Small-bioreactor platform technology as a municipal wastewater additive treatment

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ABSTRACT

The bioaugmentation treatment approach presents both an economical and environmentally friendly solution for wastewater treatment. However, the use of exogenous bacterial cultures presents several limitations: negative interaction between microorganisms and adaptation to new physical and chemical composite environment. These selective forces create a significant challenge for the introduced culture to achieving the required biomass in order to conduct the target biological treatment. Small-bioreactor platform (SBP) technology is aimed at introducing exogenous bacterial culture with some protection to reduce some of the natural selection process. The current study was aimed at validating the use of SBP technology to improve biological treatment, especially during a stress period, by using macro-encapsulated bioaugmentation treatment. The study results indicate that the use of SBP technology elevates the stability of biological treatment, improving operational factors such as the reduction of foaming phenomena and sludge accumulation. Still, a significant study needs to be conducted to understand the potential of this technology; especially the impact on biological treatment by using different types of microorganisms for different types of wastewaters and the relationship between the biomass within the SBP capsules and the natural microorganisms.

Key words | activated sludge, bioaugmentation, biodegradation, encapsulation, microbial diversity, wastewater treatment

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INTRODUCTION

As industry becomes more complex and abundant, environmental regulations are constantly being legislated, and the treatment of wastewater becomes more challenging; thus research and innovation in advanced wastewater treatment technology is required. Increasing environmental awareness and tighter government policies demand new, environmentally friendly ways to clean up contaminants using low-cost methods and materials.

In activated sludge treatment systems the wastewater's indigenous microorganisms may suffer from shock loads of organic matter or penetration of anti-microbial agents, which can induce significant damage to the biological process of wastewater treatment (Rittmann & Whiteman 1994). Therefore, extensive ongoing research is being focused on finding specialized microorganisms and adapting them to hostile conditions such as wastewater environments. This usually refers to bioaugmentation, which is used in wastewater and contaminated soil treatment sites in order to benefit biological purification processes (Van Limbergen *et al.* 1998; El Fantroussi & Agathos 2005).

The commercial market offers a wide range of special microbial products for different types of contaminant reduction. Nevertheless, the main challenge of the bioaugmentation treatment approach is the survival and maintenance of a sufficient exogenous culture biomass in unstable conditions such as wastewaters (Vogel 1996; Boon *et al.* 2000, 2003). The technologies employed in bioremediation using exogenous bacterial cultures are varied. Studies have pointed out that encapsulated microbial bioaugmentation can be a novel alternative solution for increasing microbial diversity. Encapsulation of exogenous bacterial cells might be a solution for generating protective barriers around microorganisms. Encapsulation, using gellan gum micro-beads, alginate, agar, polyacrilamide, hydrogel (Moslemy *et al.* 2002), and techniques resembling encapsulated bacteria within electrospun core-shell

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microtubes (Klein *et al*. 2009), has emerged as a promising solution for overcoming the practical limitations of using cell formulations (for more information see Moslemy *et al*. (2006)).

Alongside ongoing studies, a novel technology recently was developed and presented as the 'small bioreactor platform' (SBP) (Menashe 2010). Briefly, SBP technology integrates engineering and microbiology into a solution that enables high microorganisms concentrations as well as growth and viability inside the capsule (Figure 1) in a suspended state.

SBP technology is based on a specially designed capsule (2.5 cm long and 0.8 cm in diameter) which physically separates introduced microbial culture inside the capsule from the natural microbial flora within wastewater streams. The SBP was designed to bring about a significant reduction in natural selection forces by the prevention of direct contact and interactions between the microorganisms inside and outside the capsule membrane.

The main aim of the SBP technology is to increase the wastewater microorganisms' diversity, in order to achieve removal of specific contaminants and to increase the stability of the biological process within the wastewater treatment process by developing an additional microbial biomass and path to the bioreactor microbial flora. The postulated platform provides a tailor-made microenvironment for the active microorganisms to conduct the desired purification process without physical exposure either to the SBP culture or the wastewater natural cultures to each other.

