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A laboratory study of the biodegradation of MTBE solubilised in water by a microbial consortium entrapped in a water-in-oil-in-water double emulsion

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ABSTRACT

A stable water-in-oil-in-water ($W_1/O/W_2$) double emulsion containing in its inner aqueous phase a microbial consortium (60 $mg_{protein}\ L^{-1}_{double\ emulsion}$) known to degrade methyl *tert*-butyl ether (MTBE) was prepared. The primary emulsion (W_1/O) had a volume disperse phase (ϕ_1) of 0.3 and was stabilized with 8% Span 80. The double emulsion had a volume disperse phase (ϕ_2) of 0.3 and was stabilized with 0.2% polyacrylic acid (PAA). A maximum degradation rate of 12.4 $mg_{MTBE}\ g^{-1}_{protein}\ h^{-1}$ was achieved in a microcosm that contained an initial concentration of 146 $mg_{MTBE}\ L^{-1}_{double\ emulsion}$. This degradation rate was significantly lower ($P < 0.05$) than that exhibited by the free cells (48.7 $mg_{MTBE}\ g^{-1}_{protein}\ h^{-1}$), probably because the encapsulated cells metabolized an ingredient of the double emulsion as carbon source besides MTBE. Nevertheless, our results show that this biodegradation technique has great applicability potential.

1. Introduction

Methyl *tert*-butyl ether (MTBE) is an oxygenating agent that is added to gasoline to reduce toxic pollutant emissions by automobiles. Water pollution by MTBE is usually caused by gasoline leaks, and the chronic exposure of humans to this compound via ingestion of contaminated water, has had detrimental effects on their health, as MTBE is a neurotoxic and is catalogued as a possible human carcinogen agent [1]. MTBE is miscible in gasoline and is soluble in water, alcohol and other ethers, has high water solubility (23.2–54.4 g L^{-1}), and a low octanol/water partition coefficient (log K_{ow} 0.94–1.16). Once in the groundwater, MTBE can move at virtually the same velocity as water, whereas benzene and other petroleum constituents tend to adsorb to soil particles and be degraded. Therefore, once MTBE gets into groundwater, an MTBE plume develops rapidly which is difficult to remediate by conventional methods. More information is needed on cost-effective techniques to treat MTBE at contaminated sites [2,3]. The Environmental Protection Agency of the United States proposed an advisory level of 20–40 $\mu g_{MTBE}\ L^{-1}$ in water [4].

Several technologies for treating MTBE and other fuel oxygenates have been reviewed [5] but these methods require treating relatively high MTBE concentrations to be cost efficient, and are deemed inefficient as they simply transfer the pollutant from one place to another [6]. Thus an ongoing research topic is the development of technologies for efficiently eliminating contaminants from water. Biological methods for MTBE degradation are a good alternative for bioremediation of polluted sites [2,7] including the preferred use of aerobic [7–11] microorganisms although some have been reported under anaerobic conditions [12,13].

Nevertheless, these microorganisms are not present naturally in sufficient quantity, so they must be artificially added in sufficient quantity to the contaminated site. The entrapment of a microbial consortium (MC) within a water-in-oil-in-water ($W_1/O/W_2$) double emulsion seems to provide an excellent technological option for eliminating efficiently contaminants in water. These systems have been used in the removal of heavy metals [14,15] and inorganic salts [16] from water. $W_1/O/W_2$ double emulsions can be defined as an organic liquid phase interposed between two aqueous liquid phases. When double emulsions are dispersed in a water body polluted by a given contaminant, a large surface area arises between the contaminated water body and the emulsion droplets, and even a relative larger surface area between the emulsion droplets and the encapsulated the inner aqueous

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phase. When different and specific thermodynamic conditions exist between the contaminated water body and the inner aqueous phase of the double emulsion, an accelerated mass transfer process of contaminants from the bulk of outer aqueous phase to the inner aqueous phase of the system can be achieved [17]. It is the last mechanism which makes double emulsions potentially suitable for decontamination purposes.

However, the use of these systems in practical applications has occurred only in the last few years, as double emulsions are inherently very unstable systems [18]. Nevertheless, in recent years the stability of these systems has been much improved by using biopolymers as stabilizing agents in the outer aqueous phase [19,20]. The use of stable double emulsions allows for: (1) co-encapsulation of nutrients with the microbial consortium in the inner aqueous phase; (2) their use in conditions where high shear rates exist; (3) achieving a good dispersion of the emulsion in the contaminated water, facilitating an intimate contact with the pollutant and (4) easy recovery of the double emulsion from water by density difference.

