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Augmenting Lagoon Process Using Reactivated Freeze-dried Biogranules

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Abstract This study investigated the feasibility of using freeze-dried biogranules in lagoon basins. The effect of different operational conditions on treatment performance and detention time of granule-based lagoons was examined in a series of laboratory-scale batch studies. Optimal granule dosage was 0.1 g/L under anaerobic condition, resulting in 80–94% removal of 1000 mg/L chemical oxygen demand (COD) in 7–10 days. Under aerobic condition, granule dosage of 0.2 g/L achieved the best result for identical COD concentration. However, adequate amount of nutrients (optimal COD/N/P ratio of 100/13/0.8) should be supplied to encourage the growth of aerobic species. At optimal COD/N/P ratio, aerobic treatment interval significantly reduced to 2–3 days with corresponding COD removal efficiency of 88–92%. Inhibition of high concentrations of COD (5000 mg/L) and ammonia (480 mg/L NH_4-N) was observed on microbial activity and treatment capacity of the biogranules. Mixing was a crucial measure to overcome mass transfer limitation. Onetime inoculation of lagoon with fresh granules was the best approach to achieve a satisfactory treatment efficiency. This study suggested

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Highlights
- Application of reactivated biogranules in lagoon process was studied for the first time.
- Impact of attached growth in the form of granules was investigated on the treatment interval in lagoons under different operational conditions.
- Aerobic oxidation and anaerobic decomposition in granule-based lagoons were optimized.
- Required detention time for degradation of high concentration of organics was significantly decreased below 3 and 10 days in the presence and absence of oxygen, respectively.

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that utilization of the biogranules is a feasible and sustainable technique for augmenting lagoon plants in terms of improved effluent quality and reduced retention time.

Keywords Biological wastewater treatment · Detention time · Lagoon · Nutrients · Reactivated biogranules · Treatment augmentation

Introduction

Lagoons are known as low-cost wastewater treatment processes in terms of installation, operation, and maintenance. These processes are suitable for small municipalities where limited budget and skilled worker are decisive factors in selecting the appropriate wastewater treatment method [1]. Well-designed lagoon plants could effectively remove a wide range of contaminants such as nutrients [2, 3], heavy metals [4], emerging contaminants [5], and pathogens [6]. Owing to these features, lagoon processes have been realized as attractive and cost-effective upgrade alternatives.

However, large space requirement has restricted the application of lagoons to rural communities where sufficient land for accommodating lagoon basins is available and inexpensive [7]. Footprint of a wastewater treatment plant (WWTP) is a function of hydraulic residence time (HRT) and sludge accumulation rate [8]. Due to the lack of sludge management system, HRT and solids retention time (SRT) are coupled in lagoon processes. As a result, the HRT of lagoon plants should be extended to meet the required SRT [9]. For instance, typical HRTs of 20–50, 10–40, and 3–10 days have been suggested for anaerobic, aerobic, and aerated lagoons, respectively. Depending on the ambient temperature, detention time of 25 to 180 days has been advised for facultative lagoons. Sludge accumulation in lagoon basin worsens the situation and increases the volume requirement. It has been advised that up to 5% of the volume of lagoon basin should be reserved for sludge deposits in cold regions where the highest net sludge accumulation rate has been observed [10]. In a lagoon basin, the biomass could either settle in the lagoon or discharge with the effluent, depending on its density. Hence, improving the settling property of the sludge can be a strategy to enhance the biomass retention in the system. This method can potentially disintegrate the SRT and HRT, and subsequently decrease the footprint of the process.

Granulation technology has been recognized as a promising technique in dealing with high-strength wastewater and toxic compounds [11, 12]. This technology involved the formation of dense and multispecies microbial aggregates which could significantly improve the settling property of sludge, thus, the biomass retention time. As a result, sludge volume index (SVI), known as an indicator of sludge settleability, decreased from 270 mL/g for flocculent inoculum to 12–35 mL/g for phosphorus-accumulating granules [13]. de Kreuk et al. [14] could reach the long SRT of 71 days in a granular sequencing batch reactor (SBR) without any separate sedimentation step, while a short HRT of 5.6 h was maintained. These features allowed integration of biological reaction and sedimentation in a single reactor, resulting into a compact footprint [15]. Although the granules could only form under the strong selection pressure of an upflow hydraulic regime [16], its storage capacity proved the feasibility of mass production and subsequent utilization in the systems with different hydraulic flow patterns such as lagoons [17, 18]. Thus, the granulation technology can be a potential solution for the long detention time and large footprint of lagoon systems.

However, application of biogranules for augmenting lagoon processes has not received attention so far. In this study, granulation technology and lagoon treatment were linked using proprietary biogranules. The objective of the study was to investigate the effect of different operational conditions on treatment performance of granule-based lagoons with a focus on detention time. A series of laboratory-scale batch studies were conducted using synthetic wastewater. Aerobic and anaerobic treatment competency of biogranules in removing organic matter and ammonium nitrogen were optimized. The effect of granule dosage, initial concentration of organic matter, inoculation method, acclimatization, and the presence of sufficient mixing, air, and nutrients were monitored on treatment performance of lagoon.

Materials and Methods

Experimental Setup and Operational Condition

The laboratory-scale experimental setup (Fig. 1) consisted of bioreactors (18.7 cm diameter × 20 cm height) with individual working volume of 5.0 L. Scale of the setup was selected based on the study of Alcántara et al. [2] where the authors investigated nitrous oxide emission from lab-scale high-rate algal ponds (HRAPs) with the working volume of 7 L. The bioreactors were operated under batch mode. Such operational condition could provide an insight into the treatment capacity of the biogranules at different detention times, only by extending the duration of the experiment. Synthetic wastewater was introduced to the bioreactors onetime at the beginning of the experiment. Then, the wastewater was stirred in the bioreactors by means of mechanical mixers (Laboratory mixer Stir-Pak, Model 04555-00), equipped with three-bladed propeller. The mixers were operated at the speed of 1500 rpm which could maintain granules in suspension. At lower speed, the granules were settled down at the bottom of the reactors. When aerobic condition was supposed to prevail, air was continuously diffused into the reactors using fine bubble diffusers. The level of dissolved oxygen (DO) was not controlled at a

