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Biocompatibility of Low Molecular Weight Polymers For Two-Phase Partitioning Bioreactors

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ABSTRACT: Two phase partitioning bioreactors (TPPBs) improve the efficiency of fermentative processes by limiting the exposure of microorganisms to toxic solutes by sequestering them into a non-aqueous phase (NAP). A potential limitation of this technology, when using immiscible organic solvents as the NAP, is the cytoxicity that these materials may exert on the microbes. An improved TPPB configuration is one in which polymeric NAPs are used to replace organic solvents in order to take advantage of their low cost, improved handling qualities, and biocompatibility. A recent study has shown that low molecular weight polymers may confer improved solute uptake relative to high molecular weight polymers (i.e., have higher partition coefficients), but it is unknown whether sufficiently low molecular weight polymers may inhibit cell growth. This study has investigated the biocompatibility of a range of low molecular weight polymers, and compared trends in biocompatibility to the well-established "critical log P" concept. This was achieved by determining the biocompatibility of polypropylene glycol polymers over a molecular weight (MW) range of 425–4,000 to *Saccharomyces cerevisiae* and *Pseudomonas putida*, two organisms which have been previously used in TPPB systems. The lower MW polymers were shown to have lower average log P values, and showed more cytotoxicity than polymers of the same structure but with higher molecular weight. Since polymers are generally polydisperse (i.e., polymer samples contain a distribution of MWs), removal of the lower MW fractions via water washing was found to result in improved polymer biocompatibility. These results suggest that the critical log P concept remains useful for describing the toxicity of polymeric substances of different MWs, although it is complicated by the presence of the low MW fractions in the polymers arising from polydispersity.

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Introduction

In situ product removal is a strategy used in bioreactor design to limit the accumulation of toxic fermentation products, thereby increasing process efficiency. One of the most successful approaches to in situ product removal has been the use of two phase partitioning bioreactors (TPPB) (Dafoe and Daugulis, 2014). This approach uses a non-aqueous phase (NAP) in order to extract product as it is being produced by a microbial population, thereby reducing cytotoxicity. Work in the field of TPPBs has largely focused on the use of organic solvents as the extracting phase and has been shown to enhance efficiency in a number of biosynthetic processes utilizing both prokaryotic and eukaryotic cells (Bruce and Daugulis, 1991; Barton and Daugulis, 1992; Brennan et al., 2012; Heipieper et al., 2007; Newman et al., 2006; Vrionis et al., 2002; Yang et al., 2009). TPPBs utilizing an organic solvent NAP have also been shown to be effective in promoting the efficient degradation of various toxic substrates (Darracq et al., 2012; Quijano et al., 2009; Yeom and Daugulis, 2011). The major challenge that has limited progress in the field of TPPB research has been selecting liquid partitioning NAPs which are non-toxic to the microorganisms involved. A clear relationship has been established between the logarithm of the octanol-water partitioning coefficient (log P, or log K_{OW}) of an organic solvent and the toxicity of that solvent to microorganisms (Bruce and Daugulis, 1991; Inoue and Horikoshi, 1991). Solvents with low log P values tended to be toxic, while compounds with higher log P tended to be biocompatibile. The log P value at which solvents are biocompatible to a given microorganism has been termed the "critical log P", and this phenomenon has been experimentally demonstrated for a number of cell types (Bruce and Daugulis, 1991; Barton and Daugulis, 1992; Khan and Daugulis, 2010; Inoue and Horikoshi, 1991). Biocompatible alternative NAPs that have been investigated include silicone oils (El Aalam et al., 1993) or ionic liquid extractants (Quijano et al., 2010a,b), which have tended to be prohibitively expensive. A challenge in selecting liquid NAPs for TPPBs has therefore been in identifying a material that is low in cost, rapidly uptakes the target compound, and has a log *P* value which is above the critical log P of the producing organism (Bruce and Daugulis, 1991).

Recent efforts of our group have focused on using polymers as NAPs in TPPB applications. Polymers tend to be non-toxic to organisms, non-volatile, low in cost, non-biodegradable, and have superior handling qualities. Materials such as DuPont's Hytrel® and styrene/

butadiene rubber have been shown to be effective in improving productivity of a number of bioreactor systems via in situ product removal (Amsden et al., 2003; Khan and Daugulis, 2010; Morrish and Daugulis, 2008). Recycled automobile tires, which have a near-zero cost, have also been shown to enhance productivity in certain TPPB applications (Tomei et al., 2012). Given the demonstrated efficacy of polymeric NAPs, we have recently developed theoretical models to assist with rational polymer selection (Bacon et al., 2014; Parent et al., 2012; Poleo and Daugulis, 2014). One outcome of this work was the demonstration that low molecular weight (MW) polymers tend to have higher solute uptake (i.e., have higher solute affinity) than an equivalent polymer of a higher MW (Bacon et al., 2014) and these results suggest that low molecular weight polymers should be further explored as NAPs for bioreactor systems. Experimental work has shown that reducing the MW of a poly(n-butyl acrylate) from an average of 100,000 to 258 improved the partitioning coefficient of n-butyl acetate and benzene by over 150%, which clearly demonstrates that the gains in solute uptake by using low MW polymers are often substantial (Bacon et al., 2014)