The objective of this work was to obtain a stable $W_1/O/W_2$ double emulsion entrapping in its inner aqueous phase an aerobic microbial consortium known to biodegrade MTBE, and to determine the biodegradation rate of MTBE when put into contact in a microcosm with the $W_1/O/W_2$ double emulsion containing the microbial consortium.

2. Materials and methods

2.1. Microbial consortium

The MC used in this work was one that had been previously isolated from upper soil layers contaminated by gasoline in Mexico [9]. The MC was suspended in the enriched mineral culture medium (ECM) described by Fortin and Deshusses [21], using 146 $mg_{MTBE}\ L^{-1}$ as the only carbon source, in a 1.5 L reactor, with stirring at 130 rpm and at 25 °C. Propagation of the biomass was done in pro-culture flask (1 L) in sterile conditions, by placing in it 400 mL_{ECM} and by inoculating the MC withdrawn from the reactor. MC pellets were obtained by centrifuging the ECM with biomass at 9000 rpm for 20 min at 25 °C. The cells were washed twice with mineral medium free of MTBE. The biomass was re-suspended in ECM to obtain an aqueous suspension (AS) with a concentration of 500 $mg_{protein}\ L_{ECM}^{-1}$. Protein content was determined by Lowry's et al. [22] method.

2.2. Formation of the $W_1/O/W_2$ emulsions

The double emulsions were prepared using the two-stage method [20]. In the first stage water-in-oil emulsions (W_1/O) were prepared in which the aqueous phase (W_1) was made up by AS, which on turn were dispersed in the oil phase made up by white mineral oil with Span 80 as lipophilic emulsifier with the help of a high shear homogenizer Silverson L4R (Silverson Machines, Ltd., Waterside, Chesham, Buckinghamshire, England) operated at 8000 rpm during 10 min. Different Span 80 concentrations (0.5, 1, 2, 4, 8, 10, 16 and 30%, w/w) and dispersed-phase volume fractions (ϕ_1) of 0.1, 0.2, 0.3 and 0.4 were used. The concentration of droplets in an emulsion is usually described in terms of the dispersed-phase volume fraction (ϕ), which is equal to the volume of emulsion droplets (V_D) divided by the total volume of the emulsion (V_E): $\phi = V_D/V_E$ [23]. In order to determine the Span 80 concentration and ϕ_1 providing the W_1/O emulsion most stable for our study, we studied the size variation of the droplets with time. The mean droplet size can be calculated statistically in several ways from the size distribution data obtained experimentally considering the properties of the emulsion under study. For our W_1/O emulsions, the total surface area of the droplets containing the microbial consortium for a given volume concentration of disperse phase is important, so that the mean diameter is derived from the mean initial surface–volume diameter [24]:

$$D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

where n_i is the number of droplets with diameters d_i.

The selected W_1/O emulsion was then re-dispersed in different aqueous solutions of polyacrylic acid (PAA) (Pemulen TR-1®, Noveon Inc., Ohio) with concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4% (w/w) adjusted to pH 7, to provide $W_1/O/W_2$ emulsions with a dispersed-phase volume fraction (ϕ_2) of 0.3, by stirring at 1000 rpm for 10 min in a Caframo mechanical mixer (model BDC6015, Ontario, Canada). The stability of the $W_1/O/W_2$ emulsions was determined following the change in the mean volume droplet size ($D_{3,0}$) over time:

