pH 7 and pH 12, respectively.

3.6 Short-term exposure to optimal pH conditions has a long-lasting impact with higher rates of oxalate removal in N-deficient reactor

There was a near linear improvement of oxalate removal (increasing by approximately 1.29 mg/L.h each day) after ~ 84 d of operating the N-deficient reactor during the start-up at an in-reactor pH of 9 (Weerasinghe Mohottige et al., 2017). With a decrease of in-reactor pH to 8, there was an instantaneous increase of oxalate removal rate (9.84 mg/L/h per day) (Fig. 4). As anticipated, when in-reactor pH was returned to pH 9, there was an immediate reduction in the oxalate removal rate. However, after an initial decrease, the oxalate removal continued to increase at a lower rate (4.43 mg/L.h per day) with exposure to pH 9 (Fig. 4). This improvement in reactor performance at pH 9 after a period of exposure to an in-reactor pH of 8, suggested an improvement in biological activity which was likely a result of an increase in biomass concentration, which was not quantified. After 5 days of exposure to pH 9, when the in-reactor pH was once again reduced to 8, the oxalate removal once again increased (16.9 mg/L.h per day) (Fig. 4).

This experiment hints at a strategy that could be effectively used to increase the biomass activity of an N-deficient biofilm oxidizing oxalate exposed to an unfavourable high pH such as pH 9. As previously highlighted, nitrogenase activity is compromised at high pH (Yang et al., 2014), and this likely imposes severe nitrogen limitation, affecting microbial growth. The exposure to favourable pH conditions increases nitrogenase activity reducing limitations of nitrogen for growth and as a consequence, a rapid increase of biomass can be anticipated. With prolonged exposure to unfavourable pH, the higher rate of oxalate removal is unlikely to be sustainable specifically if the level of nitrogenase activity is not sufficient to maintain the biomass in the reactor. Therefore, an intermittent exposure to a favourable pH such as 8 may be needed if the higher rate of oxalate removal is to be maintained long-term at pH 9. Alternating between a favourable and unfavourable pH

facilitates biomass management and future research should examine the development of smart operational strategies that could capitalise on this finding to reap economic benefits from using N-deficient biomass to oxidize oxalate.

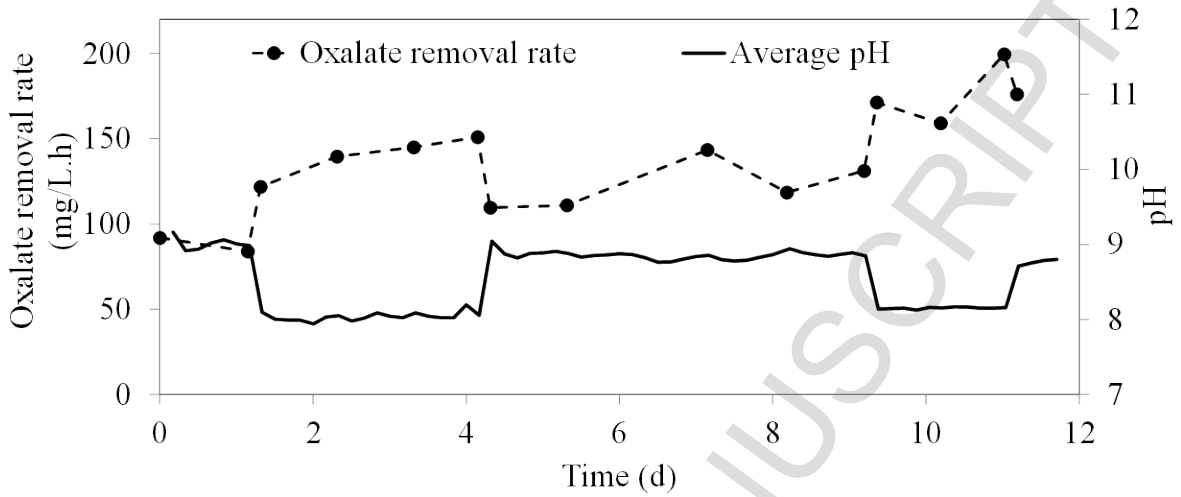


Fig. 4. Intermittent exposure to a favourable pH and its impact on the removal of oxalate after the exposed to an unfavourable pH.

3.7 Other organic impurities in Bayer liquor could potentially increase the performance of N-deficient reactor

Power et al. (2011) provides a summary of the organic compounds detectable in Bayer process liquor. Of the large number of organic compounds in Bayer liquor, oxalate imposes a major impact (due to co-precipitation with aluminium trihydroxide), while other organics tend to have a limited impact on the overall process (Brady, 2011). The increase in concentration of other organics with the recycling of process liquor, however, is of concern and requires management.