A common explanation for a polymer's biocompatibility and non-bioavailability is that it can be considered to be a very large molecular weight organic solvent, and hence be effectively inert to microorganisms. It is important to recognize, however, that a spectrum exists that spans low MW monomeric liquid organic solvents at one end, and their high MW polymeric counterparts at the other end. That is, polymers can exist over a vast range of monomeric ("mer") units and corresponding molecular weights, and that physically they can exist as liquids in the case of low molecular weight (small "mer") polymers or very hard, large molecular weight materials. Given that low molecular weight polymers begin to become more monomer-like (which may not be biocompatible), there is a potential concern that arises in the use of low MW polymers in TPPB applications. Limited work has been done to investigate the use of low MW polymers as NAPs (Barton and Daugulis, 1992) over a range of MWs, and these findings have suggested that low MW polymers (<1,000 Daltons) could be toxic to microbes, even though they have improved solute uptake, as noted earlier (Bacon et al., 2014). To the best of our knowledge, however, there has been no examination of the properties of the polymers that led to toxicity, nor any effort to correlate the toxicity of these low MW polymers to the critical log P concept. The objective of this work was to improve the understanding of the patterns of toxicity over a range of polymer MWs by determining the relationship between the log P of the polymers and polymer MW to microbial biocompatibility. Specifically, trends in the toxicity of a range of relatively low MW polypropylene glycols (PPG) to *Saccharomyces cerevisiae* and *Pseudomonads putida* (a eukaryotic and a prokaryotic organism used in TPPB applications) were examined, and methods to reduce toxicity of low MW polymers were explored.

Materials And Methods

Log P Determination of PPGs of Different MWs

The log P of the various polymer samples was estimated in order to assess the validity of the critical log P concept by determining the log P of the polymer samples and comparing this with the trends in biocompatibility. Accurate estimation of organic molecules with greater than 30 non-hydrogen atoms utilizing log P prediction software is challenging (Mannhold et al., 2009) There is also a range of molecular weights within each polymer sample as a result of the polydispersity of the polymers, making log P calculations impossible. A method that makes use of high pressure liquid chromatography (HPLC) for log P estimation of organic compounds has been previously developed (Eadsforth and Moser, 1983) which first determines the retention time of a series of organic solvents of known log P, in order to create calibration equation, from which the log P of an unknown compound can be determined. The Organization for Economic Co-operation and Development (OECD) later adopted this method (OECD, 2004). A method for the calculation of the average log P of samples with multiple peaks has also been developed (Vik et al., 1998), and was also adopted by the OECD (OECD, 2004). The method finds the log P of each peak in the chromatogram, and calculates the weighted average log P through the following equation, which was also used in this work:

$$Weight\ Average\ Log\ P = \frac{\sum_i (\log P_i)(area\%_i)}{Total\ Peak\ Area\%}$$

To estimate the log P of the various PPG samples, 0.100 g of polymer was dissolved in 1 mL of a 1:1 mixture of acetonitrile and water. A Varian HPLC with a Prostar 210 UV detector measuring at 200 nm, and a Polaris 5 C-18-A column (150 mm × 4.6 mm) were utilized. The mobile phase was a 1:1 mixture of acetonitrile and water at a pumping rate of 1 mL/min.

Cultures and Media

Saccharomyces cerevisiae, an industrial ethanol-producing yeast strain obtained from Alltech (Nicholasville Kentucky) was cultivated in a medium (Doran and Bailey, 1986) containing 20 g/L glucose, 5 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 2 g/L yeast extract, and 0.1 g/L $CaCl_2$. *Pseudomonas putida* ATCC 11172 was grown in medium described by Vrionis et al. (2002), using 5 g/L glucose, 6 g/L K_2HPO_4, 4 g/L KH_2PO_4, and 2g/L $(NH_4)_2SO_4$, augmented with a divalent salt solution (0.66 g/L MgCl2, and 0.25 g/L $CaCl_2$) and 0.5% (vol/vol) trace element solution (0.3 g/L H_3BO_3, 0.089 g/L $ZnSO_4 \cdot 7H_2O$, 0.024 g/L $NiSO_4$, 0.018 g/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.003 g/L $CuSO_4 \cdot 5H2O$, 0.050 g/L $MnSO_4$, and 0.190 g/L $CoCl_2$); 0.0006% $FeCl_3$. Cultures were grown for 24 h in 50 mL, of medium in 125 mL Erlenmeyer flasks at 30°C and 180 RPM. All reagents used in the medium formulations were obtained from Fisher Canada.

