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ABSTRACT

Increasing demand for rare earth elements (REE) is expected to lead to new development and expansion in industries processing and or recycling REE. For some industrial operators, sending aqueous waste streams to a municipal wastewater treatment plant, or publicly owned treatment works (POTW), may be a cost effective disposal option. However, wastewaters that adversely affect the performance of biological wastewater treatment at the POTW will not be accepted. The objective of our research is to assess the effects of wastewaters that might be generated by new rare earth element (REE) beneficiation or recycling processes on biological wastewater treatment systems. We have been investigating the impact of yttrium and europium on the biological activity of activated sludge collected from an operating municipal wastewater treatment plant. We have also examined the effect of an organic complexant that is commonly used in REE extraction and separations; similar compounds may be a component of newly developed REE recycling processes. Our preliminary results indicate that in the presence of Eu, respiration rates for the activated sludge decrease relative to the no-Eu controls, at Eu concentrations ranging from <10 to 660 µM. Yttrium appears to inhibit respiration as well, although negative impacts have been observed only at the highest Y amendment level tested (660 µM). The organic complexant appears to have a negative impact on activated sludge activity as well, although results are variable. Ultimately the intent of this research is to help REE industries to develop environmentally friendly and economically sustainable beneficiation and recycling processes.

KEYWORDS

Wastewater, biological treatment, microorganisms, rare earth elements

INTRODUCTION

New technologies and new industries generally lead to the generation of new wastes. Volumes and types of waste generation are important considerations in the development of any new industrial process, and can have a large impact on plant design and therefore costs. While leachates from conventional production of rare earth elements (REE) from ore are generally expected to have very low REE concentrations and impacts, new recycling or REE processing technologies will likely generate different wastes with different compositions. The environmentally sensitive handling of wastewaters may constitute a significant responsibility and expense for commercial deployers of new technologies, and will be a crucial factor in determining the ultimate economic as well as ecological sustainability of new approaches for meeting the anticipated increased global demand for strategic metals. For some industrial generators, sending aqueous wastestreams to a municipal wastewater treatment plant, or publicly owned treatment works (POTW), may be a cost effective disposal option. However, wastewaters that jeopardize the performance of the POTW will not be acceptable. The objective of this project is to assess the effects of wastewaters generated by REE beneficiation or recycling processes on biological wastewater treatment systems. Biological wastewater treatment, mediated by highly efficient microbial communities adapted for this purpose, is a critical module in modern POTWs, enabling them to meet requirements for discharge to water bodies such as oceans, lakes or rivers.

Previously we completed aerobic pure culture studies using two different strains of nitrifying bacteria challenged with synthetic wastewaters containing yttrium (Y) or europium (Eu) and the organic complexant tributyl phosphate (TBP); details are provided elsewhere (Fujita, Barnes, Eslamimanesh, Lencka, Anderko, Riman, & Navrotsky, 2015) but briefly summarized here. Nitrifying bacteria oxidize ammonia to nitrite and subsequently to nitrate, and nitrification is a critical step in the removal of nitrogen from wastewater. Eu and Y were targeted because they are major components of fluorescent light phosphor powders; fluorescent lamps have significant potential for near-term commercial recovery of REEs, due to their high content of Y, Eu and terbium (Tb), the large volume of lamps already in use, and the existence of end-of-life recycling services for the lamps (Binnemans, Jones, Blanpain, Van Gerven, Yang, Walton, & Buchert, 2013). TBP is a traditional extraction solvent used for lanthanide and actinide separations, and was used as a representative organic waste component because non-REE constituents may be just as important or more important than the REE in imparting toxicity to the wastewaters. In the pure culture experiments, we exposed the ammonia oxidizing bacterium *Nitrosomonas europaea* and the nitrite oxidizing bacterium *Nitrobacter winogradskyi* to simulated wastewaters containing varying levels of Y and Eu (10, 50 and 100 ppm), and TBP (0.1 g/L). These REE amendment levels are very high, and in fact at the initial pH used for the experiments > 98% of the REE was insoluble, yet we observed that Y and Eu additions at 50 and 100 ppm inhibited *N. europaea*. Provision of TBP with Eu increased *N. europaea* inhibition, although TBP alone did not substantially alter activity. For *N. winogradskyi* cultures Eu or Y additions at all tested amendment levels induced significant inhibition (although again virtually all of the added Eu and Y were insoluble), and nitrification shut down completely with TBP addition.