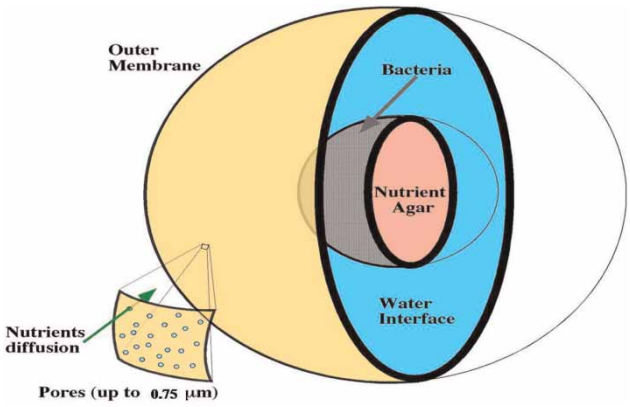

Figure 1 | The SBP capsule contains several elements: the external barrier made of cellulose acetate microfiltration membrane, the aquatic interface that includes the exogenous inoculums and supplemental nutrients in agar. The SBP capsules are marketed in a dry state (inactive product), and need to be activated by hydration. Each host bioreactor can have several thousand capsules in cartridges or perforated cages. The ratio of SBP capsules numbers to wastewater volume depends mainly on the target contaminants (type and concentration), and the hydraulic time retention.

Also, the technology is suggested as a useful method to avoid the possibility of exogenous microorganisms being diluted by the incoming waste stream. For more details see Menashe (2010, 2012).

This study presents the implementation results of SBP technology within an activated sludge municipal wastewater treatment plant.

MATERIALS AND METHODS

SBP capsules: preparation and activation

The structure, composition and validation of the SBP capsule technology are presented in detail in the patent article number PCT/IL2010/000256, and illustrated in Figure 1 (Menashe 2010). Two SBP capsules types were made and used in this study: NatiCap™$_{\text{Petroleum}}$, and NatiCap™$_{\text{Municipal}}$, which contained the commercial blend PHC type 4 and FOG type 2 respectively. NatiCap™$_{\text{Petroleum}}$ is aimed at biodegrading petroleum hydrocarbons and other components such as phenols and polyphenols, while NatiCap™$_{\text{Municipal}}$ contains a microbial blend that is adapted to municipal wastewater treatment enriched with fats and oils. All components, including microorganisms, inside the SBP capsule are in a dry state and may be activated by introducing the capsules into water, which activates the capsule's microbial biota within a period of a few hours.

Wastewater treatment pilot system design and wastewater characterization

Ha-Solelim (RANI Ha-Solelim, Israel) wastewater treatment plant (a membrane bioreactor plant) drains wastewater from the cities of Nazareth and Nazareth Illit, as well several villages nearby. These influents include mainly sanitary wastewater, periodic high concentrations of proteins, fats and oil from slaughterhouses, and episodes of inflow of olive mill wastewater (OMW) during the autumn (October to November), which is a byproduct of the olive oil extraction process. The inflow parameters for chemical oxygen demand (COD) are 1,000–2,200 mg l^{-1}, and 500–1,000 mg l^{-1} for biological oxygen demand (BOD), which is considered to be a high organic load stream for sanitary wastewater.

The pilot system was established in Ha-Solelim wastewater treatment plant and operated during a 5 week period. The system comprises the control system, representing the activated sludge treatment process, and the test

system, which demonstrates the combination of the activated sludge treatment process with the SBP technology treatment process. The test system encased 35 SBP capsules (30 $NatiCap_{Municipal}$ and five $NatiCap_{Petroleum}$) that were placed within the bioreactor, about 0.5 m below the water surface and above an air diffuser. Both systems had a common inflow feed (from the anaerobic pond) to the aerated bioreactors (350 L volume). The hydraulic retention time (HRT) of the wastewater within the bioreactors was set to be 5 days, which is considered to be long, since we aimed to magnify the bioreactor response in case of anti-microbial agent penetration and to provide sufficient time for oil and fats biodegradation. The effluents from the bioreactor continued to the sedimentation tank (250 L volume) at the same flow rate as the influent (by overflow and gravitation). Diffusers in the aeration tanks enriched the water with oxygen and agitated its contents, with a similar air flow rate. The control and test systems were activated 3 weeks prior to the experiment in order to generate sufficient activated sludge and to stabilize the biological process in both systems.

Water quality analysis

Weekly or biweekly measurements of BOD, COD, total organic carbon (TOC), and fats and oils in the inflow and the outflow of both systems were performed according to the methods recommended by *Standard Methods* (American Public Health Association 1995). Sludge sedimentation tests were performed in order to estimate the biomass volume in each bioreactor.

Study performing stages

The treatment process had two main stages: the activation stage of the SBP capsules and the treatment process itself. SBP capsule activation was done by incubating the SBPs for 48 hours in the test bioreactor. The start point of the study (day 0) was set as the following the stage of SBP capsule activation. The SBP capsules were packaged in a perforated elastic mesh bag and were immobilized in the test system.