Critical Log P Determination

The critical log P values of *S. cerevisiae* and *P. putida* were determined by utilizing a 1 mL inoculum in 50 mL of the corresponding medium described above, and 5 mL of an organic solvent (or no solvent as the control). The list of solvents used, along with supplier information and reported log *P* values can be found in Table I. Cultures were cultivated for 24 h at 30°C and 180 RPM.

Table I. Information for solvents used in critical log P experiments.

Solvent	Log P	Supplier
Gamma nonalactone	2.08	Givauden, United States
Ethyl benzenoate	2.64	Sigma Aldrich, Canada
Hexane	3.29	Fischer, Canada
Cyclohexane	3.44	OmniSolv, Canada
Octane	4.30	Acros, United States
Dodecanol	5.13	Acros, United States
Decane	5.80	Acros, United States
Dodecane	6.82	Fischer, United States
Oleyl Alcohol	7.56	Aldrich, United States
Hexadecane	8.86	Acros, United States

Polymers

Bulk polypropylene glycol (PPG) with molecular weights of 425, 725, 1,000, 2,000, and 4,000 with an unspecified polydispersity index, and PPG standards of average MW 470, 830, 1,080, 2,160, and 3,100 with polydispersity indices (M_W/M_N) of 1.07, 1.06, 1.05, and 1.08, respectively, were used in these experiments. All MW are given as the weight average (M_W). All polymers were purchased from Scientific Polymer Products.

Biocompatibility of Polymers of Different MW

These experiments were undertaken to determine both the effect of polymer MW, and the effect of the amount of polymer addition, on cell toxicity. The toxicity of the bulk PPGs of various MWs was assessed by shake flask experiments, with each shake flask containing 1, 5, or 10 g of the polymer, which are viscous liquids at room temperature. 1 mL of microbial inoculum and 50 mL of the corresponding medium (described above) was added to each 125 mL flask, which were incubated at 30°C and 180 RPM for 24 h. The addition of 5 g of polymer to 50 mL medium (10% NAP fraction) represents a "conventional" methodology when testing biocompatibility, as was also done above in determining the critical log P values of the two organisms. The use of lower (1 g) and higher (10 g) amounts of polymer NAP was intended to assess any volume dependency of toxicity. The critical log P model of toxicity is expected to be essentially volume independent, and in this study we aimed to assess if this was true in the case of polymeric NAPs. Two experiments utilizing liquid NAP (cyclohexane and dodecane, with log *P* values of 3.44 and 6.82 respectively) were also carried out in parallel for the purpose of comparison. These experiments were carried out in duplicate.

Effect of Removing Low MW Polymer Fractions

Since the bulk PPG samples contained a range of MW fractions, polymers were water washed in order to remove the water soluble fraction of the polymer sample. The fraction of polymer samples that was miscible in water would be expected to have a relatively low log *P* value, and therefore may contribute to toxicity of the polymer samples to microbes; by removing this fraction we wanted to test whether this low log P fraction had a strong influence on biocompatibility. Polymer sample washing was done by vortex mixing PPG with and an equal volume of RO water for 2 min. The phases were then separated by centrifugation at 3000 rpm for 5 min. NaCl (1 g) was added to assist in the phase separation of PPG 425 by increasing the ionic strength of the aqueous phase. Loss of mass after washing was determined gravimetrically, by measuring weight of the polymer fraction before and after the washing step. Washed bulk polymer was then used in the biocompatibility shake flask experiments as described above. These experiments utilized 1, 5, or 10 g of washed PPG with a MW of 425, 725, or 1,000 in 50 mL medium. This was done in order to determine the correlation between polymer MW and toxicity as well as the correlation between the polymer volume fraction and toxicity for the washed polymer samples compared to the unwashed polymers.

Analytical Methods

Cell growth was measured by optical density at 600 nm (OD_{600}) using an Ultraspec 3,000 spectrophotometer (Biochrom). PPG with MW of 2,000 or 4,000 formed an immiscible mixture with the growth medium that interfered with the OD measurements. In such cases NaCl (1 g) was added to assist in phase separation of 5 mL samples. If this was not sufficient, organic solvents such as hexane and isopropanol were added in sparing amounts, typically 1 mL of solvent to 5 mL of medium. This improved phase separation. Glucose was measured by HPLC (Varian Prostar, Mississauga, ON) with a PL Hi-Plex H column (300 mm × 7.7 mm) at 60°C using a refractive index detector (PS 356, Varian Prostar) and a flow rate of 0.7 mL/min with a 10 mM H_2SO_4 mobile phase.