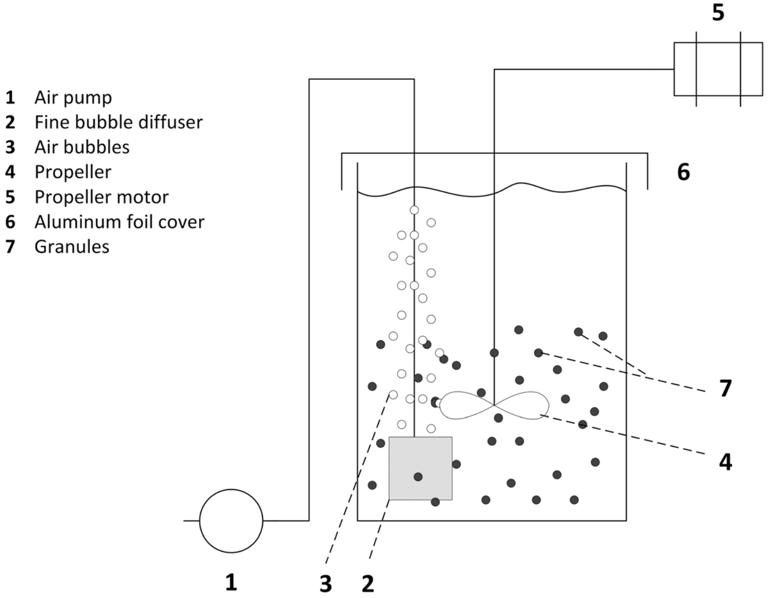


Fig. 1 Schematic of laboratory-scale experimental setup for the batch studies

specific concentration. However, the observation showed that it was constantly high enough (7.7 ± 0.5 mg/L) to ensure complete aerobic condition and to prevent from local anaerobic zones. The DO concentration was not controlled under anaerobic condition either; however, constant DO monitoring proved that the DO concentration was dominantly below 0.5 mg/L. Losses due to water evaporation were observed to be increased by applying aeration. Thus, the bioreactors were covered with aluminum foil sheets. The reactors were operated indoors at daytime temperature of 20 ± 3°C. The experiments were extended until a satisfactory treatment efficiency (COD removal efficiency ≥90%) was achieved.

The batch studies could simulate the situation of actual anaerobic as well as aerobic and aerated lagoons in terms of corresponding DO concentrations. Deep bioreactors were selected as anaerobic condition could be easily achieved in such reactors with little or no control; covering the bioreactors by aluminum foil sheets caused the anaerobic condition to happen within a short time (few hours), predominantly in the deeper zones. Whereas, the aeration could ensure constant and homogenous aerobic condition in the bioreactors. No sludge management system was devised to be consistent with full-scale practices; in actual lagoons, power input level has not been adequate to control the retention of solids in the basin. Similarly, in this study, solids could either settle down or be washed out with the effluent if the system was being operated under a continuous flow mode. However, various environmental factors can alter the hydraulic flow pattern in actual cases. Those factors included water column mixing induced by wind and/or convection, and wet and dry weather flows. Such factors could not only influence on the hydraulic regime but they could also change the composition of influent wastewater considerably [19]. Residence time distribution (RTD) studies with tracer experiments were suggested to compare flow pattern in actual lagoons and batch and continuous lab-scale bioreactors. Significant enhancement in treatment efficiency of full-scale lagoon plants inoculated by the proprietary biogranules could be considered as a proof for the result of this study (Pishgar, unpublished).

Seed Sludge and Inoculation Protocol

Proprietary bio-enhanced granular microorganisms (EGMs), also known as Hycura™, were used as inoculum in this study. EGMs were freeze-dried biogranules which have been mass produced and commercialized by Acti-Zyme Products Ltd. in Canada (Fig. 2). The EGMs consisted of aggregated and lyophilized numerous facultative and anaerobic microorganisms alongside adequate enzymes and nutrients for reactivation and biochemical activity of present organisms. The nutrients included about 70% organic matter, 40% organic carbon, and 4% Kjeldahl nitrogen. Spatial distribution of different inorganic elements in a single biogranule is shown in Fig. S1 (see Supplementary Material). The included enzymes were (units per mg granule) as follows: amylase (7.5), protease (2.36), and lipase (21.6).

Optimal dosage of granules was determined by examining a wide range of granule concentrations (0.05 to 1.0 g/L) under aerobic and anaerobic conditions. The best inoculation approach (i.e., onetime or stepwise) was investigated by addition of 0.2 g/L EGMs to two separate bioreactors; at onetime inoculation, required dose of biogranules was added to the bioreactor at the beginning of the experiment. Stepwise inoculation was accomplished in five steps; biogranule dosing scheme included (in g EGMs to 5 L of synthetic wastewater) the following: 0.25 at the beginning of the experiment, 0.05 at day 1, 0.25 at day 4, 0.25 at day 6, and 0.2 at day 8. Acclimated granules were taken from those bioreactors where degradation of 1000 mg/L COD was aerobically completed using 0.1 g/L EGMs. The inocula were

