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# Anaerobic-aerobic granular system for high-strength wastewater treatment in lagoons

Rania A. Hamza*, Oliver T. Iorhemen and Joo H. Tay

*Department of Civil Engineering, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada*

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**Abstract.** This study aimed at determining the treatability of high-strength wastewater (chemical oxygen demand, COD>4000 mg/L) using combined anaerobic-aerobic granular sludge in lagoon systems. The lagoon systems were simulated in laboratory-scale aerated and non-aerated batch processes inoculated with dried granular microorganisms at a dose of 0.4 g/L. In the anaerobic batch, a removal efficiency of 25% was not attained until the 12th day. It took 14 days of aerobic operation to achieve sCOD removal efficiency of 94% at COD:N:P of 100:4:1. The best removal efficiency of sCOD (96%) was achieved in the sequential anaerobic-aerobic batch of 12 days and 2 days, respectively at COD:N:P ratio of 200:4:1. Sequential anaerobic-aerobic treatment can achieve efficient and cost effective treatment for high-strength wastewater in lagoon systems.

**Keywords:** biological wastewater treatment; dried granular microorganisms; high-strength wastewater; lagoon systems; sequential anaerobic-aerobic treatment

## 1. Introduction

Industrial wastewater, typically referred to as high-strength wastewater, is a major source of water pollution due to its elevated organic content. High-strength wastewaters are characterized by chemical oxygen demand (COD) concentrations greater than 4000 mg/L (Chan *et al*. 2009, Hamza *et al*. 2016). The effluents of these industries need to undergo pretreatment followed by biological treatment to remove the organic matter. However, conventional biological treatment processes fail to stabilize high-strength wastewater to regulatory limits. Aerobic treatment processes are not economically feasible for the treatment of high-strength wastewater. Anaerobic processes suffer from low bacterial growth rate, high sensitivity to toxic loadings, fluctuations in environmental conditions, and require post treatment to bring the water quality within regulations (Leitão *et al*. 2006, Chan *et al*. 2009, Grady Jr *et al*. 2011, Chan *et al*. 2012).

Lagoons have been widely used for wastewater treatment. Lagoons are large shallow basins enclosed by earth embankments in which wastewater is treated using entirely natural processes involving both algae and bacteria (Mara 2004). The activities of autotrophic, phototrophic, and

*Corresponding author, Ph.D. Candidate, E-mail: rania.sayedeid@ucalgary.ca
[a]Ph.D. Candidate, E-mail: oliver.iorhemen@ucalgary.ca
[b]Professor, E-mail: jhatay@ucalgary.ca


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heterotrophic microorganisms are employed to remove wastewater pollutants (Shpiner *et al.* 2009). Lagoons offer the advantages of being very simple to construct, having low capital, operational and maintenance (O&M) costs, and exhibiting good resistance to hydraulic and organic shock loads (Mara 2003, Mara 2004). The major disadvantage of the technology is the large land requirement. However, where space is not a constraint, lagoon systems remain attractive processes (Orupold *et al*. 2000).

Lagoon systems have been employed to treat high-strength wastewaters (Rakkoed *et al.* 1999, Rajbhandari and Annachhatre 2004, Arbeli *et al.* 2006, Shpiner *et al.* 2009). Anaerobic lagoons are typically employed to treat high-strength wastewaters. However, the treatment efficiency in anaerobic lagoons is limited to only 60% and therefore, they are followed by facultative lagoons to provide the required treatment (US EPA 2002). The pathways for pollutants removal in lagoon systems are sedimentation and biodegradation (Rajbhandari and Annachhatre 2004).

The structure of microorganisms responsible for biodegradation plays an important role in the treatment process. Since lagoons employ naturally-occurring microorganisms, the removal efficiency is limited to that offered by flocculent sludge. To enhance the performances of biological treatment processes, a novel biotechnology - granulation - has emerged. Granules are aggregates of microorganisms that form through microbe-to-microbe self-immobilization in the absence of any biocarrier (Beun *et al.* 1999, Liu and Tay 2004). Granular sludge offers distinct advantages such as dense and strong microbial structure, high biomass retention time, tolerance to toxicity and resistance to shock loading when compared to suspended cultures (Ergüder and Demirer 2005, Adav *et al.* 2008, Maszenan *et al.* 2011). These granules are dense microbial communities containing millions of organisms per gram of biomass (Tay *et al.* 2009), which individually are not capable of completely degrading wastewaters, but complex interactions among the resident species can achieve rapid treatment of wastewater (Liu and Tay 2002, Liu and Tay 2004).