$$D_{3,0} = \sqrt[3]{\left(\frac{\sum n_i d_i^3}{\sum n_i}\right)} \quad (2)$$

Properties such as the viscosity at high shear rate can be better correlated with $D_{3,0}$ [24]. Both $D_{3,2}$ and $D_{3,0}$ were estimated with an image analysis system (Optic Microscopy Olympus BX 45 and Olympus C-3030 Digital Camera, Olympus Optical Co. Ltd., Tokyo, Japan; Image-Pro Plus version 4.5 Software, Media Cybernetics Inc., Silver Springs, MD). The stability of the W_1/O and $W_1/O/W_2$ emulsions was quantified by determining the rate of droplet coalescence [25], using the criteria where the rates of coalescence in the range of $1 \times 10^{-3}\ s^{-1}$ indicate unstable emulsions, of $1 \times 10^{-7}\ s^{-1}$ stable emulsions and of $1 \times 10^{-12}\ s^{-1}$ very stable emulsions [26]. Emulsions were subjected to a constant high shear rate (100 s^{-1} for 15 min) with MCR 300 Physica rheometer using a cylindrical double gap measuring system (Paar Physica, Messtechnik, Stuttgart, Germany), in order to emulate the mechanical stress to which they are exposed when injected into aquifers. $D_{3,2}$ of the inner droplets and $D_{3,0}$ of the outer droplets were determined before and after the test in order to determine if significant changes in droplet size of the emulsions occurred.

2.3. Kinetic experiments and analytical techniques

Microcosms were prepared in 125 mL serological bottles sealed with Mininert standard gas-tight valves (Supelco, Sigma–Aldrich Canada Ltd., Oakville, Ontario, Canada) under aerobic conditions, in order to determine the entrapped MC activity, and compared to that of free cells. Each flask contained 10 $mL_{W1/O/W2\ emulsion}$ which included entrapped MC (0.06 $mg_{protein}\ mL_{double\ emulsion}^{-1}$). Two microlitres of MTBE were injected directly into the liquid phase in each bottle to give a concentration of 146 mg L^{-1} [9]. The bottles were incubated in a rotary shaker (130 rpm, 30 °C). Cells were deactivated by autoclaving at 121 °C for 15 min before entrapment or when used as abiotic control. An experiment was performed without MTBE to evaluate CO_2 production. MTBE, O_2 and CO_2 concentrations were measured from a 100 μL headspace gas sample, 24 h after injection of MTBE into shaking bottles and analyzed by gas chromatography (GC). The variation of total peak area of the gases was monitored during the incubation period (288 h).

MTBE concentration was quantified daily by injecting the sample with an air-tight syringe to a GC equipped with a flame ionization detector (PerkinElmer Instruments Autosystem XL, Waltham, MA). A silica capillary column (HP-5, 5%, 30 m, 250 μm × 0.25 μm) and N_2 at a flow rate of 50 mL min^{-1} as carrier gas was employed. The oven temperature was 100 °C, of the detector 220 °C and of the injector 200 °C. O_2 consumption and CO_2 production were quantified similarly but using a GC equipped with a thermal conductivity detector (Gow-Mac Series 550, Bethlehem, PA) and a concentric column (CTR1 Alltech Inc., Kentucky) and with He as carrier gas with a flow rate of 65 mL min^{-1}. The oven, detector and injector temperatures were 40, 150 and 130 °C, respectively. MTBE consumption or CO_2 production rates were fitted with the Gompertz model as previously described [27]. All the experiments were done in triplicate.

2.4. Statistical analyses

The variables $D_{3,2}$, $D_{3,0}$ and rates of coalescence of droplets of emulsions were analyzed using a completely random block design, where time was blocked. Parameters of degradation of MTBE for free or entrapped MC were arranged in a completely randomized design. Data were analyzed by analysis of variance. Significant differences between treatments were determined by Tukey's test at $P < 0.05$ using the NCSS version 5 statistical software (Wireframe Graphics, Kaysville, UT) (NCSS, 2001).

3. Results and discussion

3.1. Formation of the $W_1/O/W_2$ emulsions

Droplet size and rate of droplet coalescence were initially determined in the W_1/O and $W_1/O/W_2$ emulsions without entrapped MC. Based on the stability of these abiotic emulsions, a $W_1/O/W_2$ double emulsion including MC was made afterwards. Evaluation of the W_1/O emulsions consisted on determining the effect of Span 80 concentration at a constant ϕ_1 of 0.1 on $D_{3,2}$ after 1 h of manufacture. As Span 80 concentration increased, $D_{3,2}$ decreased (Fig. 1). The W_1/O emulsions with concentrations between 0.5 and 4% Span 80 showed significantly higher ($P < 0.05$) droplet sizes than those with concentrations in the range of 8–30%. Furthermore, the former group of emulsions tended to sediment after 5 days due to their relatively large droplet size, and in