Although primarily acclimatized to only oxidize oxalate, both N-supplemented and N-deficient reactors demonstrated the ability to simultaneously oxidize other organic compounds introduced alongside oxalate (Fig. 5). The impact of other organic compounds on the oxidation of oxalate

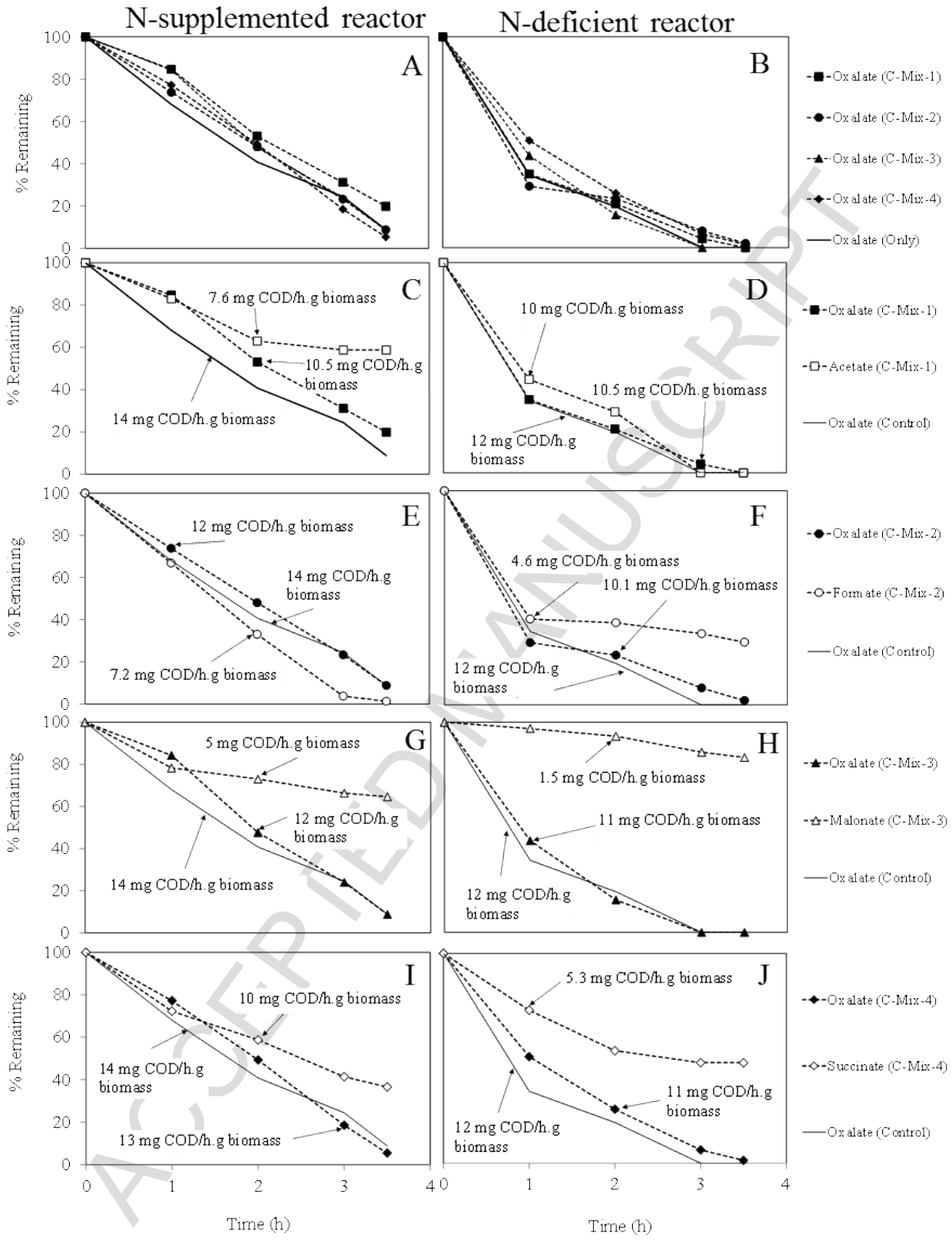
appeared negligible with both reactors, as the recorded oxalate oxidation rates remained similar even with the presence of other organics (Fig. 5).