As a follow-up to the pure culture studies, we have examined the effects of the same REE synthetic wastewater components on the biological activity of activated sludge obtained from the Idaho Falls Wastewater Treatment Plant. Nitrification is one of the critical functions carried out by the activated sludge, and we wanted to determine if the effects observed with the pure cultures would be reflected in the complex mixed microbial communities present in an actual wastewater treatment plant. Our approach and the findings are presented below.

METHODS

Chemicals

Unless specified otherwise, all chemicals used were ACS reagent grade. Yttrium oxide (99.99%) was purchased from Research Chemicals (Phoenix, AZ) and europium chloride (99.99%) from Strem

Chemicals, Inc. (Newburyport, MA). Tributyl phosphate was provided within a commercial isoparaffin matrix (Isopar^{TM} L, from ExxonMobil), as a 30:70 (v/v) mixture.

Microbial Inoculum

Activated sludge collected from or near the exit of the aeration tank of the Idaho Falls Wastewater Treatment Plant (IF WWTP) was used as inoculum. The sample was collected by IF WWTP personnel using sterile containers provided by the INL, refrigerated, and transported to the INL on ice within a few hours. Activated sludge (AS) for the experiments described here was collected two different days: on March 16, 2015 for the Eu experiments ("AS batch 1") and on April 22, 2015 ("AS batch 2") for the Y experiments. Once at the INL we determined the total suspended solids (TSS) concentration as described below and dispensed the activated sludge slurry (while stirring to maintain the suspension) in 20 or 25 mL aliquots (volume depending on the TSS) in 50 mL centrifuge tubes that were then immediately frozen at -20°C. For the experiments, the activated sludge aliquots were thawed immediately prior to addition of the entire aliquot to the experimental vessel.

Synthetic REE Wastewater Composition

The synthetic wastewater consisted of 0.1 M HCl containing Eu or Y at levels that resulted in concentrations of 0, 6.6, 66, or 660 µM REE (equivalent to 0, 1, 10, or 100 ppm Eu and 0, 0.59, 5.9 and 59 ppm Y) in the final test matrix. After dispensing the acidic solution into an individual reaction vessel, we adjusted the pH to 7.5±0.5 using NaOH. For some of the experiments, TBP (as a 30:70 mixture (v/v) with the isoparaffin solvent Isopar^{TM} L) was added directly (i.e, not autoclaved or pretreated) to the test mixture and tested at a final concentration of 0 and 0.1 g/L in the test matrix.

Synthetic Sewage

A synthetic sewage medium was included in the experiments with composition as described in Organization of Economic Cooperation and Development Test Guideline method 209 (OECD, 2010) and listed in Table 1. Aliquots (10 mL) of filter-sterilized medium were stored frozen at -20 °C. Prior to use, aliquots were thawed and the pH checked and if necessary adjusted to pH 7.5±0.5.

