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# Application of immobilized and granular dried anaerobic biomass for stabilizing and increasing anaerobic bio-systems tolerance for high organic loads and phenol shocks

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## HIGHLIGHTS

• Dried anaerobic bacteria can be immobilized in hydrophilic polyurethane foam.

• This unique matrix enabled fast recovery of biomass methanogenic activity.

• These cure medium improved biomass tolerance to high hydraulic and organic loads.

• PAC in the immobilization structure mitigated the effect of phenol.

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# ABSTRACT

This study focuses on the stability and tolerance of continuous-flow bioreactors inoculated with anaerobic methanogens in three different configurations: (R1) dried granular biomass immobilized in PACenriched hydrophilic polyurethane foam, (R2) dried granular biomass, and (R3) wet granular biomass. These systems were tested under two different organic loading rates (OLR) of 6.25 and 10.94 ( $g_{COD}/(L_{reactor}*d)$ ), using a glucose-based synthetic mixture. The effect of an instantaneous shock load of phenol (5 g/L for three days), and of phenol inclusion in the feed (0.5 g/L) were also tested. At the lower OLR, all reactors performed similarly, however, increasing the OLR lead to a significant biomass washout and failure of R3. Biomass in R1 was more tolerant to phenol shock load than R2, though activity was recovered in both systems after about one month. PAC provided protection and shortened the adaptation time for 0.5 g/L phenol that continuously was fed.

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# 1. Introduction

Spectrum of anaerobic technologies has been applied to treat various types of wastewater owing to their reduced energy consumption, mitigated production of excess sludge, and potential production of energy-yielding gases such as methane (Sekiguchi et al., 2001; Smith et al., 2012). The application of anaerobic technologies could be applied for high-strength industrial organic wastewater (Barrera et al., 2014; Jang et al., 2014), as well as for low-strength domestic wastewater (Bae et al., 2014; McCarty et al., 2011), and complex wastewater containing persistent and

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toxic chemical compounds, such as from petrochemical refineries and chemical compound production plants, contain phenolic compounds that affect process stability and performance (Cabrol and Malhautier, 2011; Chen et al., 2008; Rebhun and Galil, 1988; Rosenkranz et al., 2013). In general, anaerobic treatment of such wastewater is considered more energy-efficient than aerobic processes because of the reduced oxygen consumption and the added value of valuable biogas. In addition, these processes produce less biomass and can handle higher organic loads in comparison to aerobic processes (Lettinga, 1996). In practice, however, operational stability obstacles still limit wide application of commercialized anaerobic technologies for wastewater treatment (Dupla et al., 2004). Moreover, anaerobic processes are highly vulnerable to organic and hydraulic load fluctuation, suffer active biomass washout from reactors, are sensitive to inhibitors, and require lengthy





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periods of acclimation to achieve efficient biodegradation (Chen et al., 2008; Dereli et al., 2012). To overcome the principal limitations of conventional and unstable processes, granular-based anaerobic systems incorporating an immobilized biomass have been proposed as an alternative technology for complex wastewater treatment (Wu et al., 2008). Systems with immobilized biomass facilitate the use of compact units operating without recirculation or separation systems. Compared to suspended growth processes, immobilized microorganisms possess several advantages, including high metabolic activity rates and strong resistance to toxic chemicals (Dwyer et al., 1986; Massalha et al., 2007; Wu et al., 2008). The effective control of sludge retention time, potentially high biomass concentration and, consequently, the option of applying low hydraulic retention times have encouraged the adoption of immobilized biomass technology (Fazolo et al., 2007). Moreover, immobilization of biomass increases its tolerance to hydraulic or quality shocks, providing a secure environment for efficient activity (Gao et al., 2011).

Based on previous developments of biomass drying procedures and unique immobilization techniques using hydrophilic polyurethane foam (HPUF) (Massalha et al., 2015), this study is aimed at comparing various configurations of anaerobic reactors for treatment of organic synthetic wastewater. The influence of biomass immobilization within a matrix enriched with powdered activated carbon (PAC) was examined. In addition, the effect of high organic loading rate (OLR) and sudden addition of phenol was also tested. The information obtained here is expected to be useful for development of tolerant, stable and environmentally-effective anaerobic systems for treatment of organic wastes containing problematic constituents.