Results

Critical Log P

Figure 1 shows the cell density of *S. cerevisiae* and *P. putida* relative to control, grown in the presence of various organic solvents spanning a range of log *P* values, using optical density measurements and glucose residuals as metrics for cell growth. These experiments were conducted using a conventional ratio of solvent: aqueous phase volumes of 10%, or 5 mL solvent in 50 mL aqueous broth. The results show an approximate critical log P of 5.0 and 3.5 for *S. cerevisiae,* and *P. putida*, respectively. These values are similar to those previously reported for these organisms (Barton and Daugulis, 1992; Vrionis et al., 2002). In practical terms, this suggests both that the presence of NAPs whose log P values are greater than 5.0 and 3.5, respectively, would be anticipated to be biocompatible, and also that *P. putida* is more tolerant to the presence of NAPs as its critical log P value is lower than that of *S. cerevisiae*.

Log P of Different MW PPGs

Figure 2 shows the HPLC chromatogram for unwashed and washed polypropylene glycol (PPG) 1,000 samples for the "bulk" grade of the polymer. Figure 2a distinctly demonstrates that PPG 1,000 as received from the manufacturer is a mixture of different MW PPG polymers, as evidenced by the numerous peaks seen in the chromatogram. As noted earlier, even "pure"

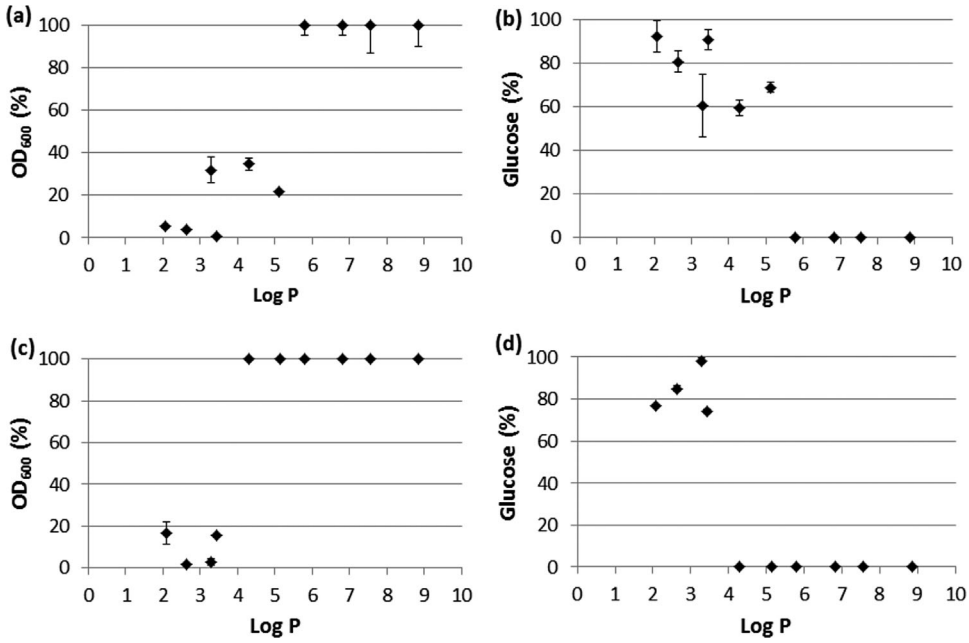

Figure 1. Biocompatibility versus log P of solvents after 24 h of growth. (**a**) Optical density at 600 nm of *S. cerevisiae* relative to control. (**b**) Remaining glucose for *S. cerevisiae* relative to initial concentration. (**c**) Optical density at 600 nm of *P. putida* relative to control. (**d**) Remaining glucose for *P. putida* relative to initial concentration. Error bars indicate 1 standard deviation.

polymers will have a certain degree of polydispersity, or MW distribution about some average MW value, and this is in contrast to simple, pure solvents, which would be expected to have a single HPLC chromatogram peak. The data in Figure 2a also suggest that the various MW constituents in PPG 1,000 would have different log P values, ranging from relatively low values (e.g., log P of 2.0 for the lower MW PPG species) to higher ones (and higher MW). The polymer fractions with lower log P values might be expected to inhibit cell growth, while higher log P polymers could be expected to be biocompatible according to the critical log P model of toxicity. Thus although PPG 1,000 as received from the manufacturer can have on overall estimated log P value (see Table II), it appears to have a range of components some of which would likely be biocompatible and some that would not.

