Anaerobic biological treatment of methylparaben in an expanded granular sludge bed (EGSB)
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ABSTRACT
This study evaluated the behavior of an anaerobic expanded granular sludge bed system using different methylparaben (MPB) concentrations. The assay was conducted for 268 days and was divided into seven stages of operation, which included the starting stage and subsequent stages where the MPB concentration was increased. The inoculum that was used was a mixture of anaerobic granular sludge with flocculent active sludge that contained 21.7 g/L of total suspended solids and 17.4 g/L of volatile suspended solids, resulting in an organic content of approximately 80%.

The MPB removals after applying concentrations of 300 mg/L, 500 mg/L and 1,000 mg/L during the different stages and adding glucose to the influent were 94 ± 2.4%, 84 ± 5.8% and 88 ± 7.4%, respectively. For phases without glucose, the results were 97.4 ± 0.4%, 96 ± 1.6% and 98.2 ± 0.3%, respectively. The results showed a high pollutant removal and good progress in terms of the physical and biological characteristics of the granular biomass, which showed no change in the presence of the compound or a concentration increase.

Key words | anaerobic treatment, expanded granular sludge bed (EGSB), methylparaben (MPB), removal

INTRODUCTION

The impact of wastewater and chemical pollution on the environment is an issue of global importance, primarily due to the large variety and quantity of compounds, many of which are toxic and discharged uncontrollably into the environment. In many cases, the impact of these compounds on the environment and human health is not clearly known (Brausch & Rand 2011).

Parabens are a type of personal care products that are widely used as preservatives. Parabens are esters of 4-hydroxybenzoic acid and are anti-microbial agents that are used in cosmetics, toiletries, pharmaceuticals and food (Brausch & Rand 2011) due to their broad spectrum of activity against molds and yeasts (Aubert et al. 2012). These compounds are released continuously into urban wastewater at relatively high levels (González-Mariño et al. 2011). The presence of these compounds has been reported at concentrations of nanograms per litre in surface water samples (González-Mariño et al. 2011; Brausch & Rand 2011). In raw and treated wastewater, average values of 4,200 ng/L and 25 ng/L have been measured, respectively (González-Mariño et al. 2011).

A growing concern has emerged regarding the possible long-term effects of parabens on wildlife and humans (Lin et al. 2009). By testing in vitro and in vivo, it has been found that parabens (including methyl) have estrogenic activity (Boberg et al. 2010) and have also been classified as xenoestrogen compounds, a class of endocrine disruptors, whose chemical structure is closely associated with their estrogenicity (Vo et al. 2010). Like other xenoestrogens, parabens can act as estrogen mimics with the potential to alter target tissues, such as the breast and uterus in either a beneficial or detrimental way (Guadarrama et al. 2008).

Recently, several reports have suggested a relationship between these compounds and the occurrence of breast cancer (Lin et al. 2009; Vo et al. 2010; González-Mariño et al. 2011).

Several physico-chemical and biological technologies to remove these contaminants have been evaluated. Among the physico-chemical technologies, advanced photochemical processes (Sánchez-Martín et al. 2013), photocatalysis by TiO₂ (Lin et al. 2009) and photocatalysis by green hydrothermal synthesis of ZnO nanotubes (Lam et al. 2013) have been evaluated. However, the low operating costs and high removal efficiencies of biological technologies are attractive, justifying their use. Aerobic treatment systems...
such as activated sludge systems (González-Mariño et al. 2011) or aerated pebble-bed biofilm systems (Fan & Wang 2012) have achieved high removals of methylparaben (MPB). However, studies of anaerobic systems in which its removal is evaluated have not been reported yet. Expanded granular sludge beds (EGSBs) are an effective wastewater treatment technology, which is an improvement of the upflow anaerobic sludge blanket (UASB) (Fuentes et al. 2011). In EGSB treatment, the recirculation of the effluent is provided throughout the system, which promotes mixing within the system and optimizes the contact between the biomass and the substrate to be degraded. An improved efficiency in the organic matter removal process is thereby achieved. Therefore, EGSB technology is an attractive tool to treat wastewater (Wang et al. 2011). The objective of this study was to evaluate a pilot EGSB system for MPB removal at different concentrations. The efficiency of the system at removing pollutants and any possible destabilizing or inhibitory effect of the biomass and/or the biological system were also determined.