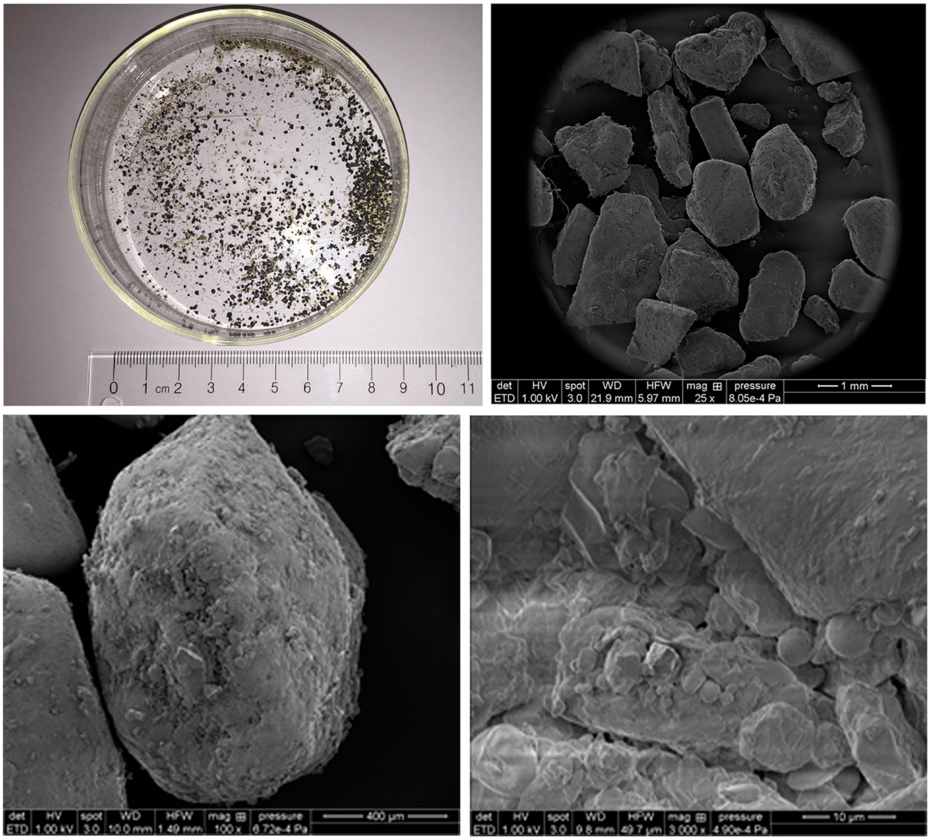


Fig. 2 SEM images of freeze-dried biogranules (EGMs) at dry state with different scales and magnifications

withdrawn from the reactors after bringing granules into the suspension. Two inoculum concentrations of 20 and 80% (*v*/*v*) were examined. Dry biogranules were hydrated in lukewarm water for 20 min prior to usage.

Wastewater Composition

Nutrients and enzymes were included in the granules (see Supplementary Fig. S1). Such amount of nutrients was presumed to be sufficient for reactivation and performance of present microorganisms. Thus, addition of nutrients has not been advised by the manufacturer for commercial applications. To be consistent with actual cases, extra nutrients were not supplemented at the initial stages of the experiment. Wastewater was first synthesized by addition of sodium acetate to deionized water. Initial COD concentration was fixed at either 1000 or 5000 mg/L, depending on the type of the experiment. The aim of this study was to simulate high-strength municipal wastewater with COD concentration above 1000 mg/L [20] and high-strength industrial effluents with over 4000 mg/L [21]. To observe the effect of additional nutrients on the treatment performance, nitrogen (N) and phosphorus (P) were further supplemented to the wastewater. At this stage, COD of synthetic wastewater was maintained at 1000 mg/L, and the concentrations of ammonium nitrogen (NH_4-N) and phosphate (PO_4-P) varied between 25–480 and 2.7–15 mg/L, respectively. Desired concentrations of ammonium nitrogen and phosphate were adjusted by the addition of NH_4Cl and potassium phosphate buffer (KH_2PO_4 and K_2HPO_4 with molar ratio of 1:1). Fig. S2 (see Supplementary Material) illustrates the matrix of optimization for nutrient concentrations, with the corresponding COD/N/P ratios. Initial pH of the wastewater was maintained at neutral condition (6.8 to 7.0).

Analytical Methods

Concentrations of soluble COD (sCOD) and mixed liquor volatile suspended solids (VSS) were determined using standard methods [22]. Dissolved organic carbon (DOC) was measured using combustion total organic carbon (TOC) analyzer (Teledyne Tekmar, Apollo 9000) equipped with an autosampler (Folio Instrument Inc., model Kitchener 519-748-4612). An empirical correlation ratio between DOC and sCOD was established and repeatedly controlled. Thus, fast and convenient DOC method was used to determine the soluble organic content and oxygen demand of the wastewater. Concentrations of ammonium nitrogen (NH_4-N) and phosphate (PO_4-P) were analyzed by ion chromatography (Metrohm Compact IC Flex equipped with conductivity detector and autosampler). DO concentration and pH were monitored by portable DO meter (YSI, 550A) and pH meter (VWR symphony SP70P), respectively. Samples were withdrawn on a daily basis. The samples were filtered using 0.45 μm filters and diluted by the factor of 10 prior to the analysis. Scanning electron microscopy (SEM) was performed on dry biogranules using FEI Quanta 250 FEG field emission scanning electron microscope.

Specific oxygen uptake rate by heterotrophic bacteria $(SOUR)_h$ was measured at the end of the batch experiments in accordance with standard methods [22]. The utilized respirometer (Respirometer Systems and Applications, LLC PF-8000) was equipped with a water bath to maintain a constant temperature of 30 °C during the test. The oxygen uptake rate was recorded at intervals of 1 min. The substrate was composed of acetate with COD concentration of 500 mg/L. At the end of the batch experiments, the mixed liquors were allowed to settle for 30 min, and 1 L of the settled liquor was retained. Then, the settled liquor was homogenized, and 50 mL of that was inoculated into $(SOUR)_h$ flasks as a representative of the microbial community in the bioreactors. The tests were performed in duplicate.

Results and Discussion

Optimization of Anaerobic Treatment

Optimal Concentration of Biogranules

Figure 3 depicts the effect of granule dosage on the detention time required for anaerobic degradation of 1000 mg/L COD. Reactivation performance of the biogranules in lagoon process was noteworthy. All the applied dosages resulted in 73 to 80% COD removal in 7 days. The COD removal efficiency improved by time and reached 83–94% at day 10, while the highest efficiency corresponded to 0.1 g/L. When the experiment period was extended to 14 days, the biodegradation rate of organic matter further enhanced and achieved a high value of 99% by the dosage of 0.1 g/L. Inoculation concentration of 0.1 g/L presented the best treatment efficiency and the fastest degradation rate within a period of 7–10 days. However, dosages of 0.05 and 0.2 g/L could also result in