The chromatogram shown in Figure 2b is for a sample of PPG 1,000 that had been water-washed to remove the more water soluble (lower MW) constituents, and a clear shift can be seen between the washed and unwashed samples, where many of the peaks with short retention time have become smaller or have disappeared completely. This suggests that the washing process has removed low log P compounds, which would be expected to lead to an increase in average log P for the treated PPG sample.

Figure 3a represents the chromatogram from the analysis of a PPG 1,080 standard, with low polydispersity, and this chromatogram shows a tighter distribution of peaks, as would be expected in a sample with low polydispersity. Figure 3b is the chromatogram for a washed standard PPG 1,080 sample, which shows that the relative intensity of the peaks does not shift when washing the standard samples. This suggests that a smaller fraction of the polymer sample is water soluble. This would be expected in a sample with very little low MW polymer content.

The shift in the polymer MW distribution seen in Figure 2 is shown quantitatively in the calculated average log P values given in Table II. The log P values for the washed polymer samples are higher than unwashed samples, as a result of the low log P polymers being removed by washing, thus increasing the average log P. The increase in log P is correlated with loss of mass upon washing, as seen in Table II. Substantial losses in mass are seen in PPG 425 and 725 (20%, and 17.5% respectively), while PPG 2,000 and 4,000 seem unaffected by the wash. This suggests low MW polymers have a larger water soluble fraction that can be lost to washing. Calculated average log P for PPG standards is given for comparison.

Biocompatibility of PPG

Figure 4 shows the growth of *S. cerevisiae* and *P. putida* as measured by the percentage of optical density relative to a control, using 5 g of the various MW PPG polymers in 50 mL aqueous medium. A clear trend can be seen for both cell types: low MW PPGs appear to inhibit growth, while higher MW polymer appears to be biocompatible. In both *S. cerevisiae* and *P. putida*, uninhibited growth is seen in samples with molecular weights exceeding 1,000. This mirrors previously reported results seen for *Clostridium acetobutylicum*, which suggested that 1,200 molecular weight PPG and higher were biocompatible (Barton and Daugulis, 1992). This work therefore shows that three separate organisms are biocompatible to 1000–1200 MW PPG and above. It is also of note that *P. putida* exhibits signs of NAP tolerance at lower molecular weights than does *S. cerevisiae*. This is consistent with the