Table 1 - Synthetic sewage medium (OECD 209, 2010)

Component	Concentration (g/L)
Peptone	16
Meat extract	11
Urea ($CO(NH_2)_2$)	3
NaCl	0
$CaCl_2 \cdot 2H_2O$	0.4
$MgSO_4 \cdot 7H_2O$	0.2
K_2HPO_4	2.8

REE and TBP Exposure Experiments

The general testing conditions are listed in Table 2. We set up each treatment condition in a 500 mL flask containing 50 mL of test mixture. Each test mixture contained 20-25 mL activated sludge, 1.6 mL synthetic sewage, 10 mL simulated wastewater and deionized water to bring the volume up to 50 mL. The amount of sludge added to each test mixture provided ~ 1.5 g /L of TSS; the sludge and synthetic sewage fractions are based on examples of test mixtures described in OECD method 209. The amount of wastewater represented 20% (v/v) of the final test volume. This corresponds to the percentage of wastewater used in the pure culture studies as well as that reported by others for assays of ammonia and nitrite oxidation inhibition by industrial wastewaters (Grunditz, Gumaelius, & Dalhammar, 1998).

For each flask, we added the wastewater first, adjusted the pH to 7.5 ±0.5 using NaOH, and then added all of the other components except for the activated sludge. For the treatments with TBP (0.1 g/L), 17.2 µL of the 30:70 (v/v) stock mixture of TBP in Isopar^{TM} L was added to the test mixture prior to adding the inoculum. Then sludge aliquots were added sequentially at 15- 20 minute intervals to each flask. We placed each flask in a 20 °C incubator for 3 hours with orbital shaking at 175 rpm (This shaking rate was shown in preliminary studies to keep the sludge in suspension and maintain the DO above 60-70% saturation per OECD recommendations). The 15 minute interval between individual treatments allowed completion of time-sensitive assays while maintaining a consistent incubation time between treatments.

Table 2 - Experimental test conditions

Test ID	Test condition
A	Endogenous activity
B	6.6 µM REE
C	66 µM REE
D	660 µM REE
E	TBP (0.1 g/L)
F	6.6 µM REE + TBP (0.1 g/L)
G	66 µM REE + TBP (0.1 g/L)
H	660 µM REE + TBP (0.1 g/L)

After 3 hours of incubation, we immediately processed the test mixtures in a biological safety cabinet. The pH was measured using pH test strips, and samples were taken for chemical oxygen demand (COD), ammonia, and nitrate. A total of approximately 5 mL was removed. The remainder was used immediately for measurement of oxygen utilization.

Analytical Methods

Total suspended solids (TSS) of the activated sludge was measured using American Public Health Association (APHA) Method 2540 D (APHA, 1989). Briefly, 2 mL of activated sludge suspension was filtered onto a glass fiber filter, which was then dried at 103 °C-105 °C overnight or until a constant weight was observed.

COD was measured using 0.2 mL aliquots diluted 10X according to Hach method 10236, with Hach TNT 825 or Orbeco TT207-10 reagents. Both products are Hg-free and provided in premeasured reaction vials. The analyses were performed following manufacturer instructions using a benchtop spectrophotometer. NH_3 was measured using an ammonia gas sensing electrode (Thermo Scientific) according to manufacturer instructions. Nitrate was measured using Orbeco Method 265, and Lovibond reagent vials (Kit #535580) with an Orbeco colorimeter (MC 500 Multiparameter).

Oxygen utilization rates were measured in a 50 mL self-standing vial in which a calibrated dissolved oxygen (DO) probe (Orion 081010MD) could be inserted along with a small magnetic stir bar. Once the test mixture solution was added to the vial and the probe inserted and cap tightened, the system was protected from additional air (oxygen) input. The unit was positioned over a magnetic stir plate and mixed at a low speed. The DO concentration was monitored continuously (5 s intervals) over a period of 5 minutes using a benchtop meter (Thermo Scientific Orion Versastar) or until the DO fell below 2 mg/L. The oxygen utilization rate was calculated using the measured values in the linear range between 2.0 mg/L and 7.0 mg/L, as described in OECD method 209 (OECD, 2010). The Specific Oxygen Utilization Rate (SOUR) was calculated as described in EPA method 1683 (EPA, 2001).

RESULTS

Activated Sludge Characterization

The TSS values determined in our laboratory for the activated sludge samples were 5.11 g/L and 3.85 g/L for AS batch 1 (March 16, 2015) and AS batch 2 (April 22, 2015), respectively. These values were comparable to those determined by IF WWTP staff for separately collected samples taken from the aeration basins on the same days; they measured 3.74 g/L for the March sample and 3.21 g/L for the April sample.