MATERIALS AND METHODS

Description of the EGSB reactor

The investigation was performed with an EGSB laboratory-scale reactor made from acrylic with a useful volume of 15 L and a headspace volume of 4 L. The EGSB consisted of three units: an upper portion with a 23.5-cm diameter equipped with a gas-collecting hood to promote solid–liquid–gas separation, a thin cylinder with a diameter of 6.4 cm, where expansion of the granular sludge occurred, and a chamber formed by a perforated plate that allowed the flow to uniformly enter. The total height of each system was 190 cm. The reactor was equipped with two Masterflex peristaltic pump systems (6–600 rpm) to control the feed rate and recirculation system.

Inoculum

The reactor was inoculated with an anaerobic sludge mixture that consisted of low-activity granular sludge and activated floculent sludge from the anaerobic treatment systems of a dairy farm and a meat company (Santa Rosa de Osos, Antioquia, Colombia), respectively. Characterization data for the mixture indicated the slurry contained 21.7 g/L of total suspended solids (TSS) and 17.4 g/L of volatile suspended solids (VSS), where VSS was approximately 80% of the TSS, which indicates a high organic material content. The sludge volume index (SVI) presented a value of 32.7 ± 2.8 mL/g, and the size distribution of the granules ranged from 0.1 to 0.6 mm.

Operational strategy

The anaerobic EGSB reactor was operated at different stages depending on both the MPB load used and glucose addition. Initially, at the start of the extended system, a boot stage was used until stable conditions were obtained. Later, MPB loads were increased, and the effect of glucose on the removal was evaluated. The duration and the main characteristics of each operating stage are summarized in Table 1.

Composition of synthetic wastewater

Fifteen litres of synthetic wastewater consisting of 15 g of anhydrous glucose, 10 g of bicarbonate, 0.5 g of urea and 1 mL of macronutrients and micronutrients per litre of feed were prepared daily. In addition, the influent was enriched with MPB at the working concentration (300, 500 or 1,000 μg/L), which had been previously diluted in methanol. The ratio of nutrients used was chemical oxygen demand (COD)/N/P = 600/7/1 (Guo et al. 2008). Preparation and dosing of these nutrients and other trace compounds were performed according to Londoño et al. (2012).

Batch experiments

Batch experiments were performed to determine the anaerobic biomass response based on the increase of the pollutant concentration under study. To assemble the test, 60-mL amber glass bottles were fitted with rubber stoppers and aluminum seals to obtain the correct seal for each experimental unit with a working volume in the bottles of 30 mL. The culture medium had a similar composition to that of a continuous system; however, 0.3 g/L of yeast extract and 1 mL of resazurin solution at 0.1% were added. Subsequently, after boiling and cooling, 0.5 g of cysteine and 2 g of NaHCO₃ were added, and the pH was adjusted to be between 6.8 and 7.0. Experimental units with active biomass were used to evaluate biological degradation of MPB and experimental equivalent sterilized units were used to evaluate abiotic removal mechanisms of MPB. All bottles, samples and controls were incubated at 30 °C at 100 rpm.
Images from the scanning electron microscope

The sample preparation for the scanning electron microscope (SEM) images was based on the method of fixation with glutaraldehyde (Bhattacharyya & Singh 2011). Biomass pellets were washed with a phosphate buffer solution of 0.1 mol/L at a pH of 7.0, fixed with 2.5% glutaraldehyde for 2 hours and then washed with water for 5 minutes. Dehydration was performed by passing the slurry through ethanol at 25, 50, 70, 80, 90, 95 and 100%. Lastly, a thin gold coating was added to the samples prior to their analysis in the SEM.