However, the cultivation of granules is carried out in an upflow reactor that requires controlled loading and operational strategy; and, it is influenced by a variety of factors such as reactor start-up, seed sludge, substrate composition, organic loading rate, feeding strategy, reactor design and hydrodynamics, settling time, exchange ratio and aeration intensity (Tay *et al.* 2001, Liu and Tay 2004, Adav *et al.* 2008, Show *et al.* 2012). These conditions do not apply to lagoons. Dried granules can provide a practical solution for commercial and industrial applications due to the convenient storage and handling, in addition to making the process entirely passive.

The present work investigated the application of dried granules (proprietary engineered granular microorganisms - EGMs) in treating high-strength wastewater in lagoon systems under aerobic and sequential anaerobic-aerobic conditions. It has been hypothesized that the effluent of anaerobic treatment contains solubilized organic matter suitable for subsequent aerobic treatment because of its reduced organic strength and enhanced amounts of nitrogen and phosphorus (Chan *et al.* 2009, Chan *et al*. 2012).

## 2. Materials and methods

### *2.1 Experimental design and operation*

5 L jars were used as batch reactors to depict the lagoon system in aerobic, anaerobic and sequential anaerobic-aerobic operations. Mechanical mixers were employed to provide gentle

mixing of the wastewater. Air was supplied into the aerated batch through fine-pore ceramic diffusers. The jars were covered to minimize losses by evaporation. A schematic diagram of the aerobic and the anaerobic system is shown in Fig. 1. Dry EGMs, provided by Acti-Zyme (Hycura) (Fig. 2), were applied at a dose of 0.4 g/L. Acti-Zyme EGMs are bio-augmentation products that include over six billion microbes and enzyme per gram. Energy Dispersive Spectroscopy (EDS) analysis showed that EGMs are composed of 60-65% (wt) carbon, traces of sodium, silica, calcium, magnesium, aluminum, potassium and iron oxides. The experiment was conducted at room temperature (21±2°C). The reactors were operated without pH control.

### *2.2 Wastewater*

Synthetic wastewater, using sodium acetate as carbon source, was used in the experiments. Nitrogen ($NH_4Cl$) and phosphorus (buffer solution of $KH_2PO_4$, $K_2HPO_4$) were supplemented to provide a COD:N:P ratio in the desired range. In the literature, it has been indicated that a COD:N:P ratio of 100:5:1 is required for aerobic treatment and 700:5:1 to 250:5:1 for anaerobic treatment (Droste 1997, Chan *et al.* 2009, Metcalf and Eddy Inc. *et al.* 2014). However, the variations in the removal efficiencies and the observed biomass yield are important factors that