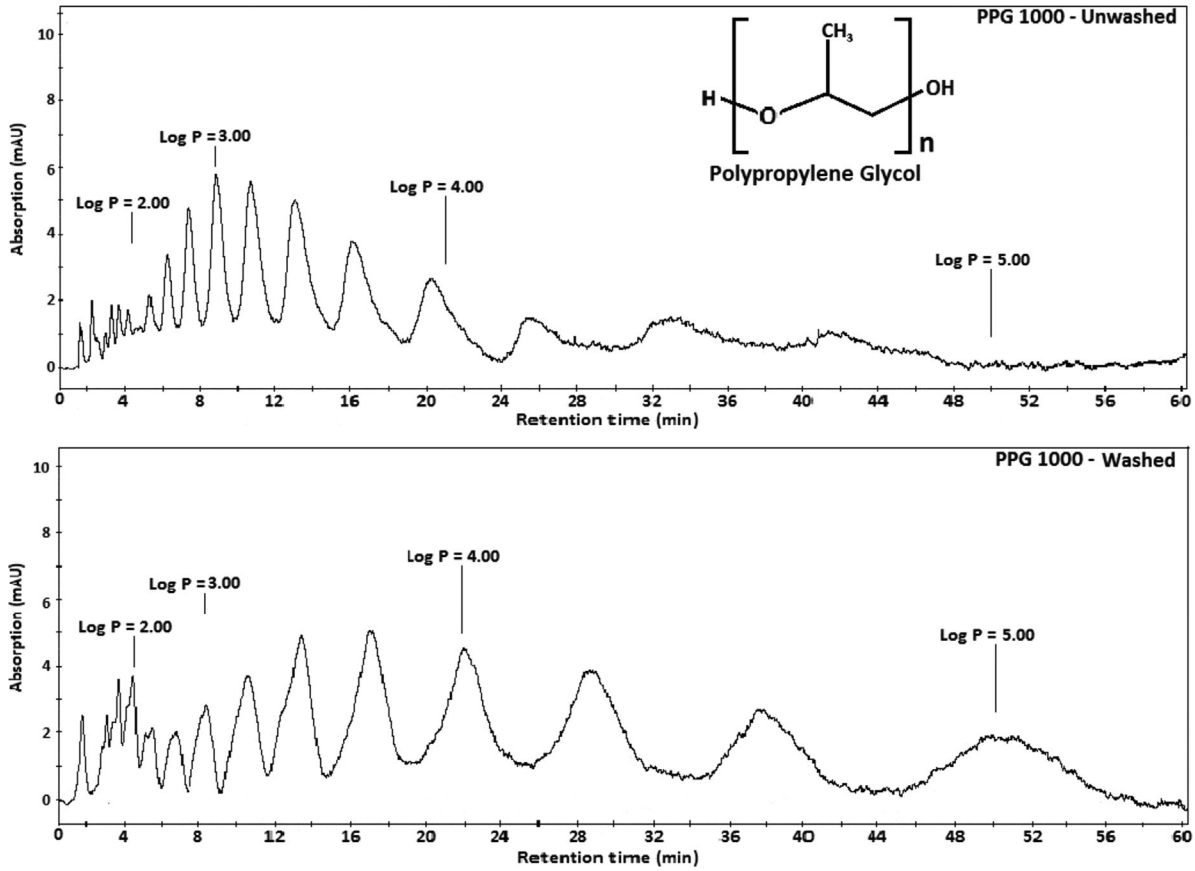

Figure 2. HPLC chromatograms of 1,000 molecular weight polypropylene glycol (**a**) as received—unwashed and (**b**) washed.

critical log P model of toxicity, as *P. putida* has been shown to have a lower critical log P, as shown in Figure 1, which suggests that the critical log P approach may also be applicable to polymeric NAPs.

Figure 5 shows the biocompatibility of *S. cerevisiae* and *P. putida* over a range of 1–10 g of NAP addition using PPG 425, 725, and 1,000, as well as dodecane and cyclohexane. As noted in the materials section, the reason for using different amounts of NAP was to determine the polymer fraction dependency of toxicity. That is, since low MW fractions are present in PPG (which may be cytotoxic), the addition of larger amounts of these polymers to the cultures could result in sufficient low molecular weight material in order to cause toxicity. The low molecular weight polymers clearly show a correlation between the amount of polymer added and cell biocompatibility, with higher polymer addition resulting in reduced cell growth. PPG samples with MWs above 1,000 do not show this trend, and appear to be biocompatible regardless of the polymer fraction added. This experiment was repeated for PPG 2,000 and 4,000, although growth remained at approximately 100% of the controls under all conditions (data not shown). Trials were also preformed utilizing the organic solvents dodecane and cyclohexane. The log P of dodecane is approximately 6.8, which exceeds the critical log P of both *S. cerevisiae* and *P. putida*, suggesting that total biocompatibility would be expected at any volume, which is seen in these results. Cyclohexane, by contrast, has a reported log P of 3.4 and should therefore be expected to strongly inhibit growth, as the log *P* value is below the critical log P of both *S. cerevisiae* and *P. putida*. This is observed in these experiments at all levels of cyclohexane addition. The (expected) volume independence of organic solvents is a noticeable different relative to the volume dependence seen in the polymeric NAP experiments.

Table II. Calculated average log *P* values for PPG samples.

MW of PPG samples	Average log P Unwashed	Average log P Washed	% Loss of mass after washing	MW of PPG samples Standards	Average log P Standards
425	1.19	1.29	20.0%	470	1.74
725	2.69	3.24	17.5%	830	2.45
1000	3.28	4.07	7.6%	1080	3.17
2000	3.39	–	<1%	2160	2.74
4000	4.05	–	<1%	3100	3.02

Washed PPG Experiments

Figure 6 compares the biocompatibility of washed and unwashed PPG samples. As noted earlier, PPG samples were