With the exception of succinate, the oxidation rates of other organic compounds were remarkably lower (approximately half) compared to the oxidation rate of oxalate in the N-supplemented reactor. The oxidation rate of succinate (10 mg-COD/h.g biomass) in the N-supplemented reactor was only marginally different to the oxidation rate of oxalate (14 mg-COD/h.g biomass). Similar results were recorded with the N-deficient reactor. However, instead of succinate, the oxidation rate of acetate (10 mg-COD/h.g biomass) was only marginally different to the oxidation rate of oxalate (12 mg-COD/h.g biomass). Formate was the only carbon source that was completely oxidized in the N-supplemented reactor during the 4 h experiment, whereas acetate was the only carbon source that was completely oxidized in the N-deficient reactor.

The oxidation profiles of oxalate and most other organic carbon sources showed a parallel trend to each other (Fig. 5C–J) in both of the N-supplemented and N-deficient reactors. This implied a concomitant oxidation of the two substrates (i.e. oxalate and the additional carbon source). Co-metabolism refers to a concomitant oxidation of a non-growth substrate during the growth of a microorganism on an utilisable carbon and energy source (Wackett, 1996) and in this instance, further studies are needed to confirm whether the oxidation of other organic compounds was a result of co-metabolism.

The N-deficient reactor showed an overall lower concentration of biomass. While an inhibition of nitrogenase activity at high pH may have resulted in a lower concentration of biomass, the higher energy demand of nitrogen fixation may also have limited the availability of carbon to facilitate the growth of microorganisms in the N-deficient reactor. The energy gain from the oxidation of oxalate ($\Delta G° = -608.46$ KJ mol^{-1}) is 1.5 times lower than what could be gained from acetate ($\Delta G° = -925.88$ KJ mol^{-1}) and 2.9 times lower than what could be gained from succinate ($\Delta G° = -1740.57$ KJ mol^{-1}).

519 Accordingly, if the high demand of energy for nitrogen fixation (Hill et al., 1972) was impacting the
520 biomass growth in the N-deficient reactor, this impediment could potentially be eliminated by the N-
521 deficient biofilm's ability to co-oxidize other organic carbon sources present in Bayer liquor. Future
522 research is needed to consolidate fundamental knowledge about slow growth of biomass in the N-
523 deficient reactor and to examine the effectiveness of the discussed strategies to overcome this
524 limitation.



525

526 **Fig. 5.** The efficacy of N-supplemented and N-deficient biofilms to oxidize organics other than

527 oxalate and the influence of other organics on oxalate removal.

3.8 Implication of the findings

As demonstrated, the biological oxidation of oxalate could be facilitated by using both N-supplemented and N-deficient reactors. The tested systems have the potential to effectively treat similar oxalate loads. There are obvious operational savings that could be achieved using N-deficient systems (due to no requirements of an external nitrogen source), but in order to realise these savings, current operational strategies need to be revisited to specifically favour the N-deficient treatment systems. This study highlights some beneficial strategies that could be considered for optimising the efficiency of N-deficient systems, for example by capitalising on the carbonate produced during oxidation of oxalate to impose an in-reactor fluctuation of pH.

The ability of both reactors to co-oxidize other organics present in Bayer liquor is not only beneficial but has the potential to increase the activity of an N-deficient reactor. The extra energy and carbon available as a result of co-oxidation may enable the N-deficient reactor to better respond to the extra energy demand required for nitrogen fixation. In contrast, the N-supplemented reactor could get hampered with an excessive increase of biomass growth, which could lead to operational challenges such as oxygen mass transfer limitation and a build-up of anaerobic pockets (could lead to methane emissions due to the fermentation of oxalate and other organics). In summary, this comparative study provides insight on N-deficient systems and highlights opportunities for the alumina industry to embrace N-deficient systems to remove oxalate from Bayer liquor.

4. Conclusions

This study for the first time examined the impact of pH and other organics of Bayer liquor on two biofilm reactors under N-supplemented and N-deficient conditions. Based on the results of this study, N-supplemented reactor performs better in oxalate removal rate and has much shorter start-up period compared to an N-deficient reactor at pH 9 that the biomass was acclimatized. However, when exposed to optimal pH conditions, the N-deficient and N-supplemented reactors achieved similar

552 oxalate removal rates. The N-supplemented reactor was irreversibly impacted when exposed to an
553 in-reactor pH exceeding 11 and the oxalate removal inhibition at pH 7 however, was reversible. A
554 short-term exposure of the N-deficient biofilm to an optimal pH 8 induced higher oxalate removal
555 rates at pH 9. Accordingly, the carbonate produced during oxidation of oxalate could be used to
556 impose an in-reactor fluctuation of pH, in order to expose the biofilm intermittently to an optimal pH
557 environment promoting growth of biomass. Other organic impurities in Bayer liquor had a negligible
558 effect on oxalate removal rates and potentially could increase the performance of the N-deficient
559 reactor.