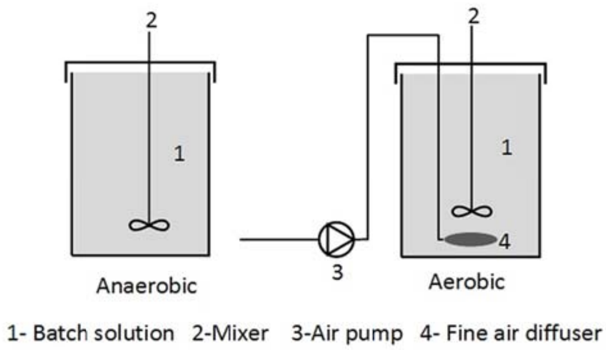


Fig. 1 Schematic diagram of the anaerobic and the aerobic jars

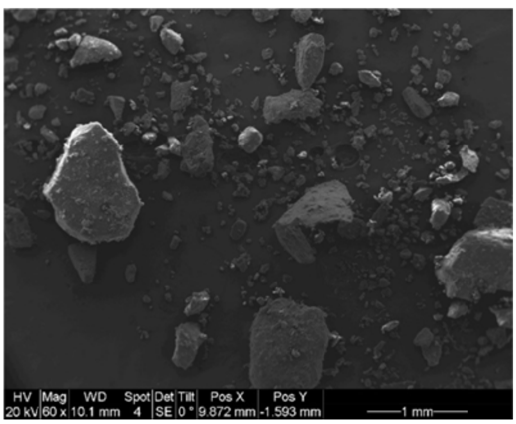


Fig. 2 Scanning Electron microscope (SEM) image of EGMs

determine the appropriate COD:N:P ratio for each type of wastewater. Ammary (2004) found that a ratio of 900:5:1.7 for anaerobic treatment of olive mills wastewater could achieve 80% COD removal and that a ratio of 170:5:1.5 in an aerobic treatment for pulp and paper wastewater achieved a COD removal of 75%. Generally, based on the universally accepted biomass chemical formula ($C_5H_7NO_2P_{0.074}$), the phosphorus requirement is approximately one-fifth that of nitrogen on a weight basis. Based on this, in the present study, various COD:N:P ratios were investigated, considering nutrient-abundant conditions as well as nutrients-scarce conditions. Initial COD concentrations, COD:N:P ratios, pH, dissolved oxygen (DO), operational conditions and hydraulic retention time (HRT) for the batch experiments are detailed in Table 1.

### *2.3 Procedures*

Batch experiments were designed to determine treatment efficiency at COD>4000 mg/L in aerobic, anaerobic and sequential anaerobic-aerobic processes. Aerobic, anaerobic, and sequential anaerobic-aerobic conditions were tested (Table 1). Solutions were inoculated with a dose of 0.4 g/L dried granules divided into two equal doses: at the beginning of the experiment and on the 7th day. The treatment processes were monitored for two weeks.

### *2.4 Analytical methods*

Samples were withdrawn daily. The withdrawn samples were filtered using 0.45 µm syringe filter. Samples were analyzed for residual soluble COD (sCOD), $PO_4^{3-}$, TN, TKN, $NH_3$ using HACH kits. COD was measured using COD USEPA reactor digestion method 8000 (HR). TN, TKN and nitrate + nitrite were measured using simplified TKN TNT 880. Ammonia was measured using ammonia TNT 832 (HR). Phosphorus was measured using phosphorus reactive, molybdovanadate method 8114 (HR). The pH and the dissolved oxygen (DO) were monitored throughout the duration of the experiments.

Table 1 Initial COD concentrations, environmental conditions and HRT

| Batch ID | B1 | B2 | B3 | B4 | B5 | B6 |
|---|---|---|---|---|---|---|
| Initial COD (mg/L) | 5800 | 5660 | 5655 | 5575 | 5495 | 5650 |
| condition | O[a] | A/O[b] | O[a] | A/O[b] | O[c] | A/O[b] |
| COD: N: P | 100:4:1 | 100:4:1 | 200:4:1 | 200:4:1 | 300:4:1 | 300:4:1 |
| Initial pH | 7.1 | 6.7 | 7.2 | 7.0 | 7.0 | 7.3 |
| DO conc. (mg/L) | 7-8 | 0.2-0.4[d]<br>7-8[e] | 7-8 | 0.2-0.4[d]<br>7-8[e] | 7-8 | 0.2-0.4[d]<br>7-8[e] |
| HRT (days) | 14[e] | 12[d] + 2[e] | 14[e] | 12[d] + 2[e] | 14 | 12[d] + 2[e] |

a. O: oxic or aerobic (gentle mechanical mixing and one aerator)
b. A/O: sequential anaerobic-aerobic
c. O: oxic (two aerators, no mechanical mixing)
d. Anaerobic operation
e. Aerated operation

## 3. Results and discussion

### *3.1 The pH and dissolved oxygen (DO) concentration*

The pH in the non-aerated batches increased from a starting value of approximately 7.0 to 8.4, while in aerated batches it reached 9.5. The pH increase was higher in the batches with higher COD removal rates. The pH increase has been reported in previous research. Uzal *et al.* (2003) observed an increase in pH when the substrate was consumed by the microorganisms in anaerobic digestion, with effluent reaching pH 9.4. The stoichiometry for the aerobic oxidation of acetate can be as represented in Eq. (1) (Metcalf and Eddy Inc. *et al.* 2014)

$$0.125CH_3COO^- + 0.0295NH_4^+ + 0.103O_2 \rightarrow 0.0295C_5H_7O_2N + 0.0955H_2O + 0.0955HCO_3^- + 0.007CO_2 \quad (1)$$