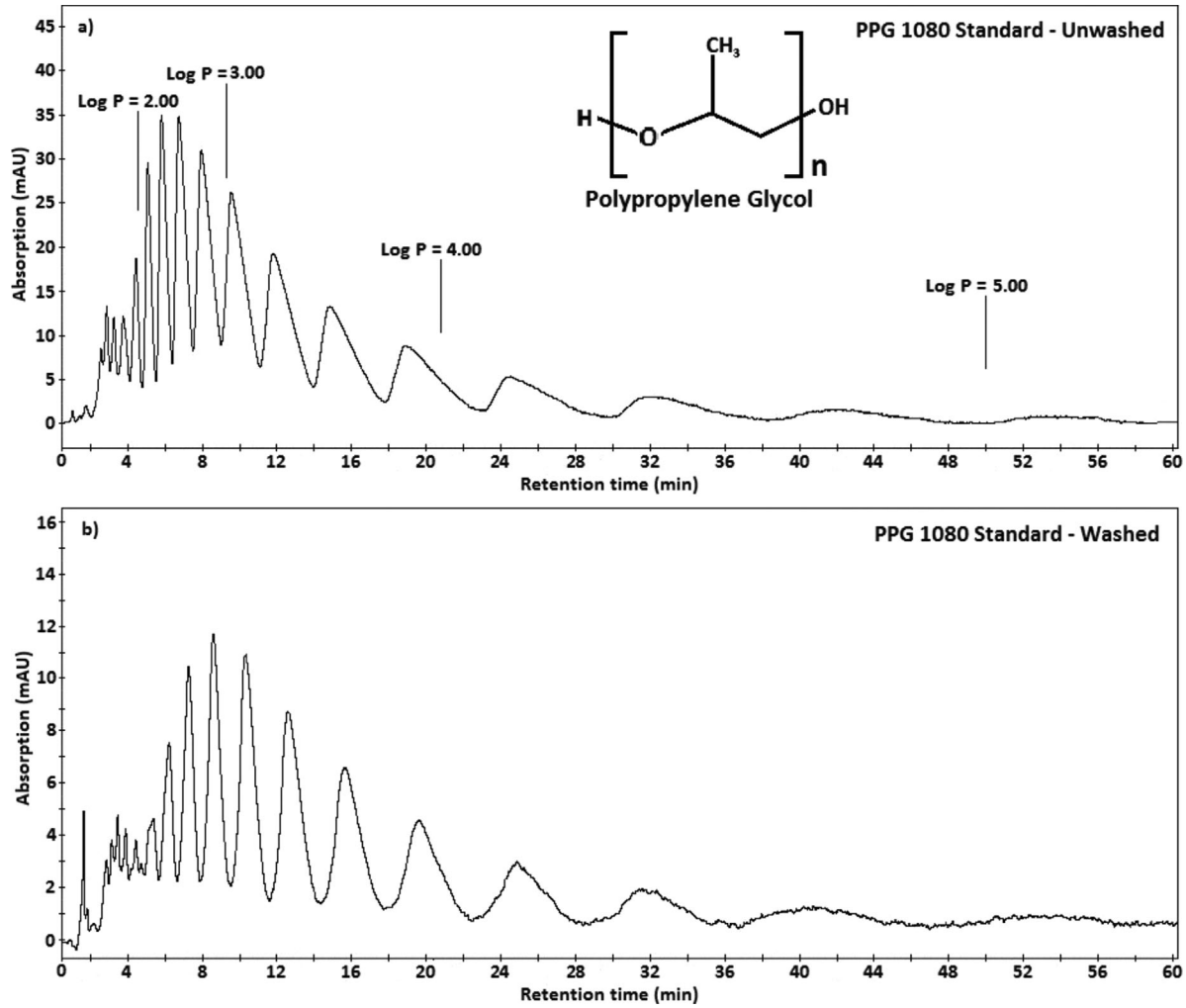

Figure 3. HPLC chromatograms of 1,080 molecular weight polypropylene glycol standard (**a**) as received—unwashed and (**b**) washed.

washed with water in order to remove the low log P water soluble fraction. It is clearly seen in Figure 6 that the presence of washed polymer resulted in higher cell growth than in the case of unwashed polymer samples. This implies that the low molecular weight fraction that is removed as a result of washing was the chief cause of toxicity, and that washing polymer samples may be an effective means by which biocompatibility can be improved for relatively low MW polymers with high polydispersity.

Figure 6 also shows that *P. putida* growth improved more than *S. cerevisiae* as a result of polymer washing. In panel (a) it can be seen that the average growth of *S. cerevesiae* when exposed to 10 g of unwashed PPG 425 was 28% of the control, while the culture with washed PPG 425 was 40% of the control. In contrast, *P. putida*, had an average growth of 14% relative to the control when exposed to 10 g of unwashed PPG 425, while growth when exposed to washed polymer was approximately 67% of control. This is seen again in the case of using 10 g of washed PPG 725, where *S. cerevisiae* growth was 88% of the control, and *P. putida* growth was identical to the control. It is of interest that *P. putida* shows more growth than *S. cerevisiae* in the presence of washed PPG, and therefore shows higher tolerance to polymeric NAP, and is as anticipated by the critical log P model of NAP tolerance. It should also be noted that many of the data points for both washed and unwashed samples in Figure 6e and 6f lie at approximately 100%, which obscures many of the data points.

Discussion

The first insight to note from these results is that the connection between polymer molecular weight and cytotoxicity appears to follow the general trend that had been demonstrated in the past—low MW polymers are less biocompatible than higher MW polymers (Barton and Daugulis, 1992). Importantly, this work establishes this trend in eukaryotic cells as well as prokaryotic cells, which has not been reported in the past.