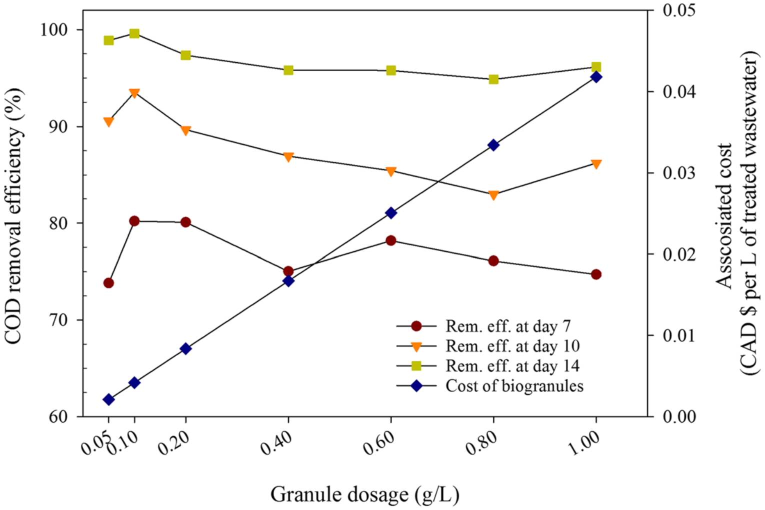


Fig. 3 Effect of granule dosage on detention time for anaerobic degradation of 1000 mg/L COD

remarkable COD removal rates of 74–90 and 80–89% in the same treatment interval, respectively. Although high dosages of the biogranules (0.4–1 g/L) resulted in satisfactory COD removal rates (82–86%) in 10 days, increase in the dosage proportionately increased the wastewater opacity which could be an indication of the residual suspended solids. Moreover, cost-effectiveness of the process plays a key role in choosing lagoons for the treatment purposes, which should not be compromised by the budget spent on quality biosolids for augmenting the system. The cost associated with each granule dosage is depicted in Fig. 3. Thus, anaerobic treatment using low granule dosages (0.05–0.2 g/L) could well meet both the economical perspectives and the effluent quality standards.

Utilization of the biogranules significantly decreased the degradation time of high concentration of organics in lagoons. Based on the definition of Metcalf and Eddy [20], 1000 mg/L COD was attributed to high-strength municipal wastewater, while typical domestic wastewater only contained 330 mg/L COD [23, 24]. Low treatment efficiencies of domestic wastewater have been reported in suspended-growth lagoons and ponds. García et al. [3] could remove 35 to 38% of 260 mg/L COD of urban wastewater in large-scale high-rate algal ponds in a period of 8–10 days. Cameron et al. [1] could achieve 34% removal of 3.62 mg/L 5-day biochemical oxygen demand (BOD_5) from the sewage lagoon effluent in 15 days in a three-cell wetland plant. Although Ewing et al. [8] could increase the COD treatment efficiency of facultative lagoon from 35–50 to 90% by introducing aeration, the initial concentration of COD varied between 120 and 140 mg/L and a considerable reduction in organic matter occurred between days 11 and 19. The results of this study revealed that inoculation of lagoons by the utilized biogranules could be a solution for rapid and efficient treatment of highly loaded municipal wastewater.

Effect of Organic Loads

High load of organic matter considerably impacted anaerobic treatment capacity of the biogranules (see Supplementary Fig. S3). Despite great competency of low granule dosages in removing 1000 mg/L COD (see Fig. 3), dosages of 0.1 and 0.2 completely failed to decompose high concentration of 5000 mg/L COD. However, granule dosage of 1 g/L could remove 18 and 60% of 5000 mg/L COD when the detention time was extended to 17 and 37 days, respectively. Although shortening treatment interval could desirably reduce the plant footprints, long detention time of lagoons has improved ammonia nitrogen removal efficiency in these systems; storage of treated swine wastewater in an anaerobic lagoon benefited the denitrification process and decreased residual concentration of total nitrogen from 241 to 2–11 mg/L within 180 days [25]. Conkle et al. [26] suggested that higher reduction rates of pharmaceutically active compounds, compared to those reported in conventional WWTPs, could be explained by the long treatment time of lagoon processes. Therefore, the process duration may have to be compromised when effective removal of refractory compounds and high concentrations of organic matter are intended in lagoons.

Mixing

Significant effect of mixing on treatment capacity of the biogranules and the rate of treatment can be seen in Fig. S4 (see Supplementary Material). Initial COD concentration and granule dosage were maintained at 1000 mg/L and 0.1 g/L, respectively. To eliminate the contribution of aeration to mixing, experiments were conducted under DO-free condition. Mechanical mixers were the only means of supplying mixing. When adequate mixing was available, a noticeable improvement in treatment performance of the biogranules was observed. Sufficient mixing shortened the treatment interval to 6 days when COD removal efficiency of 81% was achieved. Degradation of organic matter further continued and the COD removal efficiency reached 93.5% in 10 days. In lack of disturbance, the heavy biogranules settled down at the bottom of the bioreactor, and the rate of COD degradation was slowed down. Likewise, Yang et al. [27] reported a similar effect of mixing on denitrification potential of aerobic granules; substantial reduction in nitrate concentration was achieved only when adequate mixing was supplied. This phenomenon was explained by the establishment of a poor contact between the substrate and the granules in a stagnant solution.

Federation of Canadian Municipalities [7] suggested that modifying the mixing condition and flow pattern can optimize the operation of lagoon processes. The positive effect of mixing on the performance of granule-based lagoons has been proven in this study. Mixing did not only overcome the mass transfer limitation [27], but it could also alter the microbial structure of lagoons. Lovanh et al. [28] argued that water column mixing could change the biodiversity in anaerobic lagoon by circulating the biomass throughout the stratified basin and facilitating the access of organisms to various types and concentrations of substrate. Thus, the mixing could dictate the dominance of certain species with high affinity to available forms of substrate, subsequently leading to an efficient treatment performance. Agitation in lagoons can be induced by either mechanical surface mixing or air infusion which are not economical methods due to energy consumption level. Energy sources of solar and wind powers can be manipulated for the mixing purposes. However, these renewable energy sources are site specific and seasonal with specific infrastructure requirements. As an alternative, Ewing et al. [8] demonstrated that harnessing redox energy gradients of aerobic and anaerobic zones in a facultative lagoon could provide a self-powered system.