The oxidation of sodium acetate in the presence of ammonia results in the formation of bicarbonate and carbon dioxide. However, the amount of carbon dioxide is small compared to the bicarbonate. Thus, complete neutralization was not achieved and an increase in pH was observed. Therefore, under aerobic conditions, the theoretical oxidation of acetate can be presented as follows

$$CH_3COO^- + H^+ + 2O_2 \rightarrow 2CO_2 + 2H_2O \quad (2)$$

Eq. (2) shows that for each mole of acetate oxidized, one mole of hydrogen is consumed, and thus pH is increased. In anaerobic conditions, carbon dioxide functions as hydrogen acceptor and the increase in pH is inevitable. The decomposition of acetate can be expressed by the following reactions, Eqs. (3)-(4) (Show *et al.* 2012)

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (3)$$

$$CO_2 + 8H^+ \rightarrow CH_4 + 2H_2O \quad (4)$$

The pH (over 8.5) may adversely affect biochemical activity of microorganisms. However, it seems that high pH did not adversely affect the heterotrophic growth under aerobic conditions, as sCOD removal was not impacted in all aerated batches. Therefore, it can be inferred that granular biomass are capable of withstanding alkaline conditions. However, further research needs to be conducted to study the effect of pH on treatment efficiency of EGMs for higher COD ranges (COD>10,000 mg/L).

The DO concentration was fixed throughout the experiments to maintain either aerobic or anaerobic conditions. Non-aerated batches exhibited a DO concentration of less than 0.4 mg/L, while aerated batches showed DO concentration of 7-8 mg/L.

### *3.2 The effect of COD:N ratio*

The effect of COD:N ratios, as shown in Table 1, was tested in both aerobic and anaerobic conditions. The aerated batch with COD:N ratio of 100:4 showed removal efficiency of 94% in terms of sCOD, while nitrogen was limited only to 60%. On the other hand, at COD:N ratio of 200:4, only 70% sCOD removal was achieved with over 95% nitrogen removal. Ammonium –N removal can be as a result of the microbial growth requirement for the nitrogen source since neither nitrate nor nitrite was produced. Similar results were obtained at COD:N ratio of 100:5

where all ammonia removal was the result of nitrogen requirement for bacterial growth (Yang *et al.* 2005).

In anaerobic conditions, however, the effect of COD:N ratio was significant. Anaerobic digestion is characterized by low nutrient requirements (Chan *et al.* 2009) with high susceptibility to ammonia inhibition. Despite ammonium ion being an essential nutrient source by means of nitrogen for anaerobic bacteria, free ammonia has inhibitory effect since it is freely membrane permeable (Yüzer *et al.* 2012). The pH controls the speciation of ammonia nitrogen in aqueous solutions, where total ammonia nitrogen exists as either ammonium ion ($NH^{4+}$) or free ammonia ($NH_3$), according to the following equilibrium reaction, Eq. (5). The increase in the pH will cause an increase in the concentration of free ammonia

$$NH_4^+ \leftrightarrow NH_3 + H^+ \tag{5}$$

It was reported that free ammonia concentration of 80 mg/L causes initial inhibition regardless of the pH (De Baere *et al.* 1984). In the anaerobic batch with COD:N ratio of 100:4, the pH increased drastically during the anaerobic digestion from a starting value of 6.6 to 8.1 after one day and continued to increase reaching 9.0 on day 14 (aeration was introduced on day 12). Considering the initial concentration of total ammonia-N of 250 mg/L in reactor B2 (COD:N:P=100:4:1), over 75 mg/L (approximately 30% of total ammonia-N) may be present in free form, suggesting that an inhibition took place. Yüzer *et al.* (2012) pointed out that free ammonia inhibition in anaerobic treatment under mesophilic conditions has been reported in the range of 50-150 mg/L. This is reflected in the low consumption of ammonia at COD:N=100:4 (ammonium nitrogen concentration of 250 mg N/L) in anaerobic conditions, where only 38% ammonia-N was removed, with residual concentration of 173 mg/L (as $NH_3$) of total ammonia on the 14$^{th}$ day.