Both bioreactors contained an activated sludge process. In both systems (control and test) the bioreactors were fed with wastewater influents from the anaerobic pond (C110 pond, the wastewater treatment plant inflow pond) of the Ha-Solilim wastewater treatment plant (WWTP). After stabilizing both systems with similar biomass amounts, the activated SBP capsules were implemented inside the test system. Manual sludge recycling to the bioreactor was done once every 3 days and returned activating sludge (RAS) was done by returning 0.25% of the total sludge volume to the bioreactor. Water samples were taken from the upper zone of the sedimentation tank (using a sampling valve) for an analysis of TOC, BOD, COD, oils and fats. After 4 weeks, the SBP capsules were removed from the test system and capsule morphological examination and bacterial tests were performed on the inner medium of the capsules. Both bioreactors continued to be monitored for an additional week.

Microbial characterization

In order to characterize the microbial population distribution within the SBP capsules and bioreactor media, we preformed a Gram stain followed by analysis using a light microscope (x1000).

Enumeration of culturable heterotrophic bacteria

Culturable heterotrophic bacteria enumeration within the SBP capsules was performed as follows: the inner medium (100 μl) of each capsule was plated following serial dilutions in triplicate on LA plates (Difco, USA) and incubated for 48 hours at $36 \pm 1\ ^{\circ}C$.

RESULTS

Water quality results

As shown in Table 1, the organic load of the influents is relatively high compared to average municipal wastewater influents, perhaps due to the slaughterhouse wastewater and light industry (garages, metal processing and gas stations, which are abundant in that area). Figure 2 presents the study results of organic matter concentration degradation (in percentage) of each system. Similarities in both systems' performances during the first 6 days of the study was observed, an exception being the BOD. The difference between the control and test systems during the first 6 days of the study in the treatment efficacy of COD and TOC was 8–9% and 3–8% respectively (with higher degradation rates in the test system). The BOD analysis results show a higher difference between the test system and the control system: 16–22% (higher degradation rates in the test system).

After 6 days of treatment, the control bioreactor entered into a stress state which extended to day 31 of

Table 1 | Water chemical analysis (mg l^{-1})

	Inflow				Control-effluents				SBP-effluents			
Days	**COD**	**BOD**	**TOC**	**Fats & oil**	**COD**	**BOD**	**TOC**	**Fats & oil**	**COD**	**BOD**	**TOC**	**Fats & oil**
0	1360	910	528	152	391	260	37	< 10	285	56	22	< 10
3	2297	646	714	97	358	160	79	< 10	161	57	21	< 10
6	984	740	411	17	371	283	57	< 10	230	164	24	< 10
10	989	521	117	42	461	327	92	< 10	202	105	28	< 10
17	1437	842	573	57	826	553	391	< 10	167	110	28	< 10
24	1387	875	158	100	388	272	36	< 10	227	116	32	< 10
31	2289	917	796	66	275	152	18	< 10	145	59	20	< 10
Capsule removal												
38	1546	615	632	13	362	297	35	26	155	140	13	< 10

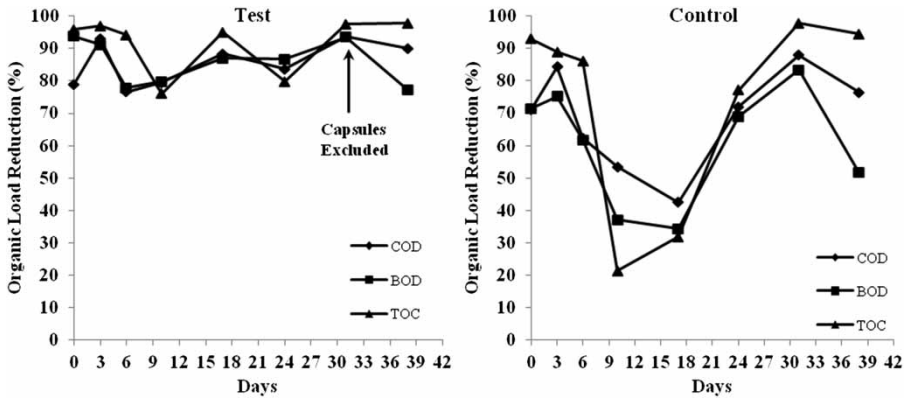

Figure 2 | Water treatment results of the test system (left) in comparison to the control system (right) over time (days). The organic load reduction rate was calculated as follows: (1-Effluents/Inflow)*100 as a function of the time from SBP's activation (start point of the trial).