Changes in Oxygen Uptake Rates

We tested the effect of Eu and TBP on activated sludge respiration on four different dates, where on each date the series of conditions listed in Table 2 were applied, one replicate each for each condition. The results for the SOUR calculations are shown in Figure 1.

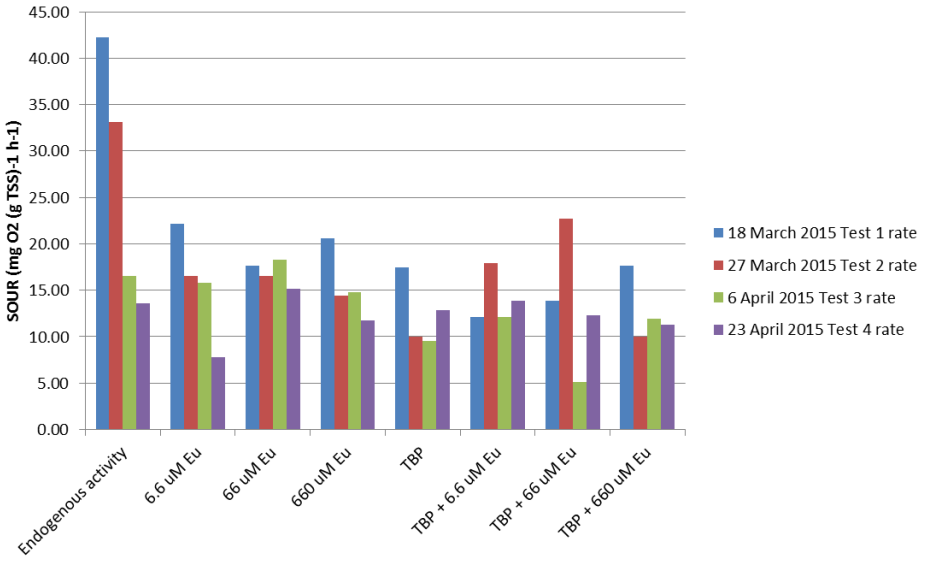

Figure 1 - Effect of Eu and TBP on specific oxygen uptake rate of activated sludge

From the endogenous activity controls, it appears clear that the activity of the activated sludge declines with storage at -20°C. The sludge used for the Eu experiments was collected on March 16, 2015, and frozen that day. By three weeks later (April 6, 2015), the endogenous activity had declined by more than 50%. In a separate test of the sludge collected April 22, 2015, the activity of the sludge after one day at -20°C was lower than the fresh (never frozen) sludge by 11.3%.

With respect to the effect of the Eu, for both of the first two test dates (within two weeks of sludge collection) the SOUR decreased significantly (48-58%) relative to the endogenous rate for all 3 levels of Eu added. It is not evident that higher Eu amendments led to greater inhibition. For the third and fourth test dates, the results were mixed; in some cases the cultures amended with Eu appeared to exhibit greater SOUR.

TBP alone also appeared to inhibit SOUR, and this was exhibited even in the later test dates (Tests 3 and 4), but it is not clear whether it enhanced inhibition by Eu, as had been observed previously with the *N. europaea* pure culture (Fujita et al., 2015). In Test 1 and Test 3, TBP appeared to enhance

inhibition by Eu, but in other cases, SOUR values were higher in samples provided with TBP and Eu compared to samples provided with just Eu at the same concentration (e.g., Test 2, with Eu at 66 µM).

The SOUR results for the experiments with added Y and TBP are shown in Figure 2. These experiments were conducted with sludge collected April 22, 2015. Three complete series were conducted within two weeks of sludge collection, and results for the endogenous sludge tests showed that they were comparable for the three test dates, although compared to the March 16 activated sludge the endogenous SOUR values were lower by about two thirds. Limited tests using the April activated sludge samples more than a month after collection (June 3) showed that activity had decreased by 50% or more, and the endogenous replicates behaved very differently from each other.