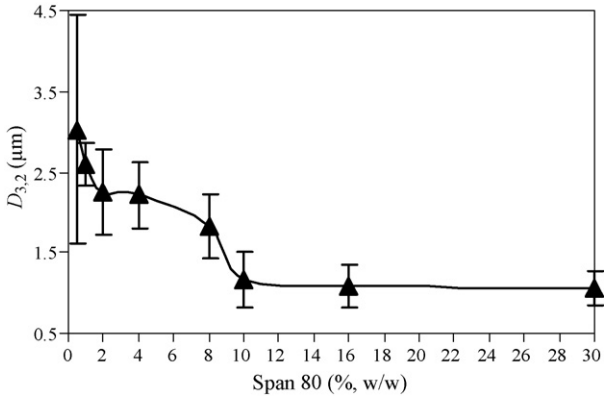

Fig. 1. Initial mean volume–surface ($D_{3,2}$) droplet diameter of W_1O primary emulsion made with different Span 80 concentrations (0.5, 1, 2, 4, 8, 10, 16 and 30 wt%) and a dispersed-phase volume fraction (ϕ_1) of 0.1.

consequence, density. Based on these results we chose a concentration of 8% of Span 80 for further experimentation, because at this concentration we obtained a sufficiently small droplet size to foster the stability of W_1/O emulsion against droplet coalescence [19], but also because excess of lipophilic emulsifier is known to destabilize $W_1/O/W_2$ double emulsions [18,28].

The W_1/O emulsions droplets with ϕ_1 of 0.1, 0.2, 0.3 and 0.4 all with 8% Span 80 showed non-significant ($P > 0.05$) differences in the rate of droplet coalescence which were 4×10^{-7}, 1×10^{-6}, 7×10^{-7} and 4×10^{-7} s^{-1}, respectively, indicating that all the emulsions had good stability. A ϕ_1 of 0.3 was selected because a large inner volume disperse phase is desired for accommodating a larger number of microorganisms. High ϕ_1 such as 0.4 greatly increased the W_1/O emulsion viscosity, so that a more drastic emulsification process was required in the second emulsification step if a homogeneous distribution of W_1/O emulsion in W_2 phase was desired. It is well known that the use of high shear rates in the second emulsification step usually result in very unstable $W_1/O/W_2$ double emulsions [28]. Thus we now proceeded to form $W_1/O/W_2$ double emulsions by dispersing a W_1/O emulsion with 8% Span and $\phi_1 = 0.3$ in aqueous solutions of PAA of different concentrations.

The effect of PAA concentration on $D_{3,2}$ of the inner droplets within the $W_1/O/W_2$ multiple emulsion formed with concentrations between 0.1 and 0.4% PAA exhibited little variation in droplet size over aging time that was non-significantly ($P < 0.05$) different among them. Mean $D_{3,2}$ of these droplets over time was around 1.49 ± 0.22 μm (Fig. 2a).

However, 0.05 and 0.025% PAA produced emulsions with $D_{3,2}$ that varied substantially with aging time (2.57 ± 1.58 and 3.36 ± 1.68 μm, respectively), that were significantly bigger than those obtained at higher PAA concentrations. $D_{3,0}$ of the outer droplets of the $W_1/O/W_2$ double emulsions as a function of aging time and PAA concentration are shown in Fig. 2b. The W1/O/W_2 emulsion containing 0.2% PAA exhibited the least variation in mean $D_{3,0}$ (9.41 ± 1.09 μm) over time, and was significantly different ($P < 0.05$) from that by the double emulsions made with 0.1% PAA (11.76 ± 1.59 m) and with 0.4% PAA (12.42 ± 1.38 μm).

Thus, from a qualitatively point of view, the best formulation for yielding a stable $W_1/O/W_2$ double emulsion was the one made up with 8% Span, $\phi_1 = 0.3$, 0.2% PAA and $\phi_2 = 0.3$.