the study. During that stress period, the control system biodegradation performances of COD, BOD and TOC values decreased significantly to 42.5, 34.3 and 21.3% respectively. In contrast, during that period the test system presented a stable biological performance, with a slight reduction and recovery over time. Therefore, these results indicate that the test system presented a superior biodegradation performance and some resistance to environmental changes. On day 31 of the study, the control system returned to the same initial activity observed during days 0–6 of the study. On day 31 the SBP capsules were removed, and both systems continued to run for an additional week. The chemical analysis results of the test system on day 38, a week after removing the SBP capsules, indicated a decrease in the system's organic degradation performance, which was also seen in the control system performance, but not with the same intensity. The overall chemical parameters (effluent organic load residence: 2% TOC, 10% COD and 23% BOD) of the test system were mainly kept steady (COD and TOC). However, the BOD value presented a significant biodegradable reduction. Nonetheless, it seems that the test system starts to present a similar behavior pattern for its biodegradable performance to the control system (effluent organic load residue: 6% TOC, 23% COD and 48% BOD). These results indicate that SBP treatment termination may result in a progressive return to normal system performance capability and response. Both systems achieved almost complete biodegradation (leaving less than 10 mg l^{-1}) of fats and oils.

Sludge sedimentation tests results

Sludge sedimentation tests of the mixed liquor were performed on day 21 of the study in order to estimate the performance of each bioreactor by the ratio of biomass to

effluents. The sludge sedimentation volume (as a percentage of total volume) after 30 min of both bioreactors was similar, and the test bioreactor had only 2.5% more sludge than the control bioreactor in average (data are not shown). An additional sludge sedimentation test was performed on the day 31 of the study, prior to the SBP capsule removal. The test results present a reversal of the biomass volume within the bioreactors: the biomass volume of the control bioreactor was 22.5%, while the biomass within the mixed liquor of the test bioreactor was 10%.

Operational factors: sludge formation and bioreactor overflows

An interesting result was observed during the study period. During the operation of the pilot, we had measured a significant sludge reduction within the test sedimentation tank, in comparison with the control. The reduction was 40–60% in volume.

Foaming and overflow incident variation between the test and control systems bioreactors were observed especially during the period of stress (days 6–31). Wastewater foaming and overflow phenomena of the control system occurred several times, on days 3–6, 10–17, 17–24, 21–31 and 31–38 of the study. In contrast, within the test bioreactor we observed a single foaming and overflow incident, on days 3–6 of the study.

Bacteriological analysis results

All of the 35 SBP capsules remained physically unspoiled with the same shape and structure as observed at the start point of the experiment. Also, the membrane of the capsules remained physically functional and no cracks were detected. It can be considered that the semi-permeable outer membrane in all the SBP capsules remained stable and functional during one month of operation.

In the bacterial viability counts of the inner medium of the NatiCap™$_{\text{Petroleum}}$ SBP capsules we observed a bacterial concentration of 5×10^9 CFU ml^{-1} (colony forming units/ml), and within the medium of NatiCap™$_{\text{Municipal}}$ capsules, we observed a bacterial concentration of 5×10^8 CFU ml^{-1}. The test bioreactor flora medium (suspension) had a bacterial concentration of 3×10^8 CFU ml^{-1}.

In order to obtain a basic morphologic characterization of the dominant microbial populations in each system, we performed a Gram stain on the SBP capsule medium and on the bioreactor medium (control and test). The microscopic visualization indicated a significant dissimilarity in microorganism composition between the SBP microorganism culture and the natural flora of the bioreactor, mainly within the NatiCap™$_{\text{Municipal}}$ formulation, which contains mainly a population of Gram-negative rod-shape bacteria. The suspended microbial population set within the test bioreactor medium was mainly Gram-positive spherical bacteria (cocci), both single and paired, as well as Gram-negative filaments. A microbial dissimilarity was observed between both formulations of the SBP capsules that were introduced into the test bioreactor. Both bioreactor (test and control) medium flora contained a similar profile of dominant bacterial populations: Gram-positive single-cell cocci, Gram-positive paired cocci and Gram-negative filaments. These results suggest that the membrane is indeed separating the microorganism cultures inside the capsules, and thus no microorganisms could cross the SBP membrane. Furthermore, the separation of the inserted microorganism cultures within the SBPs results in increased microbial diversity within the bioreactor, which is expected to lead to a better biodegradation process.