Optimization of Aerobic Treatment

Optimal Granule Dosage

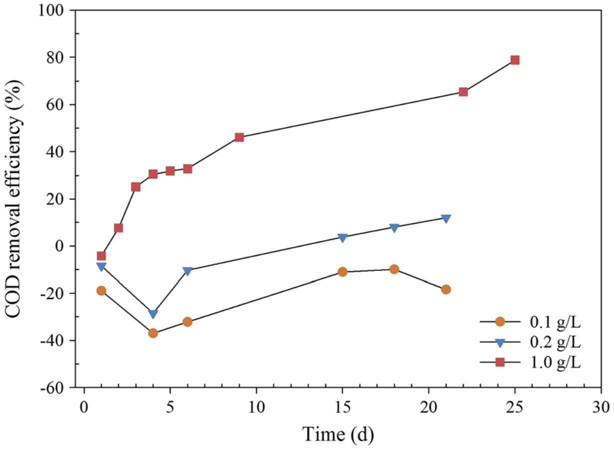
Fig. S5 (see Supplementary Material) shows treatment competency of different granule dosages for aerobic degradation of 1000 mg/L COD. Performance of the optimal dosage (0.1 g/L) was not affected by the presence of oxygen, compared to that observed under anaerobic degradation (see Fig. 3). Yet, aeration disrupted the treatment efficiency of 0.05 and 0.2 g/L biogranules. Using 0.05 g/L, aerobic treatment could retain COD removal rate of 89% in a long period of 13 days. When dosage of 0.2 g/L was applied, relatively low COD removal rate of 79 was obtained in 7 days. Besides, a lag phase of 2–3 days was observed in aerobic biodegradation profiles of low granule dosages (data not shown). In contrast, high granule dosage of 1 g/L demonstrated considerable treatment capability in the presence of ample oxygen. Although low dosages could remove less than 20% of COD concentration within a short time of 3 days, 1 g/L biogranules could degrade 59% of organic matter within the identical period. When detention time was extended to 6 days, low dosages could only remove 68% of 1000 mg/L COD on average, whereas 92% of COD concentration could be removed by the dosage of 1 g/L up to day 6 of the experiment.

Although mechanical aeration has been advised as an alternative for optimizing the performance of lagoon plants [7], aeration showed adverse effect on treatment performance of low granule dosages in this study. This phenomenon could be explained by the microbial structure of the utilized biogranules and the nutrient availability. Camacho et al. [29] categorized microbial diversity of the EGMs into obligate anaerobic and facultative bacteria. An unpublished report demonstrated that anaerobic population in EGMs exceeded 6.5 billion colony-forming unit (CFU) per gram, which was almost twofold of the number of included aerobic species (4 billion CFU/g EGMs). Therefore, sufficient bacteria were available for favorable anaerobic activity. Yet, low number of aerobic bacteria along with inadequacy of nutrients in the granular units delayed the aerobic growth and prolonged the treatment interval. However, when aerobic and facultative bacteria subsequently attained sufficient population, rapid aerobic activity offsets the initial reduction in treatment efficiency. Treatment behaviors of low and high dosages of the EGMs confirmed this argument. The lag phase, observed in aerobic degradation profiles of low granule dosages (data not shown), implicitly indicated the inadequacy of microbial population for completing the aerobic process. However, COD removal efficiency was further improved by time, indicating adjustment in the microbial selection. For instance, identical COD removal rates (95%) were achieved under both aerobic and anaerobic conditions by 0.2 g/L biogranules at day 15 of the experiment (data not shown). In contrary, high dosage of 1 g/L potentially introduced enough population of facultative and aerobic species to the system. Thus, aerobic oxidation was immediately started and the treatment interval was considerably shortened (data not shown).

Effect of Organic Loads

Increase in COD concentration to 5000 mg/L suppressed aerobic treatment capacity of low granule dosages of 0.1 and 0.2 g/L (Fig. 4). Although 65 to 79% of 5000 mg/L COD could be removed using high concentration of the biogranules (1 g/L), it was achieved in a long time of 22 to 24 days, corresponding to a slow degradation rate. However, treatment capability of 1 g/L of the biogranules was less affected by high load of organic matter under aeration, compared

Fig. 4 Aerobic treatment of 5000 mg/L COD at a wide range of detention times using different granule dosages



to that realized in anaerobic system (see Supplementary Fig. S3); identical degradation rate of 60% was achieved in 22 and 37 days under aerobic (see Fig. 4) and anaerobic (see Supplementary Fig. S3) conditions, respectively.

Inoculation Method

Fig. S6 (see Supplementary Material) shows the effect of different inoculation methods on aerobic degradation of 1000 mg/L COD, especially at short detention times. When 0.2 g/L biogranules were inoculated at a time, satisfactory COD reduction rates of 79 and 90% were achieved within 8 to 13 days, respectively. In contrary, a comparable COD removal efficiency could be achieved at a longer detention time when the same dosage was stepwise added. Stepwise inoculation prolonged the lag phase and increased COD concentration at initial days of the treatment (days 1 to 4). Increase in COD concentration could be correlated with the release of organic carbon by the fresh granules at each step of inoculation. In an actual case of rehabilitating a failed pond, a long recovery time was observed while 0.02 g/L of the biogranules was stepwise applied (data not published); a steady-state performance in removing 180 mg BOD_5/L was achieved in 4 months. According to findings of this study, the long recovery time could be explained by stepwise addition of a low granule dosage to the failed pond. Hence, onetime inoculation of lagoon seemed to be the optimal method to achieve a desirable treatment performance, specifically at low detention times.

Acclimated Biogranules

Fig. S7 (see Supplementary Material) illustrates the effect of acclimatization on aerobic treatment capacity of the biogranules. Previously used biogranules were used as acclimated consortia. Inoculum concentrations of 20 and 80% (*v*/*v*) were applied to remove 1000 mg/L COD under aerobic condition. To verify the effect of mixing on acclimatization, sufficient mixing was supplied to the bioreactor with inoculum concentration of 80% by both aeration and the mechanical mixer. At inoculum concentration of 20%, aeration was the only means of supplying mixing which resulted into an inadequate mixing condition.