Nevertheless, double emulsions are notoriously complex systems, which may undergo multiple instability mechanisms, so that a more quantitative parameter for selecting the right formulation is desirable. Given that our two indicators for droplet stability over time were $D_{3,2}$ for the internal droplet and $D_{3,0}$ for the

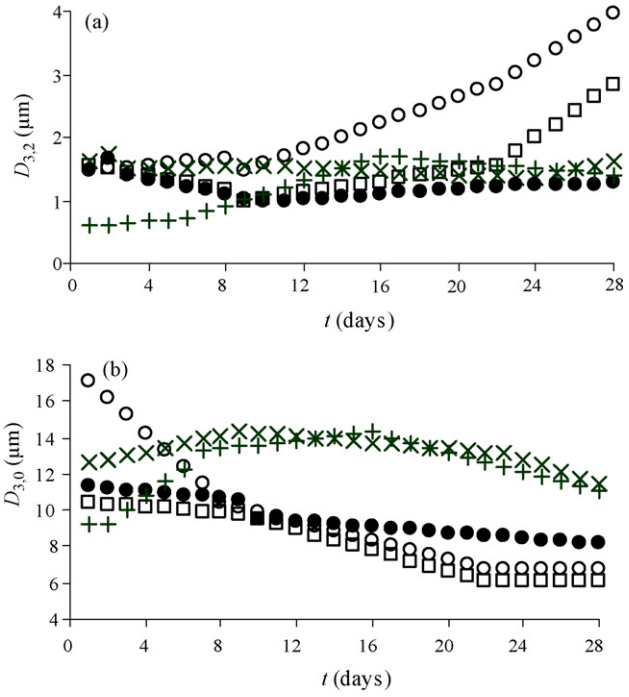

Fig. 2. Variation with aging time of (a) mean surface–volume diameter of inner droplets and (b) mean volume diameter of outer droplets of water-in-oil-in-water double emulsions made with different polyacrylic acid concentrations (○ 0.025, □ 0.05, × 0.1, ● 0.2, + 0.4%, w/w).

external droplet, and that $D_{3,2}$ was much more smaller than $D_{3,0}$, we propose that the evolution of the $D_{3,2}/D_{3,0}$ ratio gave a better insight regarding the global stability of the $W_1/O/W_2$ double emulsion than either parameter individually. For the time being, we proposed arbitrarily that $W_1/O/W_2$ double emulsions exhibiting $D_{3,2}/D_{3,0}$ in the range of 0–0.34 can be considered stable, in the range of 0.35–0.49 fairly stable and in the range of 0.5–1 unstable. As the $D_{3,2}/D_{3,0}$ ratio tends to zero, and this value is maintained over aging time, it means that the emulsion is highly stable as the superficial area of the inner droplets in relation to the volumetric size of the outer droplets is maintained constant over time. The $D_{3,2}/D_{3,0}$ ratios for $W_1/O/W_2$ emulsions made with different concentrations of PAA over a time period of 43 days are given in Fig. 3. In general terms, the lowest $D_{3,2}/D_{3,0}$ ratios over time were exhibited by the $W_1/O/W_2$ double emulsion made with 0.1 and 0.2% PAA. However, in opposition to the W_1/O emulsions where an excess of lipophilic emulsifier is detrimental to the stability of the

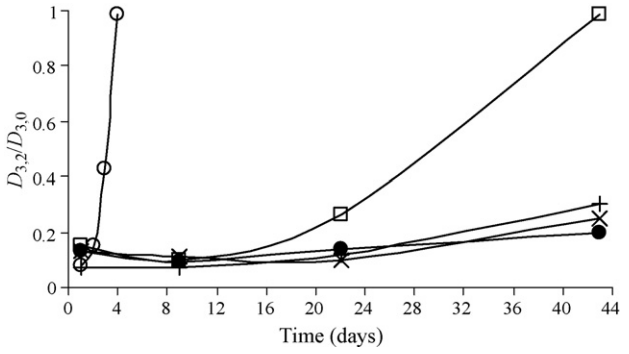

Fig. 3. Mean surface–volume diameter of W_1O primary emulsion droplets to mean volume diameter of $W_1/O/W_2$ double emulsion droplets ($D_{3,2}/D_{3,0}$) ratio as a function of aging time and different concentrations of polyacrylic acid (○ 0.025, □ 0.05, × 0.1, ● 0.2, + 0.4%, w/w).

$W_1/O/W_2$ double emulsion, higher concentrations of polymeric emulsifier in the outer aqueous phase are desirable as thicker adsorbed layers occur that provide the $W_1/O/W_2$ double emulsion with higher stability [20].

