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ORIGINAL PAPER

# Biodegradation of polyalcohol ethoxylate by a wastewater microbial consortium

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**Abstract** Polyalcohol ethoxylate (PAE), an anionic surfactant, is the primary component in most laundry and dish wash detergents and is therefore highly loaded in domestic wastewater. Its biodegradation results in the formation of several metabolites and the fate of these metabolites through wastewater treatment plants, graywater recycling processes, and in the environment must be clearly understood. Biodegradation pathways for PAE were investigated in this project with a municipal wastewater microbial consortium. A microtiter-based oxygen sensor system was utilized to determine the preferential use of potential biodegradation products. Results show that while polyethylene glycols (PEGs) were readily degraded by PAE acclimated microorganisms, most of the carboxylic acids tested were not degraded. Biodegradation of PEGs suggests that hydrophobe–hydrophile scission was the dominant pathway for PAE biodegradation in this wastewater community. Ethylene glycol (EG) and diethylene glycol (DEG) were not utilized by microbial populations capable of degrading higher molecular weight EGs. It is possible that EG and DEG may accumulate. The microtiter-based oxygen sensor system was successfully utilized to elucidate information on PAE biodegradation pathways and could be applied to study biodegradation pathways for other important contaminants.

**Keywords** Biodegradation · Nonionic surfactant · Microorganisms · Wastewater · Metabolism · Pathway · Pollution · Trace contaminants · Water

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## Introduction

Monitoring the fate of recalcitrant and slowly biodegradable compounds in the environment has become of increasing importance in recent years due to the potential for accumulation in the food chain. Specifically, components present in personal care products have gained much attention. Surfactants are complex organic compounds that are ubiquitous in municipal wastewater due to their presence in household cleaning and personal hygiene products. Polyalcohol ethoxylate (PAE), an anionic surfactant, is one of the six surfactants that make up 60% of total consumption (Brown 1995). PAE is the most common active ingredient in both dish wash and laundry detergents (Lange 1999), accounting for its high use rate of 0.5 million tons per year (Brown 1995). Identification of biodegradation pathways for PAE is essential since metabolites should be assessed for risk to human health and ecosystems.

Several researchers have published biodegradation pathways for PAE under aerobic conditions (Balson and Felix 1995; Kravetz 1990; Steber and Berger 1995). Steber and Wierich (1985) analyzed PAE biodegradation intermediates in effluent of a continuous flow wastewater treatment plant and identified two major pathways (Fig. 1). These pathways have been confirmed by other researchers (Balson and Felix 1995; Kravetz 1990). The first pathway involves hydrophobe–hydrophile scission and results in the formation of an alkyl chain and polyethylene glycol (PEG). The second pathway involves alkyl chain degradation resulting in the formation of coboxyalkyl-PEG. Biodegradation of the carboxyalkyl-PEG metabolite results in formation of carboxylic acids, which are eventually mineralized to carbon dioxide. Biodegradation of PEG has been well studied and several pathways have been suggested (Kawai 1987). However, the most accepted pathway involves successive oxidation to aldehyde and monocarboxilic acid (Kawai 1987). Finally, ether cleavage results in formation of PEG reduced by one glycol unit (Fig. 2). Glyoxilic acid (GOA) is a byproduct of the ether cleavage (Kawai 1987). Steber and Wierich (1985) proposed a new pathway for biodegradation of PEG metabolites, ethylene glycol (EG), and glycolic acid (GCA), involving oxidation to GOA, oxalic acid (OA), and formic acid (FA) (Fig. 3).

To assay the metabolic pathway for PAE in this study, microbial populations acclimated to degradation of the parent surfactant were exposed to metabolites and oxygen uptake was monitored over time.