Lag phase of COD degradation was prolonged to 3 to 4 days using acclimated biogranules (see Supplementary Fig. S7), compared to treatment performance of fresh granules (see Supplementary Fig. S5). Acclimatized consortia with inoculum concentration of 20% showed a slow COD degradation rate. COD removal of 52 to 68% was achieved in 6–7 days, respectively. At high inoculum concentration of 80%, degradation rate of organic matter was improved and COD removal rate reached 70% in 6 days. This phenomenon could be explained by proportionate increase in the number of microorganisms in bioreactor due to increase in inoculum concentration. However, enhanced treatment capacity could also be attributed to improvement in mixing condition at inoculation volume of 80%. As proposed earlier, sufficient mixing could facilitate the access of microorganisms to the substrate and, thus, could increase their growth rate. At relatively long detention time of 10 days, both inoculum concentrations resulted in identical degradation efficiency of organic matters (84–85%).

Adapting the available microbial community to prevailing condition improved the treatment capability of microorganisms in a previous study; Jain and Mattiasson [30] could achieve high methane production efficiency of 67% at low pH via acclimatizing methanogenic bacteria to pH of 5. Although, in this study, acclimatized consortia could satisfactorily treat the wastewater at short treatment times of 6 to 10 days, the treatment capacity was not comparable to that of fresh granules (see Supplementary Fig. S5). Supposedly, withdrawing the inocula at endogenous phase when the oxidation process had been completed affected the treatment capability of the culture. Since the microorganisms were at the autolysis state, a lag phase was required for their reactivation. As evaluation of the viability of adapted microorganisms can be difficult and complicated in practice, inoculation with fresh granules should be considered for actual applications.

Effect of Sufficient Nutrients

Unlike anaerobic treatment, it was observed that included amounts of nutrients in the EGMs were insufficient for the aerobic activity of microorganisms. Thus, the effect of additional nutrients on aerobic degradation of organic matter and removal of nitrogen were investigated.

Aerobic Degradation of Organics

Fig. S8 (see Supplementary Material) indicates the effect of various nutrient concentrations on aerobic treatment process. Respective N and P concentrations of 25–480 mg/L NH_4-N and 3–15 mg/L PO_4-P were considered, whereas the COD concentration was maintained at 1000 mg/L. Dosages of 0.1 and 0.2 g/L biogranules were selected to verify the optimal granule concentration under the new condition. The presence of extra nutrients enhanced aerobic treatment capacity of biogranules and significantly shortened the treatment interval to below 6 days. Both dosages of 0.1 and 0.2 g/L substantially reduced the organic matter concentration in 3–4 days. However, granule dose of 0.2 g/L presented a steadier treatment performance throughout the experiment. The optimal condition could be achieved by granule dosage of 0.2 g/L and COD/N/P ratio of 100/13/0.8. Under this condition, aerobic treatment of organic matter was completed in a short detention time of 2–3 days, corresponding to COD removal efficiency of 88–92%, respectively.

In general, the ranges of ammonium nitrogen (NH_4-N) and phosphate (PO_4-P) concentrations of 80–130 and 5–8 mg/L considerably influenced the aerobic treatment performance (see Supplementary Fig. S8). An efficient and stable operation was observed at these ranges of nutrient concentration. It was observed that decrease in granule dosage should be concomitant with increase in N concentration to maintain the degradation efficiency of organic matter. For instance, identical COD degradation trends were observed at COD/N/P ratios of 100/4/0.3 and 100/13/0.3 and granule dosages of 0.2 and 0.1 g/L, respectively. Similar behaviors were observed when granule dosage was decreased from 0.2 to 0.1 g/L while N concentration was concurrently increased from 100/2.5/0.5 to 100/13/0.5 and from 100/8/0.8 to 100/13/0.8, respectively.

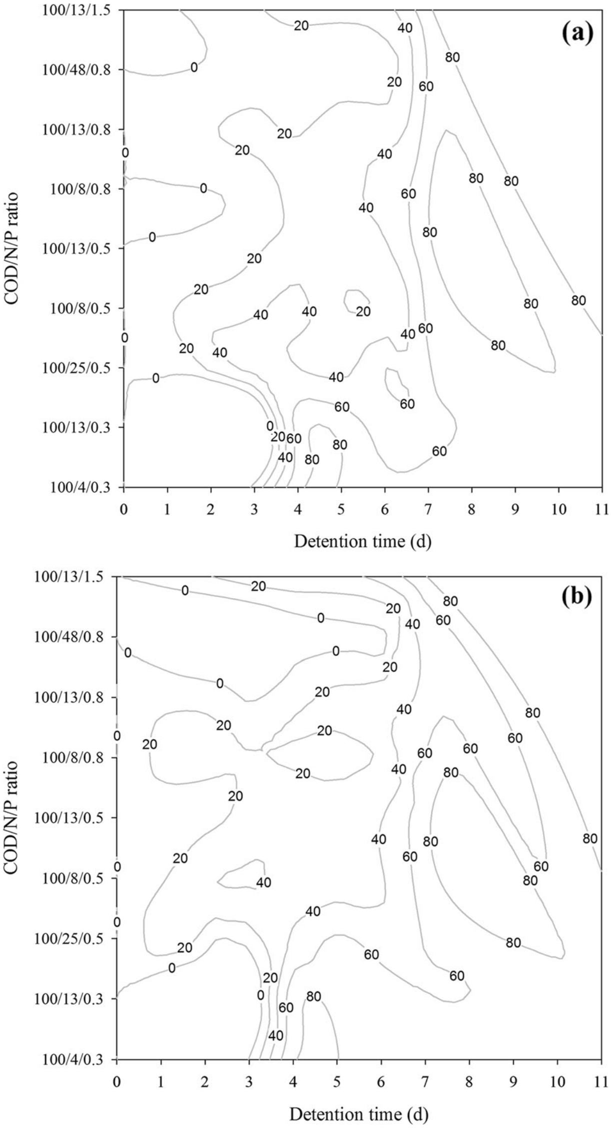
Nevertheless, extremely high concentrations of N deteriorated the treatment capacity of the biogranules (see Supplementary Fig. S8). When nitrogen concentration raised to 480 mg/L NH_4-N, aerobic treatment was severely disrupted, and COD removal efficiency considerably decreased to 68–72% by both applied dosages in ~7 days. Nonetheless, dosage of 0.2 g/L could endure this situation the best. The effect of high NH_4-N concentration could be described by the inhibition effect of non-ionized form of ammonia, also known as free ammonia (FA), on the microbial activity. FA concentration has been correlated with ammonium concentration, pH, and temperature, according to the equation proposed by Anthonisen et al. [31]. High pH value of 8.6 to 9.1 between days of 3 and 11 (see Supplementary Fig. S9) potentially shifted ammonium/ammonia equilibrium toward ammonia production. High NH_4-N concentration of 480 mg/L could theoretically produce 196 mg/L FA at pH of 9, which was drastically higher than the toxic FA concentration reported in the literature. Yang et al. [32] found out that FA concentration of over 23.5 mg/L totally impeded aerobic granulation process. Erşan and Erguder [33] defined toxic concentration of 38 to 46 mg/L FA for heterotrophic and nitrification activities of aerobic granules. Yang et al. [32] observed that respirometric activity (i.e., SOUR) of heterotrophic bacteria decreased by a magnitude of 5 when FA concentration raised from 2.5 to about 40 mg/L. Thus, very high concentration of ammonia should be avoided in aerobic granule-based lagoons as well.