The $W_1/O/W_2$ double emulsion (8% Span, $\phi_1 = 0.3$, 0.2% PAA and $\phi_2 = 0.3$) entrapping the MC exhibited even lower $D_{3,2}/D_{3,0}$ ratios with aging time than its counterpart without MC. $D_{3,0}$ (18.14 ± 1.47 μm) for the biotic emulsion was twofold that of its abiotic counterpart. Similar results were reported for active cells of *Flavobacterium* spp. ATCC 39723 entrapped in alginate and agar matrices [29].

The application of biodegrading cells in contaminated aquifers requires the production of large quantities of carrier with micrometric sizes that allow the hydraulic distribution of the encapsulated cells into the aquifers. The encapsulated cells can be then dispersed within the contaminated zone and degrade mobile contaminants [30] such as MTBE. In general, aquifers consist of several layers of silt, sand and gravel, with the pore diameter of these materials falling between 8 and 100 μm. This diameter range permits the transport of globules under 50 μm [31]. Thus it is important that the encapsulated degrading cells maintain their integrity when subjected to high shear rates when injected into the aquifer, in order to comply with the above requirements. Our $W_1/O/W_2$ double emulsion sizes satisfied both criteria: they exhibited adequate droplet size for achieving good particle filtration and showed good droplet coalescence stability as they did not exhibit significant ($P > 0.05$) droplet size changes when subjected to a high constant shear rate of 100 s^{-1} for 15 min.

3.2. Degradation of MTBE by microbial consortium entrapped in $W_1/O/W_2$ emulsion

The MTBE biodegradation activity of the entrapped cells was compared with that of the free cells and the results are presented in Fig. 4 for an initial MTBE mass of 1.46 mg. Free cells showed strong initial degradation and then a decrease in their MTBE-removal capacity after 120 h when around 85% MTBE had been consumed. This value was significantly higher ($P < 0.05$) than that displayed by the entrapped cells (60% after 288 h). Both results were better than those reported by Liu et al. [32] in the bioremediation of MTBE-contaminated groundwater, which was about ~50% in 800 h of operational period.

The MTBE degradation rate by the MC entrapped in the $W_1/O/W_2$ emulsion was 12.4 $mg_{MTBE}\ g^{-1}_{protein}\ h^{-1}$. This degradation rate was lower ($P < 0.05$) than that exhibited by the free cells (48.7 $mg_{MTBE}\ g^{-1}_{protein} h^{-1}$) (Table 1). Deactivated entrapped cells showed no degradation activity, indicating that the biological activity of living cells in the liquid phase is responsible for the depletion of MTBE in the gas phase.

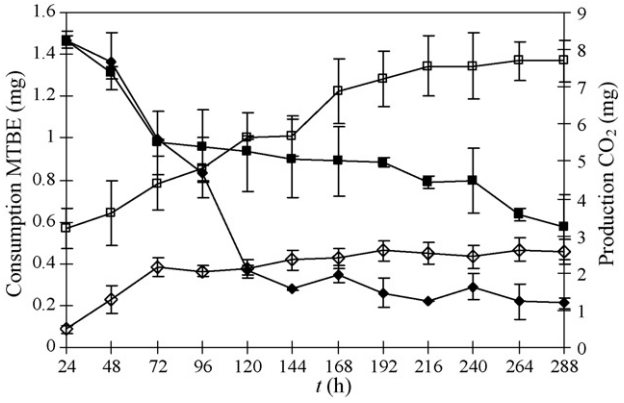

Fig. 4. Evolution of MTBE utilization and CO_2 accumulation for (◆) (◇) free and (■) (□) entrapped microbial consortium in a double emulsion. Error bars show the average deviation of triplicate runs.

Table 1
Kinetic results for the entrapped microbial consortium with and without the presence of MTBE and free cells with MTBE

Parameters	Entrapped cells with MTBE	Entrapped cells without MTBE	Free cells with MTBE
MTBE degradation rate ($mg\ g^{-1}_{protein}\ h^{-1}$)	12.4 ± 0.007^a	ND	48.7 ± 17^b
O_2 consumed (mg)	4.91 ± 0.47^a	3.37 ± 0.02^b	2.84 ± 0.25^c
CO_2 produced (mg)	6.44 ± 0.19^a	4.45 ± 0.09^b	$2.24 \pm .27^c$
Respiratory coefficient	0.76 ± 0.05^a	0.81 ± 0.04^a	0.57 ± 0.04^b

Respiratory coefficient (RC) = moles CO_2/moles O_2; ND = not determined. Superscripts with different letters in rows indicate significant differences ($P < 0.05$).