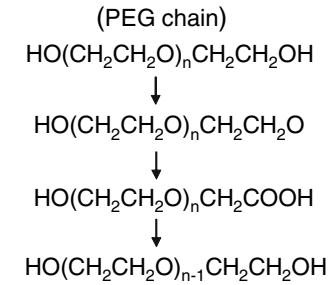


**Fig. 2** Biodegradation pathway for PEG (Kawai et al. 1978)

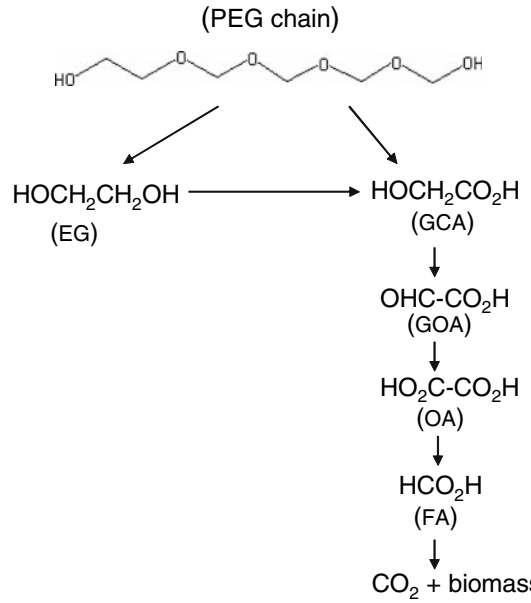


**Fig. 3** Proposed biodegradation pathway for ethylene glycol (*EG*) and glycolic acid (*GCA*) (Steber and Wierich 1985)

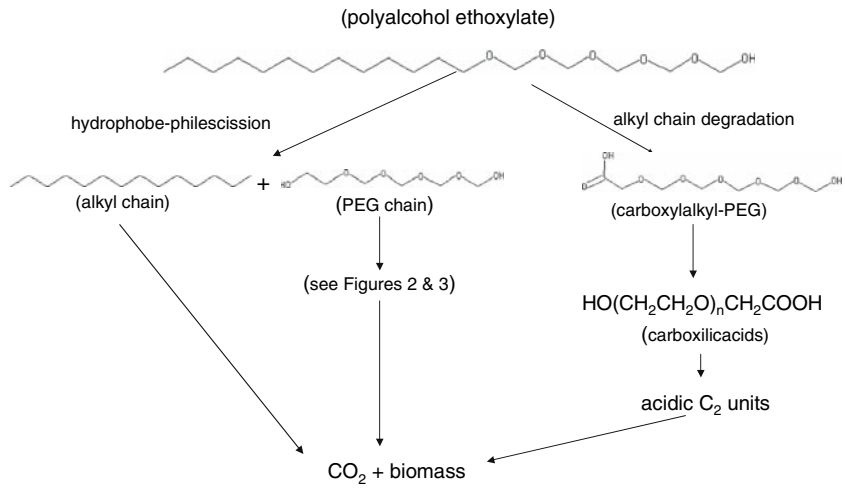


**Fig. 1** Biodegradation pathway for PAEs (Steber and Wierich 1985)

Biodegradation without a lag time, as indicated by instantaneous oxygen uptake, suggests that the population has previously been exposed to the compound and thus that the compound is likely a biodegradation metabolite of the parent compound. The micotiter-based, BD Oxygen Biosensor System allows for rapid screening of the microbial respiration of a variety of substrates (Garland et al. 2003), and was adapted for use in this study to investigate a variety of potential biodegradation pathways. This assay offers advantages over more cumbersome traditional methods of examining biodegradation pathways such as respirometry and analytical chemistry methods.

The purpose of this study was to elucidate information on the dominant biodegradation pathways for PAE and examine the efficacy of the biosensor system to examine biodegradation pathways. Despite numerous research efforts, the biodegradation pathway for PAE and one of its known metabolites (PEG) is still unclear. Specifically, two potential biodegradation pathways for PAE have been identified (Steber and Wierich 1985) and several pathways have been suggested for PEG biodegradation (Kawai 1987; Steber and Wierich 1985). PEG compounds including octaethylene glycol (OEG), hexaethylene (HEG), triethylene glycol (TEG), diethylene glycol (DEG), and EG were utilized to test for the hydrophile-hydrophobe scission pathway (Steber and Wierich 1985). The second known pathway for PAE degradation results in formation of carboxylated PEGs, which are not commercially available. However, biodegradation of carboxylated PEG has been found to form carboxylic acid metabolites (Steber and Wierich 1985). Therefore, several carboxylic acids that were found to be present in systems degrading carboxylated PEG (Steber and Wierich 1985) were utilized as substrates including GA, GOA, OA, and FA. These carboxylic acids were also utilized to examine the potential for the biodegradation pathway for PEG suggested by Steber and Wierich (1985; Fig. 3).