The optimized COD/N/P ratio was close to the composition of domestic wastewater [20]. It could be suggested that EGMs are ideal biosolids for treating domestic wastewater which introduce enough nutrient amounts into the system for aerobic growth of the microorganisms. In practical applications, addition of nutrients should be devised for aerobic treatment of industrial effluent which generally suffers from lack of nutrients.

Nitrogen Removal

Figure 5a, b represents ammonium-N removal efficiency using 0.1 and 0.2 g/L EGMs, respectively. Reduction in NH_4-N concentration could be correlated with nitrifying activity of the biogranules. By both granule dosages, less than 60% of NH_4-N concentration was removed in 6 days throughout the experiments. Extending the detention time beyond 6 days represented positive effect on NH_4-N removal efficiency. As a result, the nitrification capacity of biogranules enhanced, and average ammonium removal rate of 73% was achieved at substrate ratios of 100/13/0.5 and 100/13/1.5 at day 7. Within 11 days, ammonium removal rate reached 74 and 79% at respective nutrient compositions of 100/4/0.3 and 100/13/0.3. The results were consistent with the study of Baskaran and Farago [34] where the attached growth was encouraged by adding plastic carrier to the multi-cell pond system. Thus, aggregation of microorganisms in the form of biofilm or granules can enhance nitrogen removal capacity of lagoon plants.

Fig. 5 NH_4-N removal efficiency at various substrate compositions using granule dosages of (**a**) 0.1 g/L and (**b**) 0.2 g/L



Profiles of 100/2.5/0.5 and 100/8/0.5 proved that low ammonium concentration favored the nitrification activity. It could be correlated with low production of FA at low ammonium concentration. High FA concentration could frustrate the activity of ammonia-oxidizing bacteria (AOBs) and nitrite-oxidizing bacteria (NOBs) [32, 33, 35]. Anthonisen et al. [31] reported the toxic range of 10 to 150 mg/L FA on nitrification process. Erşan and Erguder [33] observed the inhibition effect of FA on ammonium removal efficiency of aerobic granules in a narrow concentration range of 38–46 mg/L FA. Yang et al. [32] found out that SOUR of nitrifying bacteria decreased by 2.5 times when FA concentration increased from 2.5 to 40 mg/L. In this study, FA concentration of 4.6 to 67 mg/L was calculated based on NH_4-N concentration of 25–130 mg/L and pH of

8.6–9.1, which fell within the reported toxic range. Ammonium concentration of 480 mg/L could produce extremely fatal FA concentration of 196 mg/L at pH of 9; it could explain the complete inhibition of nitrification activity at 480 mg/L NH_4-N.

It was observed that the removal of ammonium N was not started unless COD degradation was completed (Fig. 5a, b). Delay in nitrification process could be correlated with the competition between heterotrophic and nitrifying bacteria for DO uptake in the presence of ample organic matter. Fast-growing heterotrophic bacteria outcompeted the growth of nitrifying organisms during the feast condition; thus, nitrification was inhibited at primary days of the experiment. Nitrification activity started upon entering the famine condition at day 6. Yang et al. [36] confirmed that organic matter must be completely removed so that slow-growing AOBs and NOBs could dominate in the system. Moreover, sensitive nitrifying bacteria are more likely to be active in a narrow pH range of 7.5–8.6 [35]. A drastic increase in pH was seen from 8 at day 1 to 8.6–9.1 between days 3 and 11 (see Supplementary Fig. S9) when nitrification was supposed to occur. All these phenomena could simultaneously contribute to disrupted nitrification activity.

Microbial Activity of Granular Culture

Figure 6 depicts the effect of aeration, initial COD concentration, and the presence of nutrients on microbial activity in terms of $(SOUR)_h$. Since the samples for $(SOUR)_h$ analysis were taken at the end of each batch assay, the $(SOUR)_h$ values could imply the population of survived facultative bacteria which could function both aerobically and anaerobically. Switching from anaerobic to aerobic condition reduced the $(SOUR)_h$ of low granule dosages (0.1 and 0.2 g/L) about 3.2 times on average, from 2.6 to 1 and 1.5 to 0.4 mg O_2/g VSS/h, respectively. It implicitly indicated the low population of viable