A degradation rate of MTBE of 3.7 $mg\ g^{-1}_{protein}\ h^{-1}$ by free cells of *Fusarium solani* B1, CBS 117476, threefold times lower than the maximum rate found in this work over a similar experimental time period was reported [11]. The results obtained in this work were similar to those for co-metabolism of an MC with hexane which exhibited a degradation rate of 11.5 $mg_{MTBE}\ g^{-1}_{protein}\ h^{-1}$ [9]. Nevertheless, the MTBE degradation rate shown by the entrapped MC in a double emulsion was lower than that shown by a free MC (26.5 $mg_{MTBE}\ g^{-1}_{protein}\ h^{-1}$) [7], probably because in that work O_2 was constantly fed into the bioreactor, and in our work O_2 was only supplied at the beginning of the experiments. It has been reported that aerobic MTBE biodegradation rates are strongly influenced by oxygen concentration [33] and in situ MTBE biodegradation by native aerobic MTBE-degrading microorganisms can be stimulated and promoted by dissolved oxygen [34].

In the emulsions, oxygen transport into the inner aqueous phase, where the MC is entrapped, may be hindered by the two interfacial membranes formed at the inner and outer water–oil interfaces. On the other hand, it is unlikely that MTBE accumulated in the oil layer due to the low K_{ow} in the oil phase, and also because of the "facilitated transport" principle on which double emulsions operate. In "facilitated transport" solubility of the permeate (MTBE) in the membrane phase (white mineral oil) is not required since permeate interacts with the carrier molecule (Span 80), dissolved in the membrane phase. This carrier should be insoluble in both the feed and stripping phases and should interact with permeate. The driving force for the transport of permeate is provided by the difference in concentration of permeate in the stripping phase (inner aqueous phase) and the feeding phase (outer aqueous phase) [17]. The MTBE concentration gradient is maintained as the MC degrades the MTBE.

The entrapped cells in the presence of MTBE showed a higher CO_2 production than the produced by the control entrapped cells without MTBE (Table 1) and by the free cells (Fig. 4). CO_2 production in the control experiment means that the MC degraded a compound of our $W_1/O/W_2$ double emulsion, probably Span 80 which is more available than the mineral oil. The difference in the respiratory coefficient (RC) indicated that the MC degraded different carbon sources. For the emulsified cells without MTBE (RC = 0.81 ± 0.04) CO_2 production was related to the degradation of Span 80 (chemical formula $C_{24}H_{44}O_6$), which has a theoretical RC of 0.76 if it undergoes complete oxidation. For the case of free cells with MTBE, the RC reflects the respiration of this compound (theoretical RC = 0.66) and for the case of entrapped cells with MTBE as substrate, the RC suggests that both the oxygenated compound and other carbon source from the emulsion were used. Partial or full mineralization of MTBE has been reported in co-metabolic studies [2]. A further effect of the presence of an extra carbon source is the accrued oxygen consumption which may play a role in MTBE degradation by limiting oxygen availability.

Field studies include in situ recirculation of treatment cells that rely on pumping and reinjection to capture and treat a migrating contaminant plume. In situ co-metabolic MTBE bioremediation through bio-stimulation/bio-augmentation may be effective to treat shallow aquifers, but the presence of a deep water table could add operating difficulties. The system is more effective in aquifers composed of sand and hydraulic conductivities greater than 4–10 cm s^{-1} and when the soil is homogeneous to favour regular distribution of oxygen and gaseous alkanes. Necessary biochemical factors include the presence of microbes capable of degrading the contaminants, nutrients bioavailability and a neutral pH [2,32].

4. Conclusions

This work clearly indicates that the use of double emulsions including an entrapped microbial consortium in their aqueous phase constitute a potentially suitable decontamination technique for eliminating very low concentration contaminants (in this case MTBE) in water. Nevertheless, further studies are necessary, where attention must be paid in improving the formulation of the double emulsions so that the microbial consortium does not metabolize ingredients within it. In this way, higher biodegradation rates of MTBE should be achievable. As our capacity for formulating stable $W_1/O/W_2$ double emulsions with entrapped microorganisms increases, so does the feasibility of applying successfully this bioremediation technique at large scales. In this work, a simple procedure was established for obtaining such desired stable systems with the ability of biodegrading MTBE.

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