## Materials and methods

Polyalcohol ethoxylate, purchased in the form of Neodol 23-5® (Shell Co., Houston, TX, USA), is 100% pure PAE and is a mixture of 12 and 13 carbon length alkyl chains with an average of 5 moles ethylene oxide per mole of alcohol ethoxylate. PEG compounds (OEG, HEG, TEG, DEG, and EG) and carboxylic acids (GA, GOA, OA, and FA) were purchased from Sigma-Aldrich Inc., Tanfkirchen, Germany.

Activated sludge samples were collected from the recycle line of the activated sludge tank at the West Lafayette Wastewater Treatment Plant, West Lafayette, IN, USA and used as an inoculum. Three flasks were placed on a shaker table containing 5 mL activated sludge and 95 mL minimal salts media (MSM) solution. PAE was added to the flasks at a concentration of 100 mg/L. Five milliliters of activated sludge also was added to an additional flask containing 95 mL of tryptic soy broth (TSB; Sigma-Aldrich Inc.) to promote growth of a mixed population of bacteria to serve as an experimental control. The following constituents were dissolved in 1 L of distilled deionized (DDI) water to prepare the MSM solution: 0.68 g potassium phosphate, 1.73 g potassium phosphate dibasic, 0.10 g magnesium sulfate, 1.00 g ammonium nitrate, and 0.1 mL of trace elements per liter of MSM. The trace elements solution included: 2.00 g magnesium oxide, 0.40 g calcium carbonate, 1.08 g iron (III) chloride, 0.288 g zinc sulfate, 0.05 g cupric sulfate, 0.0056 g cobaltous sulfate, 0.0124 g boric acid, and 0.0098 g sodium molybdate. These constituents were dissolved in 190 mL DDI water and 10 mL of hydrochloric acid. Microbial communities were cultured on PAE or TSB over a period of 4 weeks prior to serving as an inoculum in oxygen biosensors. During this 4 weeks time span, flasks were replenished with substrate (100 mg/L PAE or TSB) once per week.

BD oxygen biosensors (BD Biosciences, Bedford, MA, USA) were used to assess oxygen uptake when microbial populations were exposed to the substrates listed above. The biosensors are 96 well plates containing an oxygen sensitive fluorophore, 4,7-diphenyl-1,100-phenathroline ruthenium (II) chloride, embedded in a gel matrix on the bottom of each well. Aerobic microbial degradation of the substrates and concomitant reduction in dissolved oxygen within the wells results in increased fluorescence. A volume of 100 μL of sterile MSM containing 200 mg/L of substrate was combined with 100 μL of inoculum consisting of either PAE degrading bacteria or mixed populations from a control flask containing TSB. There were three replicate flasks

containing bacteria acclimated to PAE degradation and each substrate was combined with bacteria from one of each of the three flasks. Each inoculum was added to wells containing 100 μL of MSM solution to serve as a control for quantification of background fluorescence and oxygen consumption resulting from carbon present in the inoculum solution. Glucose in MSM was added to each inoculum to serve as a positive control, ensuring that the inoculum contained active cultures and to provide a reference substrate to evaluate the relative microbial activity of the two cultures. Fluorescence was measured using a Perkin Elmer Victor 3 plate reader at a 485 nm excitation and 604 nm emission. Samples were measured every 20 min over 30 h at a controlled temperature of 22°C.

Several parameters were used for quantitative comparisons between fluorescence responses for different substrates. The first was time to initial response ($t_i$), or lag time. This was defined as the time to which fluorescence increased above levels observed in sample wells with no substrate, in normalized fluorescence units (NRFU). NRFU represent the fluorescence observed for each sample normalized to the fluorescence observed at the beginning of the assay. The highest NRFU observed in control wells with no substrate was 1.2, so $t_i$ was defined as the time at which NRFU for a sample increased above 1.2. The maximum value of NRFU observed for each sample was used to make comparisons. The third parameter used to make comparisons between fluorescence response for various substrates was the time at which the maximum value of NRFU ($t_m$) was observed. One-sided *t*-tests were used to compare average parameter values when substrates were added to wells containing PAE acclimated bacteria to wells containing mixed communities of microorganisms.