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567 **Reference**

568 American Public Health Association., American Water Works Association., Water Environment
569 Federation., 1995. Standard methods for the examination of water and wastewater, 19th ed.
570 American Public Health Association, American Water Works Association, and Water Environment
571 Federation, Washington, D.C.

572 Bahadur, K., Tripathi, P., 1976. Nitrogen fixation by microbial cultures with sodium salt of organic
573 acids as carbon source. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und
574 Hygiene. Zweite Naturwissenschaftliche Abteilung: Allgemeine, Landwirtschaftliche und
575 Technische Mikrobiologie 131, 375-377.

576 Banciu, H.L., Sorokin, D.Y., Tourova, T.P., Galinski, E.A., Muntyan, M.S., Kuenen, J.G., Muyzer,
577 G., 2008. Influence of salts and pH on growth and activity of a novel facultatively alkaliphilic,
578 extremely salt-tolerant, obligately chemolithoautotrophic sufur-oxidizing Gammaproteobacterium
579 *Thioalkalibacter halophilus* gen. nov., sp nov from South-Western Siberian soda lakes.
580 Extremophiles 12, 391-404.

581 Brady, J.P., 2011. An examination of the applicability of hydrotalcite for removing oxalate anions
582 from Bayer process solutions. Queensland University of Technology.

583 Brassinga, R.D., Fulford, G.D., Lang, R.G., McCready, R.G.L., Gould, W.D., Beaudette, L., 1989.
584 Biodegradation of oxalate ions in Bayer process Patent AU8939465-A

585 Chen, H.L., Spitzer, D., Rothenberg, A., Lewellyn, M., Chamberlain, O., Heitner, H., Kula, F., Dai,
586 Q., Franz, C., 2007. Sodalite scale control in alumina Bayer process. NACE International Corrosion
587 2007, 1-9.

588 Compaore, J., Stal, L.J., 2010a. Effect of temperature on the sensitivity of nitrogenase to oxygen in
589 two Heterocystous cyanobacteria J. Phycol. 46, 1172-1179.

590 Compaore, J., Stal, L.J., 2010b. Oxygen and the light-dark cycle of nitrogenase activity in two
591 unicellular cyanobacteria. Environ. Microbiol. 12, 54-62.

592 Dukan, S., Nystrom, T., 1998. Bacterial senescence: stasis results in increased and differential
593 oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon.
594 Genes Dev. 12, 3431–3441.
595 Gauthier, F., Neufeld, J.D., Driscoll, B.T., Archibald, F.S., 2000. Coliform bacteria and nitrogen
596 fixation in pulp and paper mill effluent treatment systems. Appl. Environ. Microbiol. 66, 5155-
597 5160.
598 Hadfield, K.L., Bulen, W.A., 1969. Adenosine triphosphate requirement of nitrogenase from
599 *Azotobacter vinelandii.* Biochemistry 8, 5103−5108.
600 Hardy, R.W., Holsten, R., Jackson, E., Burns, R., 1968. The acetylene-ethylene assay for N2
601 fixation: laboratory and field evaluation. Plant Physiol. 43, 1185-1207.
602 Hernandez, J.A., George, S.J., Rubio, L.M., 2009. Molybdenum trafficking for nitrogen fixation.
603 Biochemistry 48, 9711-9721.
604 Hill, S., Drozd, J.W., Postgate, J.R., 1972. Environmental effects on the growth of nitrogen-fixing
605 bacteria. Journal of Applied Chemical Technology and Biotechnology, 541-558.
606 Hind, A.R., Bhargava, S.K., Grocott, S.C., 1997. Quantitation of alkyltrimethylammonium
607 bromides in Bayer process liquors by gas chromatography and gas chromatography mass
608 spectrometry. J. Chromatogr. 765, 287-293.
609 Horikoshi, K., 1999. Alkaliphiles: Some applications of their products for biotechnology.
610 Microbiol. Mol. Biol. Rev. 63, 735-750.
611 Igarashi, R.Y., Laryukhin, M., Dos Santos, P.C., Lee, H.I., Dean, D.R., Seefeldt, L.C., Hoffman,
612 B.M., 2005. Trapping H(-) bound to the nitrogenase FeMo-Cofactor active site during H(2)
613 evolution: Characterization by ENDOR spectroscopy. J. Am. Chem. Soc. 127, 6231-6241.
614 Madigan, M.T., Parker, J., Martinko, J.M., 2000. Biology of Microorganisms. Prentice Hall,
615 London.
616 McKinnon, A.J., Baker, C.L., 2012. Process for the destruction of organics in a Bayer process
617 stream. Patent US2014/0051153 A1, 28.