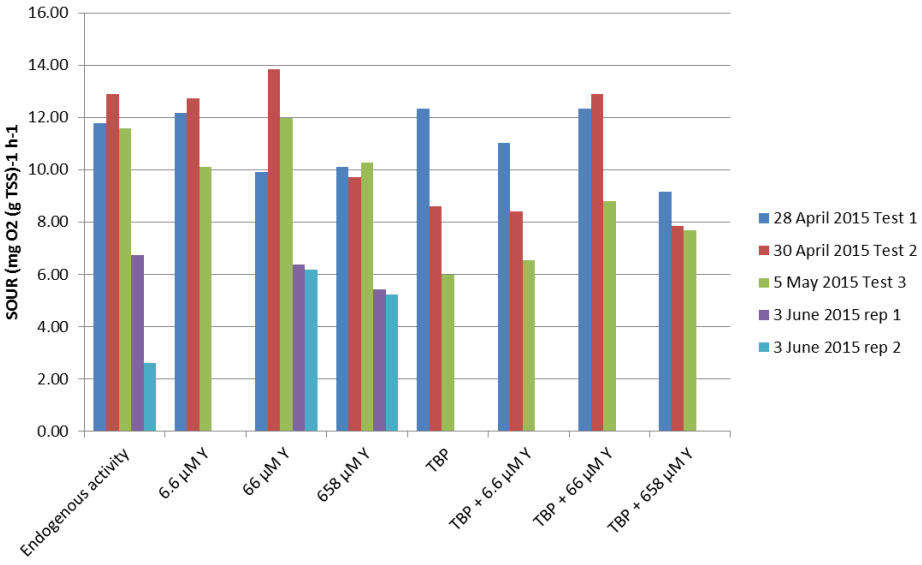

Figure 2 -. Effect of Y and TBP on specific oxygen uptake rate of activated sludge

Unlike the case for Eu, addition of Y at 6.6 and 66 µM concentrations did not appear to exert an inhibitory effect on the activated sludge; only 660 µM Y amendments resulted in reduced SOUR values (lower by 11.3 to 24.6%) relative to the endogenous SOUR on all 3 test dates.

With this sludge, the effects of TBP alone were less clear. On two out of three test dates, the addition of TBP appeared to inhibit respiration compared to the endogenous activity. In combination with Y, TBP did appear to increase the inhibition observed with 660 µM Y addition, but for the lower Y amendment levels, the measured SOUR value with TBP was sometimes higher than the corresponding SOUR without TBP.

Effects of REE on Other Parameters

Measurements of pH showed no significant difference between the treatments; final pH was consistently 6.5 across all treatments for the two sludges. COD was also not a useful diagnostic; measured values were typically on the order of 5 to 10 g/L and showed no consistent patterns. We suspected that this simply reflected the challenge of sampling a suspension, rather than a homogenous solution. Consequently we looked at whether soluble COD (following filtration through a 0.2 micron filter) was a more useful measurement; soluble COD was roughly 40-50% of COD, but we did not find any consistent pattern with soluble COD either.

Measurements of nitrogen species were expected to yield insight into effects of the amendments on nitrification activity. Ammonia was measured using a gas sensing electrode, and we found that TBP affected the membrane and therefore ammonia measurements of samples containing TBP were not reliable. For this reason we limit our presentation of ammonia results to the samples without TBP. Figures 3 and 4 show the data for ammonia concentrations in the presence of Eu and Y, respectively.

With the exception of Test 2 (March 27), the addition of Eu did appear to be associated with higher concentrations of ammonia remaining in the test mixtures (Figure 3). However, this was not necessarily reflected in the nitrate results on the various dates, which ranged from below detection (BD) to 3.3 mg-N/L and showed no consistent patterns, with REE and or TBP additions (data not shown). The addition of Y to the cultures seemed to have little or no effect on ammonia levels (Figure 4).