Analytical methods

The dissolved organic carbon (DOC), COD, TSS, VSS, pH, alkalinity and the SVI were measured in accordance with Standard Methods (American Public Health Association/American Water Works Association/Water Environment Federation 2012).

MPB quantification was performed by liquid chromatography coupled with mass spectrometry using a direct read ACQUITY UPLC BEH C18 column with a particle size of 2.1 × 50 mm and 1.7 μm. The flow was 0.3 mL/min, and the injection time was 12.7 minutes. Two mobile phases were used for the analysis of the samples, one of which was 0.01% formic acid in water and the other was 0.01% formic acid in methanol.

RESULTS AND DISCUSSION

Batch experiments

Four concentrations of MPB (300, 500, 1,000 and 2,000 μg/L) were evaluated. The inoculum for the batch tests was taken from the reactor after finishing the initial step to obtain high quality slurry for experimentation. The batch processes presented good behavior in terms of high MPB biodegradation and low sorption in the sludge. The concentration of the MPB registered for the abiotic controls (sterilized experimental units) remained constant throughout the 28 days of the assay. This indicated little relevance of other mechanisms for removal of the MPB and it suggests that adsorption/absorption did not occur as the compound was fully recovered through the aqueous phase at the end of the test. In the case of experimental units without sterilization, the MPB biodegradation was greater than 97%. The use of abiotic controls confirms that removal percentages can be attributed to biological action that occurred during
experimentation. The DOC removal rates were greater than 93% and showed no change or decrease when the MPB concentration increased, which suggests little or no inhibition of the biomass when in contact with the compound.

Continuous experiments

The EGSB system was operated for 268 days. During this time, three of the four loads studied in the batch tests were applied (300, 500 and 1,000 mg/L). For stages II, IV and VI, different MPB loads were applied using anhydrous glucose as an additional carbon source. Stages III, V and VII used the same MPB loads; however, anhydrous glucose was not added as an additional carbon source.

Tracking the alkalinity and pH during all stages of reactor operation is an important indicator to determine any system instability. The accumulation of volatile fatty acids (VFAs) produces stress conditions in anaerobic processes; thus, it is necessary to avoid their accumulation and to avoid a pH decrease, which could destabilize the system (Martín-González et al. 2013). A simple way to determine the conditions in the anaerobic system is based on determining the alkalinity supplied by the carbonate/bicarbonate groups as unprotonated forms of VFA (Martín-González et al. 2013). In this study, the alkalinity was monitored by obtaining the total alkalinity (TA), part alkalinity (PA) and the intermediate alkalinity (IA). The first term is the amount of alkalinity provided by the VFA and the carbonate/bicarbonate. The second term refers to the alkalinity provided only by the carbonate/bicarbonate. The latter term is the difference (TA–PA) and is related only to alkalinity provided by VFA (Björnsson et al. 2001). Figure 1 shows the behavior of these parameters during all stages of operation. The alkalinity analysis was based on the ratio IA/PA, where the recommended range is 0.1–0.3 to prevent unstable conditions in the system by the accumulation of VFA (Zhang et al. 2008). Primarily in phases IV, V and VI, the IA/PA ratio exceeded 0.3 (average values of 0.32 ± 0.07, 0.31 ± 0.07 and 0.31 ± 0.06, respectively), which indicates potential destruction of the buffering capacity of the bicarbonates due to possible VFA accumulation (Martín-González et al. 2013). However, the pH values were maintained between 6.5 and 8.4, which indicates no acidification of the system conditions.