DISCUSSION

In this study, a new treatment methodology, SBP technology, is presented for the implementation of exogenous bacterial culture (bioaugmentation) within a host bioreactor. The new technology enabled us to introduce a specific microbial culture or microbial blend to the host bioreactor. The SBP capsules provide a sufficiently spacious medium for long-term bacterial prosperity and biodegradation activity (Menashe 2010, 2012). We estimate that the biodegradation process is being conducted within the internal medium of the SBP capsule. Nonetheless, we expect that the introduced culture enables the synthesis of extracellular digestive enzymes (i.e. proteases, lipases), for the breakdown of large molecules (above 6,000 kDa) (Hoppe 1991; Nybroe *et al.* 1992).

The SBP capsule contains a biofilm attached to growth carriers, in order to provide two bacterial growth states: suspended and in biofilm form. According to our best knowledge, this is the first formulation (carrier, beads, macro and microencapsulation, etc.) that presents in parallel those two bacterial growth states. This unique bacterial growth combination, can promote rapid biological processes as well as resistance to hostile environmental conditions. The physical separation of the culture (Figure 1), the aquatic medium, internal nutrient supplements for bacterial

recovery, and attached biofilm growth support elements are aimed at raising the probability of rapid acclimation and bacterial culture proliferation within a confined environment (the SBP capsule) for at least several weeks, as presented in this study.

During this study we observed that the control system entered into a stress state, which was expressed as a significant reduction of the system's biodegradation performance. This stress phenomenon was observed for almost 4 weeks, starting from day 6 of the trial. Since both bioreactors present a similarity in the sludge fraction of the mixture liquor, it is suggested that the cause of the systems' stress induction was the penetration of anti-microbial agents into the bioreactors through the inflow current.

Since this study was performed during the olive harvest season, we can assume that OMW might be present within the feed water, which can explain the biological process collapse of the control system. Olive oil's phenolic compounds are the main determinants of the anti-microbial and phytotoxic actions of olive-mill wastes (Bartels *et al*. 1984; Haigler *et al*. 1992; Chang *et al*. 1993; Casa *et al*. 2003; Morillo *et al*. 2009).

Our results indicate that the SBP technology has the ability to increase the biological process stability within the WWTP host bioreactor. We assume that the culture within the SBP capsules, and especially the formulation of petroleum wastewater treatment capsules (NatiCap™$_{\text{Petroleum}}$), enabled a wider range of toxic agent biodegradation performance, including those that have an anti-microbial effect.

It is necessary to consider that some of the inflow organic matter might be absorbed to the SBP capsules; however, we estimate that this process, if it occurred, was negligible. After the capsules' exclusion from the bioreactor at the end of the study, the SBP capsules did not present structural changes (i.e. membrane crystallization, colloidal fragments inside the capsule or capsule swelling) as expected if significant absorption had occurred.

Rathore *et al*. (2013) in their review article summarized recent and historical encapsulation techniques and methods for various applications. Encapsulation, in general, is aimed at immobilizing the microbial cell in order to protect it from the environment. Immobilization may result in changes in the various physico-chemical properties of the microenvironment of the microbial cells, such as the presence of ionic charges, cellular metabolic products, reduced water activity, altered osmotic pressure, etc.

The main difference between SBP technology and other encapsulation techniques is that in the SBP technology the microorganism cells are not immobilized. The novelty of this technology is to create a protective aquatic environment for the exogenous microorganisms' cells within the SBP capsule, without the need for immobilizing the bacterial culture. The protective strategy that the SBP technology presents is the use of a constructed microfiltration membrane, instead of integrating the bacterial culture into a perforated biological and/or chemical matrix. Therefore, the bacterial cells are not attached to polymer, or other chemicals, but are suspended within the internal medium of the SBP capsule and therefore show normal cells' physiology and activity.

In this study we present the use of SBP technology as a practical tool for bioaugmentation treatment. Bioaugmentation could emerge as one of only a few environmentally friendly techniques for pollution abatement. The use of SBP technology in activated sludge treatment presents significant benefits. The increased biological process stability, which can prevent some of the biological shocks that WWTP bioreactors may experience over time, can contribute to WWTP service supplement. In addition, waste sludge has become an environmental and economic issue and carries significant operational costs. Thus, the use of the SBP technology may also decrease sludge formation, as shown in this study, and therefore presents promising economic and environmental benefits.

CONCLUSIONS

The addition of SBP capsules enables the introduction of specific microbial cultures into a bioreactor with some protection that reduces several natural selection processes, providing sufficient conditions for long-term bacterial prosperity. This study shows that the addition of specific SBP capsules brought about a more stable bioremediation process within an activated sludge pilot-scale bioreactor.

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