618 Meyers, R.A.E., 2004. Encyclopedia of physical science and technology:Third edition, Materials
619 Chapter : Aluminum. Academic Press, New York, pp. 495-518.
620 Morton, R.A., Dilworth, M.J., Wienecke, B., 1991. Biological disposal of oxalate Patent
621 WO91/12207, 12.
622 Power, G., Loh, J.S.C., Vernon, C., 2012. Organic compounds in the processing of lateritic bauxites
623 to alumina Part 2: Effects of organics in the Bayer process. Hydrometallurgy 127, 125-149.
624 Power, G., Loh, J.S.C., Wajon, J.E., Busetti, F., Joll, C., 2011. A review of the determination of
625 organic compounds in Bayer process liquors. Anal. Chim. Acta 689, 8-21.
626 Sorokin, D., Tourova, T., Schmid, M.C., Wagner, M., Koops, H.P., Kuenen, J.G., Jetten, M., 2001.
627 Isolation and properties of obligately chemolithoautotrophic and extremely alkali-tolerant
628 ammonia-oxidizing bacteria from Mongolian soda lakes. Arch. Microbiol. 176, 170-177.
629 Sorokin, I.D., Kravchenko, I.K., Doroshenko, E.V., Boulygina, E.S., Zadorina, E.V., Tourova, T.P.,
630 Sorokin, D.Y., 2008. Haloalkaliphilic diazotrophs in soda solonchak soils. FEMS Microbiol. Ecol.
631 65, 425-433.
632 Sousa, J.A.B., Sorokin, D.Y., Bijmans, M.F.M., Plugge, C.M., Stams, A.J.M., 2015. Ecology and
633 application of haloalkaliphilic anaerobic microbial communities. Appl. Microbiol. Biotechnol. 99,
634 9331-9336.
635 Sprent, P., 1990. Nitrogen fixing organisms: pure and applied aspects. Chapman and Hall, London.
636 Staal, M., Rabouille, S., Stal, L.J., 2007. On the role of oxygen for nitrogen fixation in the marine
637 cyanobacterium *Trichodesmium* sp. Environ. Microbiol. 9, 727-736.
638 Tilbury, A., 2003. Biodegradation of Bayer organics in residue disposal systems, Department of
639 Chemistry. University of Western Australia.
640 Villaverde, S., GarciaEncina, P.A., FdzPolanco, F., May 1997. Influence of pH over nitrifying
641 biofilm activity in submerged biofilters. Water Res. 31 1180-1186
642 Wackett, L.P., 1996. Co-metabolism: is the emperor wearing any clothes? Curr. Opin. Biotechnol.
643 7, 321-325.

644 Wang, H., Zhang, Y., Angelidaki, I., 2016. Ammonia inhibition on hydrogen enriched anaerobic
645 digestion of manure under mesophilic and thermophilic conditions. Water Res. 105, 314-319.
646 Weerasinghe Mohottige, T.N., Cheng, K.Y., Kaksonen, A.H., Sarukkalige, R., Ginige, M.P., 2017.
647 Oxalate degradation by alkaliphilic biofilms acclimatised to nitrogen-supplemented and nitrogen-
648 deficient conditions. J. Chem. Technol. Biotechnol.
649 Whelan, T.J., Ellis, A., Kannangara, G.S.K., Marshall, C.P., Smeulders, D., Wilson, M.A., 2003.
650 Macromolecules in the Bayer process. Reviews in Chemical Engineering 19, Pages 431–472.
651 Yang, K.Y., Haynes, C.A., Spatzal, T., Rees, D.C., Howard, J.B., 2014. Turnover-dependent
652 inactivation of the nitrogenase MoFe-Protein at high pH. Biochemistry 53, 333-343.

653

Highlights

- Two oxalotrophic haloalkaliphilic biofilms were examined at different influent pH.
- Both N-supplemented and N-deficient biofilms could tolerate broad pH range (7-10).
- At optimum pH the N-deficient system outperformed the N-supplemented system.
- The inhibition in oxalate removal at pH 7 is reversible for N-supplemented system.
- Simple organics in alumina refinery process had no effect on oxalate removal rates.