It is worth mentioning, however, that the presence of sufficient nutrients during the aerobic operation is a key factor for successful treatment. The highest removal efficiency of 96% was achieved in sequential anaerobic-aerobic treatment at a total hydraulic retention time (HRT) of 14 days (12 days as anaerobic and 2 days as aerobic) and a COD:N ratio of 200:4:1. It seems the optimum condition for organics removal in sequential treatment lies at COD:N ratio of 50:1. At higher ratio of 75:1 (as in B6), only 46% removal was achieved. This was due to the drop in nitrogen concentrations in the anaerobic batch B6, raising the COD:N ratio to 500:5 on the 12th day of anaerobic. Therefore, no sufficient nutrients were available for the aerobic process. On the other hand, the overabundance of nutrients hindered the treatment processes. At COD:N ratio of 100:4 in the anaerobic batch, ammonia inhibition took place and the subsequent aerobic treatment resulted only in slight enhancement in removal efficiency (from 20% to 28% sCOD removal in sequential anaerobic- aerobic process of 12 days and 2 days, respectively). It has been indicated that a COD:N ratio of 100:5 is preferred for aerobic treatment, and that anaerobic degradation can be successful at a ratio of 100:2.4 (Frigon *et al.* 2009). These results confirm that a COD:N ratio of 100:2 can provide suitable conditions for biological treatment of high-strength wastewater in sequential anaerobic-aerobic treatment.

### *3.3 Soluble COD removal*

The sCOD removal and the corresponding pH values with time in all the batches are shown in Fig. 3. During the first week of treatment, low removal rates were observed in all batches. After inoculating the batches with a second dose of 0.2 g/L (total of 0.4 g/L) of EGMs, residual sCOD concentrations started to decrease significantly and continued to drop until the end of the experiment. This contributed to the increase of HRT in the batch. The aerated batch at COD:N ratio of 100:4 showed sCOD removal efficiency of 94%, while in aerated batches of COD:N ratio of 200:4 and 300:4 removal efficiencies of 70% and 83%, respectively were achieved.

Minimal removals were achieved in the non-aerated batches (17-25% removal) for 12 days of