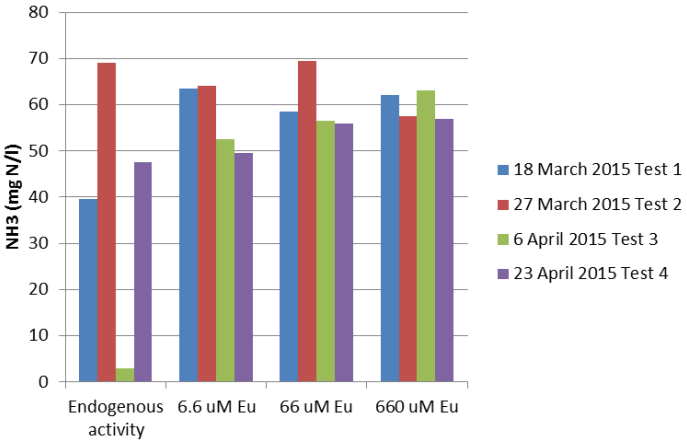

Figure 3 - Ammonia concentrations in presence of varying amounts of Eu

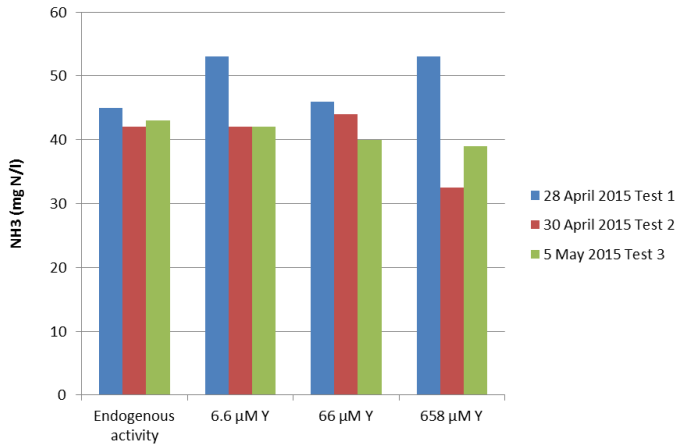

Figure 4 - Ammonia concentrations in presence of varying amounts of Y

DISCUSSION

The endogenous SOUR measurements conducted on the two different batches of activated sludge showed that the sludge activity can vary over time at a single facility; this was not unexpected, given that sewage flows into the plant and industrial inputs vary and therefore the different microbial populations in the activated sludge will vary in response (King & Painter, 1986). In addition, the IF WWTP underwent

major upgrades in February 2015, with installation of new aeration basins and associated pump and blower equipment, and it is likely that the changes in operations caused significant system fluctuations to occur prior to reaching steady state performance.

Because of the time consuming nature of the assays and because we wished to use the same inoculum for replicated experiments, from each batch of sludge we froze aliquots to use in our experiments. The results for the endogenous activity controls clearly indicated that biological activity changed with freezing and storage time. As noted earlier, a test with the April 2015 sludge indicated that the SOUR decreased by 11% after one day of freezing, compared to the freshly collected sludge. The drop in activity continued with greater storage time. For each batch of sludge, there was a large difference between the SOUR measured for the endogenous treatments on the later experimental dates compared to the early dates (soon after sludge collection). For example, for the April 2015 sludge if the endogenous treatments are compared for the first three experimental dates (within 2 weeks of sludge collection) the coefficient of variation for SOUR was 6%, while if the data for the last two experiments (6 weeks later) are included in the comparison the coefficient of variation was 48%. Replicate endogenous experiments on that date (June 3) were also widely different from each other (Figure 2). For the March 2015 sludge the coefficient of variation for the first two measurements (within 2 weeks) was 17%; if the latter two (3 and 5.5 weeks) were included the value increased to 52%. Changes to the activated sludge likely included not only reductions in the total numbers of microbial respirers but also changes in particular microbial clades (e.g., nitrifying bacteria), due to varying susceptibility to harm from freezing and thawing as well as other sample handling procedures.