With an overall average of 93%, the DOC removal process in the anaerobic system can be considered efficient. In the initial reactor period, removal percentages (Figure 2) averaged 96.4 ± 2.6%. Studies using EGSB systems have reported DOC removal efficiencies of 86 and 91% during stabilization periods (Londoño et al. 2012). The value obtained for this study showed a high operational reactor efficiency, which indicates an ideal system state to begin applying MPB loads. The application of MPB began directly after this stage, and the DOC removal efficiencies were 91 ± 2.2, 88 ± 2.7, 92 ± 4.0, 84 ± 4.4, 95 ± 2.0 and 90 ± 1.2%. Using an EGSB system, Dong & An (2011), achieved a DOC removal efficiency of 92.3% for the wastewater treatment of coke. For palm oil removal processes using a hydraulic retention time (HRT) of 5 days, efficiencies of 97% have been reported (Fang et al. 2011). The removal values obtained in this study are close to the average normal values reported for systems of this kind. In addition, Puyol et al. (2011), reported a removal of COD of 95% in an UASB system for the treatment of cosmetic wastewater. Results in our study also demonstrate that the treatment of this kind of wastewater is possible without suffering severe instability conditions despite the contact between the micro-organisms and the MPB regardless of the MPB concentration.

MPB removal was efficiently achieved using the EGSB system (Figure 2). Stages III, V and VII (without the presence of glucose) exhibited the best efficiencies, with average removal values of 97 ± 0.4%, 96 ± 1.6% and 98 ± 0.3%, respectively. For stages II, IV and VI (with added
glucose), removal efficiencies were not as high but were still significant, with average values of 94 ± 2.4%, 84 ± 5.8% and 88 ± 7.4%, respectively. These results show that MPB can be degraded in an EGSB system. The high removal percentages obtained in this study agree with those reported by González Mariño et al. (2011), who achieved MPB removals of 99.4% for activated sludge systems. In addition, using a bed of aerated biofilm, Fan & Wang (2012), achieved a MPB biodegradation removal percentage of 95.5%. These MPB removal percentages should be compared with the actual percentages of biodegradation due to the different mechanisms of elimination of the compound (sorption, biodegradation, volatilization and photo-oxidation) that may occur in the removal system. However, Carballa et al. (2005), concluded that only two mechanisms, microbial degradation and adsorption onto the suspended solids, are actually relevant in removing these compounds. In this study, it was found that the sorption of MPB into the sludge was insignificant, and therefore, the removal percentages of the compound are primarily attributed to biological action.

**Biomass analysis**

The granulation process during system operation EGSB was slow and unremarkable in increasing the diameter of the particles passing a range from 0.1–0.6 to 0.5–1.5 mm from the beginning to the end of the experimentation. However, improvements were observed in the sedimentation capacity of the biomass at the end of the experimentation with values of 7.63 ± 0.62 m h⁻¹, as compared to the initial inoculum, which showed values of 1.54 ± 0.27 m h⁻¹. In addition, the SVI always showed values <39 mL/g, providing a fully clarified effluent due to the good sedimentation rate of the sludge and low value of SVI.

A clear difference can be seen between the inoculum sludge sample and the sludge sample at the end of the EGSB operation (Figure 3). This development is characterized primarily by the specific improvement in the biological conformation of the sludge after the reactor operating time. The conditions within the system favored the growth and shape of the granular sludge with an increase in the density of the granules which went from 11.64 ± 0.59 to 30.28 ± 0.77 g/L. Consequently, this condition favored the increase in their size distribution ranges.

**CONCLUSIONS**

The operating conditions of the EGSB system established in this study allowed MPB degradation at concentrations of 200–1,000 µg/L with removal percentages above 95%. This good performance reflected adaptation of anaerobic biomass when in contact and during the degradation of the compound with significant improvements in their physical characteristics such as density, size distribution and SVI. To the best of our knowledge, there is not any similar report about MPB degradation through an anaerobic
EGSB system to date. These results presented a technological improvement for the treatment of wastewaters by establishing optimal applications in the removal of specific contaminants for the case of MPB.

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