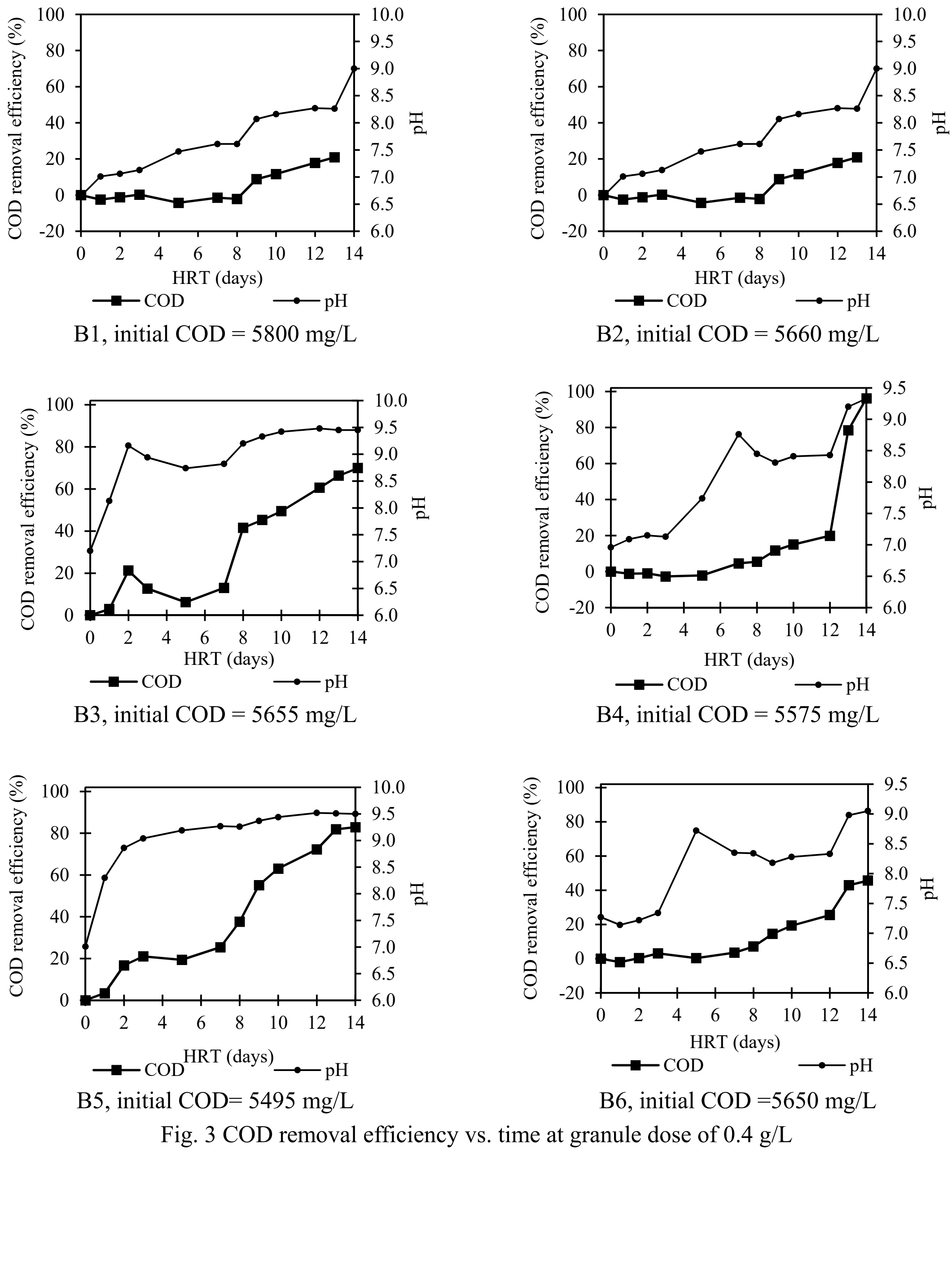


Fig. 3 COD removal efficiency vs. time at granule dose of 0.4 g/L

anaerobic operation. However, when aeration was introduced, significant removal rates were observed. It was found that although an anaerobic treatment of 12 days removed only 20% of COD, the aerobic operation of 2 days amended this low removal, so that the total removal efficiency for COD amounted to 96%. Thus, sequential anaerobic-aerobic treatment with short aerobic duration can provide better organics removal compared to aerobic treatment alone for the same total HRT. These results indicate higher removal rates in shorter aerobic operation compared to previous results found in the literature. It has been indicated that batch aerobic reactors operation for 15 days, following anaerobic digestion of 25.8 h, reduced COD concentration from 1476 to 649 mg/L (56% removal only) (Uzal *et al.* 2003). It has been suggested that prolonged anaerobic HRT and reduced aerobic reaction time is considered the best condition for COD removal (Muda *et al*. 2013). Furthermore, it has been emphasized that aerobic reaction can compensate for low COD removal rates in anaerobic reaction achieving drastic increase in overall treatment efficiency (Moosavi *et al.* 2005).

In addition, while the effect of inoculation dose of granules was not studied in this work, it seems that the granular inoculation dose is critical in the treatment of high-strength wastewater and that it is directly proportional to the strength of wastewater. In previous work (not shown), a dose of 0.2 g/L was sufficient for COD removal at initial COD concentrations of 1000-1200 mg/L and only 6 days were required for treatment achieving sCOD removals above 95%. However, at COD concentrations >5000 mg/L, a dose of 0.2 g/L provided removals less than 25% at HRT of 7 days in all batches. When a second dose of 0.2 g/L was introduced, a drastic increase in COD removal efficiency was observed in all samples, except at the anaerobic batch B2 (COD:N of 100:4), where ammonia inhibition might have taken place. Therefore, in this work, the overall HRT of the batches was extended to 2 weeks to allow for one week of treatment after the second application of the dried granules. However, further tests are required to investigate whether one-time inoculation dose of 0.4 g/L or two-step inoculation of 0.2 g/L would provide better removal efficiencies.

## 4. Conclusions

Lagoon systems were simulated in batch reactors for the treatment of high-strength synthetic wastewater (COD~5500-6000 mg/L) using proprietary EGMs.

- Removal efficiency of 94% was achieved for COD concentration >5000 mg/L at 14 days of aerobic operation at COD:N:P ratio of 100:4:1.
- Sequential anaerobic-aerobic treatment at a total HRT of 14 days (12 days as anaerobic and 2 days as aerobic) and a COD:N:P ratio of 200:4:1 provided better overall COD removal of 96%.

These findings indicate that combined anaerobic-aerobic treatment can save on energy and nutrient requirements and provide better removal efficiency for high-strength wastewater in lagoon systems.

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