## Results and discussion

A distinctive fluorescence response was observed when acclimated microorganisms were exposed to PAE within the BD microplates compared to unacclimated microorganisms (Fig. 4). Although the maximum response is similar for the two inoculums, the $t_m$, or lag, was longer in wells containing the control microorganisms compared to those containing PAE acclimated microorganisms. This data set shows

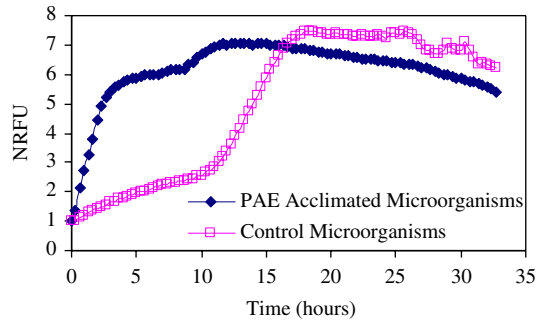


**Fig. 4** Response of PAE acclimated microorganisms and a mixed population (control microorganisms) to PAE in BD microplate assay

the importance of evaluating all three parameters; $t_i$, maximum NRFU, and $t_m$. In this case, the maximum response of the control group was the same as the PAE acclimated bacteria (Fig. 5; $p < 0.05$) but rapid response to PAE by the PAE acclimated microorganisms was observed compared to a lag in response observed in the control inoculum. While it is evident that the control community still contained PAE degrading organisms due to it's previous exposure to PAE within the wastewater treatment facility, the more rapid response observed with the PAE acclimated microorganisms demonstrates successful enrichment of PAE degrading microorganisms in PAE acclimated cultures.

The response of PAE acclimated bacteria and the control microorganisms were compared in the

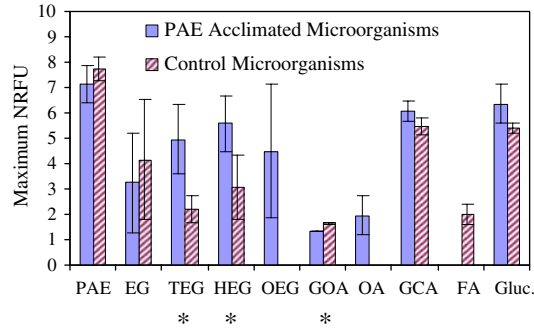


**Fig. 5** Maximum fluorescence response observed for each substrate in the presence of PAE acclimated microorganisms and a mixed population (control microorganisms). *Astericks* below substrates indicate that the maximum NRFU observed for the indicated substrate was significantly different in the presence of PAE acclimated microorganisms than in the presence of the control microorganisms at the 95% significance level. *Error bars* indicate +/− one standard deviation

presence of glucose as a substrate to compare microbial activity of the two inoculums (Figs. 5, 6, 7). The responses to glucose were very similar for the two communities with the maximum fluorescence response observed and lag time for initiation of response showing no significant difference (Figs. 5, 6; $p < 0.05$). The time to reach maximum response, $t_m$, was slightly longer for PAE acclimated bacteria than for the control microorganisms (Fig. 7). Results indicate that overall microbial activity was similar in the two cultures, indicating that differences in response to specific substrates between the two inocula reflect selective enrichment for different functional groups of microorganisms.

Most PEGs were readily degraded in the presence of PAE acclimated microorganisms, particularly higher molecular weight PEGs (Fig. 8). Response of the control bacteria are not shown in Fig. 8 for purposes of clarity. The control microorganism showed no response to OEG (as indicated by the absence of a bar in Figs. 5, 6, 7), while the fluorescence response observed in wells with PAE acclimated microorganisms was high. The maximum response observed was significantly higher for TEG and HEG in wells containing PAE acclimated microorganisms compared to control microorganisms (Fig. 5; $p < 0.05$) while $t_m$ was significantly lower (Fig. 7; $p < 0.05$). Furthermore, the lag time observed for TEG and HEG in wells inoculated with a mixed consortium was longer as indicated by larger $t_i$-values