# 2. Methods

#### 2.1. Preparation of anaerobic biomass inoculations

Anaerobic granular biomass was collected from a well operated, up-flow anaerobic sludge blanket (UASB) bio-reactor used to treat the wastewater of a citrus-based soft drink factory (PRIGAT) at Kibbutz Givat Haim, Israel. A unique aerobic drying process (Massalha et al., 2014) was conducted for this granular biomass prior to its application in three different bioreactors. The tested reactors were seeded with the same amount and source of anaerobic biomass, but after different pre-treatment and handling methods. An amount of 18.32 g net weight of dry grinded granular biomass was immobilized in 115 HPUF cubes with an average volume of 2.25 cm<sup>3</sup> per cube. These immobilized cubes were then enriched with PAC according to Massalha et al. (2015) and used to inoculate reactor R1. For inoculation of reactor R2, an amount of 18.32 g of granular dry biomass (without grinding) was used. The density of the dry granules right after the drying process was 1.36 g/mL; however after immersing the dried granules in the wastewater liquid, the density was reduced to 1.12 g/mL. For comparison, an amount of 140 g of wet granular biomass with density of 1.033 g/mL (equal to 18.32 g dry weight), was used to inoculate reactor R3. The immobilized PAC enriched HPUF cubes used to inoculate R1, and dried granules for R2, were prepared two weeks

Table 1					
Average	values	of	reactor	operation	parameters.

before starting the continuous experiments, during which they were submerged in synthetic wastewater (see below) and incubated at 37 °C.

## 2.2. Anaerobic reactors

Three identical glass-made laboratory-scale anaerobic reactors (R1, R2 and R3) with 440 mL active volume having an inner diameter of 3.8 cm and a height of 39 cm were used in this study. The reactors were equipped with cylindrical double jackets to keep a constant temperature of 37 °C by circulating heated water using a submerged pump. Each reactor has an inlet at the bottom and outlet at the top. At the top of reactors R1 and R2, a plastic screen was installed to prevent clogging of the outlet by the floating immobilized HPUF cubes. A siphon was connected to each reactor outlet enabling separation between the effluents, to be collected in a bottle, and the produced biogas to be collected into Tedlar<sup>®</sup> Air Sampling Bags (SKC Inc, USA). The reactors were fed equally with a synthetic wastewater tank kept at 4 °C by a multi head peristaltic pump.

#### 2.3. Operation conditions

Reactors R1 and R2 were inoculated and started up simultaneously under identical conditions. Reactor R3 was operated in a delay of 41 days under the same conditions of the other reactors. OLRs of the synthetic feed mixture and its composition were changed over the time of the study as can be seen in Table 1. The synthetic wastewater contained glucose as the main carbon source (1.5 and 3 g/L), phenol (0, 5, and 0.5 g/L), and essential nutrients including yeast extract and peptone which raised the influent COD (see Table 1). The daily average flow rate was  $1350 \pm 84$  mL/d. The feeding pumps were operated at a regime of 45 min on and 15 min off.

To simplify the analysis of the results, and in order to compare the performance of the different anaerobic systems, the research was divided into five experimental phases based on OLR, phenol content and mode of addition (see Table 1).

The first operational run (Phase I) lasted 105 days for R1 and R2, and 64 days for R3. The OLR was 6.25 g COD/L/d, when a steady state was reached. In Phase II the OLR was elevated to 10.94 g COD/L/d by gradual increase of influent glucose concentration by steps of 0.5 g/L per day, for a better adaption of the anaerobic systems. Phase II was aimed at testing the effect of increased OLR on the stability of the systems, and it lasted 40 days (days 105-145). The hydraulic retention time (HRT) was kept constant, while the concentration of the glucose was increased to 3 g/L. This stage proceeded until stable performance was achieved in all reactors for about one month. After a stable operation was observed in Phase II, the systems were fed an additional supply of phenol (5 g/L) for three consecutive days ("phenol shock"). The systems were examined for their stability by monitoring the methanogenic activity following this phenol shock event. Phase III (phenol sudden load) lasted 3 days (days 145-147), while keeping continuous feed of synthetic wastewater for another 54 days (Phase IV, days 147-201) until the systems were recovered and steady state was reached

	(Phase I)	(Phase II)	Phenol shock (Phase III)	Startup after inhibitor shock (Phase IV)	(Phase V)
Time (days)	0-105	105-145	145–147	147-201	201-220
Organic loading rate (g <sub>COD</sub> /(L <sub>reactor</sub> *d))	6.25	10.94		11.4	13
Glucose (g/L)	1.5	3	3	3	3
COD <sub>influent</sub> (mg/L)	2038	3585		3728	4959
Phenol (mg/L)	0	0	5000	0	500

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