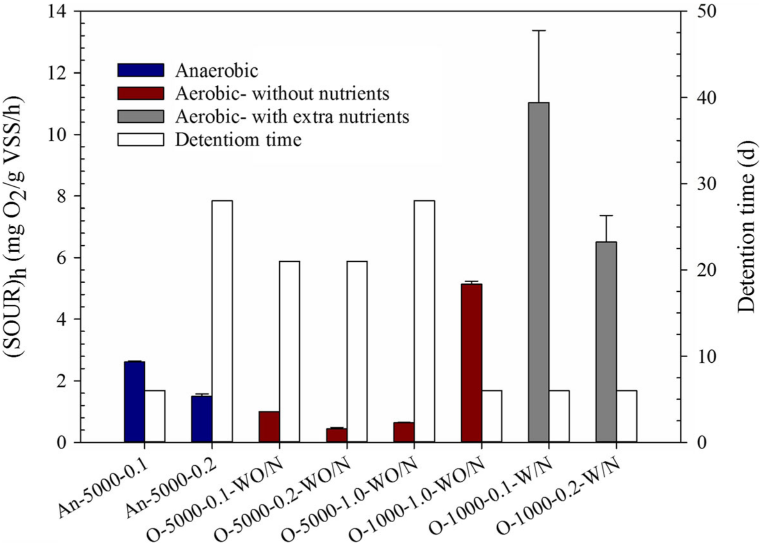


Fig. 6 Specific oxygen uptake rate by heterotrophic bacteria $(SOUR)_h$ under different operational conditions: COD concentrations of 1000 and 5000 mg/L, granule dosages of 0.1, 0.2, and 1 g/L, under aerobic (O) and anaerobic (AN) conditions, without nutrients (WO/N), and with extra nutrients (W/N) at optimal COD/N/P of 100/13/0.8

heterotrophic bacteria in aerobic culture rather than that of anaerobic culture. This finding was consistent with the fact that the treatment capacity was reduced in the presence of air. The trend of $(SOUR)_h$ values suggested that respirometric performance of granules were independent of the detention time. However, it was observed that the initiation of oxygen uptake during $(SOUR)_h$ test was delayed when the samples were taken after a long treatment time of 21 to 28 days (data not shown). This finding could also justify the prolonged lag phase using acclimated culture which was taken from endogenous phase. When initial COD concentration increased from 1000 to 5000 mg/L, $(SOUR)_h$ of high dosage of 1 g/L decreased by a factor of 3; it revealed the inhibition effect of high concentration of organic matter on the respirometric activity of the microorganisms. This phenomenon could explain the low treatment efficiency of high-strength wastewater by the utilized biogranules. In presence of sufficient nutrients during aerobic treatment, $(SOUR)_h$ of 0.1 and 0.2 g/L biogranules demonstrated considerable increase to 11 and 6.5 mg O_2/g VSS/h, respectively, which confirmed proliferation of aerobic bacteria in the presence of nutrients. Positive effect of nitrogen concentration on $(SOUR)_h$ was reported by [27]; they observed that $(SOUR)_h$ raised from 16 to 27 mg O_2/g VSS/h by an increase in N/COD ratio from 0.05 to 0.10, respectively.

Variation in pH

Fig. S9 (see Supplementary Material) depicts the time course of pH under different operational strategies. Regardless of granule dosage, pH drastically raised to the average value of 8.1 at day 1 of aerobic operation, and it remained at alkaline zone between days 2 and 11. Previous works confirmed rapid production of alkalinity due to decomposition of organic compounds to CO_2 through aerobic pathway [37, 38]. Aqueous CO_2 could reversibly react with water and form different forms of alkalinity, including carbonate and bicarbonate [20]. The lowest pH of aerobic treatment was obtained when either low granule dosage or high ammonium nitrogen concentration was applied; both conditions slowed down the oxidation rate of organic matter, and thus decreased the alkalinity production. A narrow pH range of 6.5 to 7.5 has been suggested for the optimal growth of microorganisms. Growth of methanogenic bacteria can be severely hindered at pH below 6.5. Although some bacteria can likely tolerate pH of over 9.5, their microbial activity may be disturbed [20]. Thus, increase in pH value to 9.1 might had disrupted the aerobic activity of the biogranules to some extent; long interval of 10 to 13 days required for the completion of aerobic treatment could be attributed to unfavorable pH condition. Instead, the pH moderately changed during anaerobic operation. It reached the value of 8 at day 10 and did not exceed 8.6 during 28 days of the anaerobic treatment. Montalvo et al. [37] clarified that transformation of organic matter to volatile fatty acids (VFAs) during anaerobic fermentation produced acidity. Thus, in this study, the pH was potentially counterbalanced due to equal production of VFAs and alkalinity as a result of fermentation and aerobic oxidation in a single anaerobic bioreactor, respectively.

Conclusions

Series of lab-scale batch studies were carried out to evaluate operational characteristics of granule-based lagoons:

- Dosing 0.1 g/L of proprietary biogranules (EGMs) could achieve optimal anaerobic operation without addition of nutrients. Optimal aerobic treatment was achieved by adding 0.2 g/L biogranules alongside sufficient nitrogen (N) and phosphorus (P) with corresponding COD/N/P ratio of 100/13/0.8.
- Short detention times of 7–10 and 2–3 days were obtained for complete degradation of 1000 mg/L COD under optimal anaerobic and aerobic conditions, respectively. In contrast, high concentration of organic matter (5000 mg/L COD) inhibited microbial activity of the biogranules which was demonstrated by reduction in treatment efficiency and threefold decrease in $(\mathrm{SOUR})_{\mathrm{h}}$ of 1 g/L biogranules.
- Aggregation of microorganisms in the form of granules could enhance nitrogen removal capacity of lagoon plants. Ammonium nitrogen removal rates of 60 and 73% were achieved in 6 and 7 days, respectively. Further extension of the detention time to 11 days enhanced ammonium-N removal efficiency to 74%.
- Although ammonium nitrogen concentration in the range of 80 to 130 mg/L significantly improved the aerobic degradation capacity, inhibition of free ammonia was observed on heterotrophic and nitrification activities at high ammonium concentration of 480 mg/L.
- Adequate mixing was required to overcome the mass transfer efficiency and, thus, to further modify the performance of lagoon processes. Onetime inoculation of lagoon by fresh granules was the best approach to achieve a satisfactory treatment efficiency.
- This study revealed that utilization of biogranules can be a feasible and attractive alternative for augmenting lagoon-based municipal wastewater treatment plants in practice. This technology can shorten the treatment interval and can decrease the overall land area requirement and the capital cost. The achievements of this study can be used to develop a process design guideline for practical applications of granules in lagoon treatment plants.
- Few complementary studies can be suggested to confirm the findings of this study: (1) RTD studies with tracer experiment to compare the hydraulic flow pattern in lab-scale bioreactor with that in actual lagoons; (2) microbiology of seed and reactivated biogranules; and (3) long-term integrity of biogranules under hydraulic regime of lagoon processes.

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