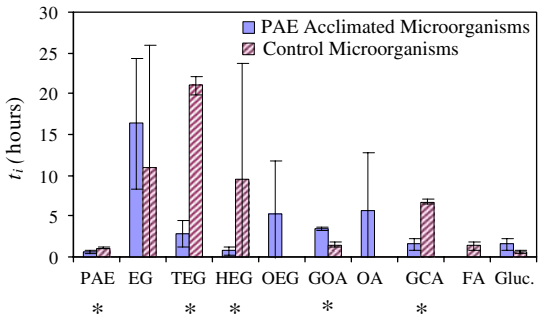


**Fig. 6** Lag time ($t_i$) for observed fluorescence response for each substrate in the presence of PAE acclimated microorganisms and a mixed population (control microorganisms. *Astericks* below substrates indicate that the $t_i$ observed for the indicated substrate was significantly different in the presence of PAE acclimated microorganisms than in the presence of the control microorganisms at the 95% significance level. *Error bars* indicate +/− one standard deviation

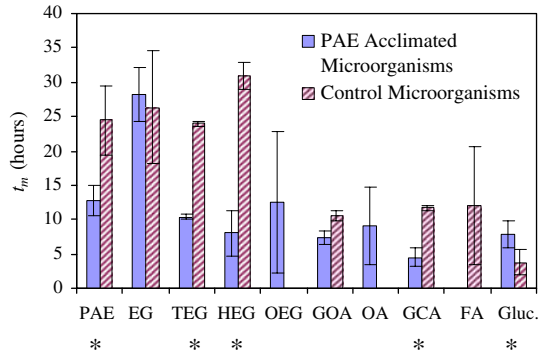


**Fig. 7** Time to maximum fluorescence response ($t_m$) observed for each substrate in the presence of PAE acclimated microorganisms and a mixed population (control microorganisms). *Astericks* below substrates indicate that the $t_m$ observed for the indicated substrate was significantly different in the presence of PAE acclimated microorganisms than in the presence of the control microorganisms at the 95% significance level. *Error bars* indicate +/− one standard deviation

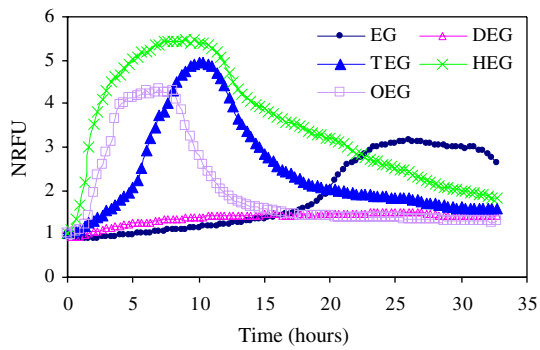


**Fig. 8** Fluorescence response in BD Oxygen Biosensor Systems when PAE acclimated microorganisms were exposed to PEGs

(Fig. 6), although the difference was not statistically significant for HEG ($p < 0.05$).

While fluorescence response of high-molecular weight PEGs was significantly higher in wells inoculated with PAE acclimated microorganisms than wells inoculated with mixed microorganisms grown in TSB, no response was observed for DEG (not represented in Figs. 5, 6, 7) and little response was observed for EG. The response of EG was not significantly different for PAE acclimated microorganisms than control microorganisms (Fig. 5; $p < 0.05$). The biodegradation pathway for PEG results in formation of acidic ethoxylates (Kawai et al. 1978), and it is possible that microorganisms

may utilize acidic ethoxylates before conversion to DEG and EG (Steber and Wierich 1985). Some researchers have suggested that the pathway for degradation of DEG and EG may be different than that for high-molecular weight PEGs (Kawai 1987). Low-molecular weight acidic ethoxylates may have been utilized rather than DEG and EG because GCA was readily degraded by microorganisms acclimated to PAE (Fig. 9). While the maximum NRFU observed was not significantly different for the two groups of microorganisms when exposed to GCA (Fig. 5; $p < 0.05$), there was a significantly longer lag time observed in the control microorganisms compared to the PAE degrading microorganisms as indicated by larger $t_i$ and $t_m$-values (Figs. 6, 7; $p < 0.05$). These results suggest that the PAE acclimated microorganisms were likely previously exposed to GCA, supporting the hypothesis that low-molecular weight carboxylic acids may be utilized rather than DEG and EG. On the other hand, it is possible that DEG and EG were not biodegraded in the system and thus may accumulate. Some researchers have observed inability of PEG degrading microorganisms to degrade DEG and EG (Cox 1978; Ogata et al. 1975; Pearce and Heydeman 1980). Potential accumulation of DEG and EG within graywater recycling systems, wastewater treatment plants, or the environment is important because EG has been identified as a toxic substance for human consumption with effects such as coma, respiratory failure, gastrointestinal upset, cardiopulmonary effects, and renal damage (ATSDR 1997). The Environmental Protection Agency has set a drinking water limit for EG of 7 mg/L for adults.