Given the observed deterioration and increased variability in endogenous activity with prolonged storage of the activated sludge inoculum, limiting the data used for evaluation of the effects of the REE and TBP additions to the experiments conducted within two weeks of sludge collection seems prudent. With this prerequisite, the Eu addition experimental data set is limited to two replicates for the entire series. Those data indicated that Eu amendments at all 3 levels reduced SOUR by approximately half; increasing the amount of Eu from 6.6 to 66 to 660 µM had no obvious effect on the extent of inhibition. In contrast, the triplicate set of experiments with Y additions indicated that only the highest amount of Y (660 µM) consistently exerted a negative effect on SOUR, resulting in reductions ranging between 11 and 25%. The apparent greater toxicity to the activated sludge of Eu compared to Y contrasts with previous studies testing Eu and Y with pure cultures of nitrifying bacteria. In those studies, Y appeared to be more toxic than Eu. For the ammonia oxidizer *Nitrosomonas europaea*, 562 µM Y resulted in a decrease in ammonia oxidizing activity of 66%, while a larger amount of Eu, 660 µM, was associated with a smaller decrease in activity, only ~20%. The difference between the two REE was less apparent but still observed, with the same bias toward greater Y toxicity, with the nitrite oxidizer *Nitrobacter winogradskyi*. *N. winogradskyi* was inhibited >95% by 562 µM Y, while 660 µM Eu was associated with a 83% decrease in activity (Fujita et al., 2015).

On each of the five testing dates completed within 2 weeks of sludge collection, the effect of TBP addition (0.1 g/L) was also tested. On four of five of those dates, TBP addition (in the absence of REE) resulted in a large decrease in SOUR, ranging from 33 to 70%. On the one date when TBP addition did not appear inhibitory (April 28, 2015), the measured SOUR in the TBP treatment was greater than the control by 5%. TBP amendment in addition to Eu or Y addition did not appear to result in either enhancing or alleviating inhibition. The results with TBP were in contrast with previous studies using *N. europaea* and *N. winogradskyi*; in those experiments TBP addition enhanced Eu toxicity to *N. europaea*, while TBP alone or in combination with Y was completely inhibitory to *N. winogradskyi* (Fujita et al., 2015).

Neither pH nor COD measurements appeared to yield useful insight into the effects of the tested amendments; pH remained unaffected, and COD was too variable, possibly due to the challenge of sampling from suspensions as opposed to homogeneous solutions. No useful diagnostic pattern could be discerned from the ammonia and nitrate levels following Eu or Y addition either; if ammonia oxidation in the activated sludge was inhibited by the REE, one might expect that ammonia would build up and nitrate would not be formed, but it is also possible that ammonia production via processes such as degradation of

urea or proteins from the synthetic sewage would be inhibited. Inferring system behavior from a single point measurement of a transient species such as ammonia or nitrate in such a complex system is difficult. Because oxygen is only utilized, and not produced in this system, tracking its rate of consumption via SOUR measurement provides a more robust measure of the activity of the microbes in the activated sludge.

CONCLUSIONS

Our experimental results indicate that europium and yttrium have the potential to negatively impact activated sludge activity. The actual SOUR values measured may have been affected by freezing of the activated sludge inocula, and this may have also altered the relative effects of the different concentrations of rare earths and TBP, but we feel that our findings with respect to the effects of Eu and Y on biological wastewater treatment consortia merit attention and further investigation. Additional experiments are being conducted to confirm some of the findings and to test other potential components of rare earth processing wastewaters. Ultimately this research will contribute to the development of environmentally and economically sustainable beneficiation and recycling processes by providing guidance for avoiding the generation of wastewaters detrimental to wastewater treatment plant performance.

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