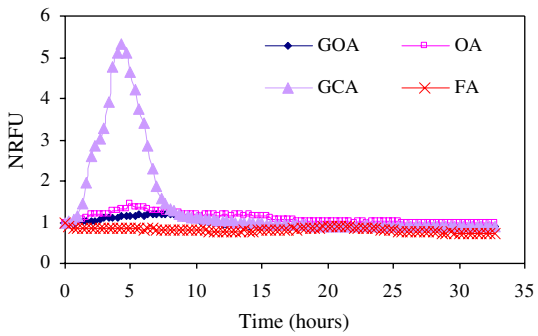


**Fig. 9** Fluorescence response in BD Oxygen Biosensor Systems when PAE acclimated microorganisms were exposed to carboxylic acids

Further, some researchers hypothesized that accumulation of PEG byproducts resulted in phytotoxicity within a graywater recycling hydroponics system (Garland et al. 2004). Because EG and DEG were not readily biodegraded in the studied system, these compounds should be screened for to determine their fate.

While PEGs were readily degraded by PAE acclimated microorganisms, most of the carboxylic acids tested were not degraded (Fig. 9). GCA was readily utilized, but this could likely be a result of the mechanism discussed above. The control inoculum actually demonstrated a higher affinity for GOA metabolism than PAE acclimated microorganisms as shown by a significantly higher maximum NRFU and lower $t_i$ (Figs. 5, 6; $p < 0.05$). The response of PAE acclimated organisms to OA was low compared to other substrates examined (Fig. 5) and PAE acclimated bacteria did not respond to FA as indicated by the absence of bars in Figs. 5, 6, 7.

Steber and Wierich (1985) showed that when PAE biodegradation is initiated by the hydrophobe–hydrophile scission, neutral PEGs are formed. Conversely, when alkyl chain degradation occurred resulting in formation of a carboxylated PEG, more than 90% of the biodegradation metabolites were carboxylic acids. Data collected in this research project suggests that PAE acclimated microorganisms were previously exposed to PEGs and not carboxylic acids. Therefore PAE biodegradation was likely initiated by the hydrophobe–hydrophile scission. Other researchers have also shown that hydrophobe–hydrophile scission is the dominant biodegradation pathway in the presence of a mixed consortium (Kravetz 1990; Steber and Berger 1995). Additionally, ready biodegradation of the tested PEG compounds indicates that the biodegradation pathway introduced by Kawai et al. (1978) involving successive oxidation of PEG resulting in a PEG reduced by one glycol unit was likely the dominant pathway by which PEGs were degraded.

Research has shown that GOA is formed when ether cleavage occurs during the biodegradation of PEGs (Kawai 1987). However, GOA was not readily degraded by PAE acclimated microorganisms (Fig. 9) even though PEG biodegradation was found to be likely. Kawai and Yamanaka (1986) reported that GOA was inhibitory to microbial growth above 5 mM. Although the level of exposure in BD

microplate assays was 100 mg/L, or 1.4 mM, which was not found to be inhibitory by Kawai and Yamanaka (1986), toxicity of GOA cannot be dismissed. Steber and Wierich (1985) proposed a new pathway for the degradation of EG and GCA where these compounds are oxidized to GA, OA, and FA. As previously discussed, PAE acclimated microorganisms did not show an enhanced ability to degrade these substrates. This proposed pathway was not likely dominant in this study.

## Conclusions

The BD oxygen biosensor systems were successfully used to generate information about the biodegradation pathways of PAE in the batch systems inoculated with a wastewater microbial consortium. Biodegradation of PEGs indicated that the hydrophobe–hydrophile pathway for PAE degradation was the dominant pathway. Several potential metabolites were identified that may not be readily biodegradable and require further investigation, specifically DEG, EG, and GOA. The microtiter-based oxygen sensor system enabled rapid screening of microbial response to multiple substrates and may be utilized to examine the biodegradation pathways for other contaminants in flow through biological processors and environmental samples.

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