Previous work had not identified the properties of the low MW polymer which lead to toxicity, while here it was shown that low MW

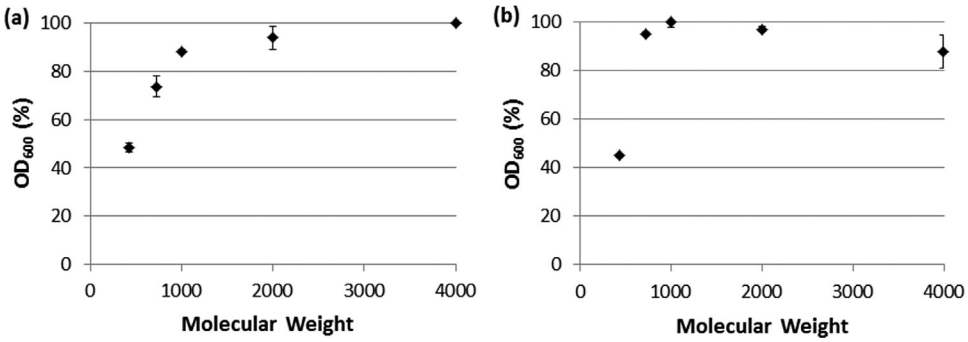

Figure 4. Twenty-four hour growth of (**a**) *S. cerevisiae* and (**b**) *P. putida* relative to control in 50 mL culture broth with 5 g unwashed PPG over a range of molecular weights. Error bars have been given to show 1 standard deviation.

polymers have low calculated log *P* values, and that the log P increases with molecular weight of the polymer. Log P has long been established as a key physical parameter for predicting toxicity (Inoue and Horikoshi, 1991) and these results show that polymeric compounds with low average log P are more toxic than are polymers with high average log P. In addition to this, by washing the polymers to remove the water soluble low MW constituents, the overall log *P* values increase, which corresponds to lowered toxicity. These results show that the critical log P model of NAP toxicity holds true to some degree in polymer systems.

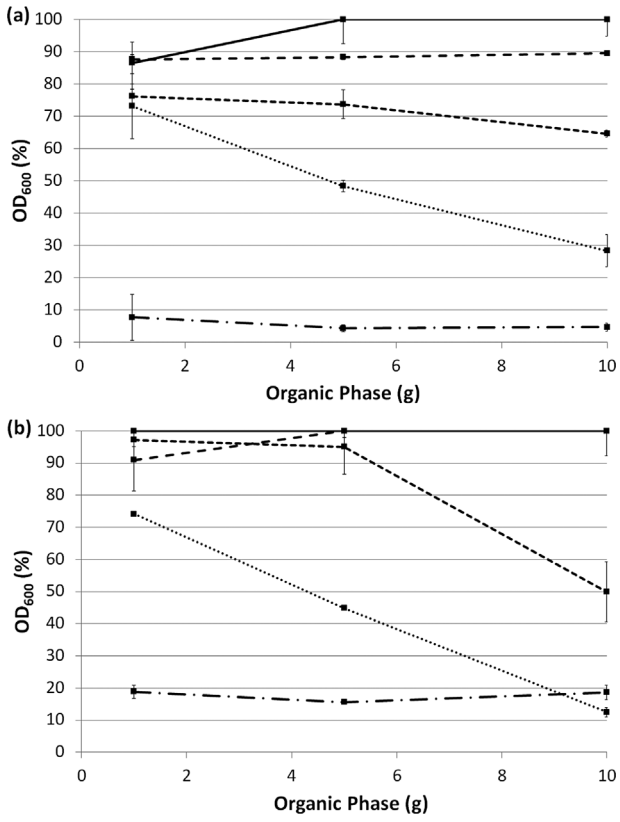

Figure 5. Twenty-four hour growth relative to control over a range of volumes. (**a**) *S. cerevisiae* (**b**) *P. putida*. (····) PPG 425 (··) PPG 725 (--) PPG 1,000 (—) dodecane and (-·) cyclohexane. Error bars have been given to show 1 standard deviation.

Although log P is clearly still an important consideration in the toxicity of short chain polymers, these initial results suggest that there are some limitations to the critical log P model as it applies to polymer systems. Here it was found that PPG 1,000, 2,000, and 4,000 are biocompatible for *S. cerevisiae*, although their weighted average log P values are below the critical log P of the organism. In addition, there appeared to be a volume dependency of toxicity for low MW polymeric NAPs, as shown in Figure 4. This effect has not been observed when using simple organic solvents, where small volumes of organic solvents with low log *P* values completely inhibit all cell growth, as is the case of cyclohexane shown in Figure 5. Therefore, although polymeric compounds seem to follow the general trend of toxicity being tied to decreasing log P, there are some additional factors that must be accounted for.

An important distinction when considering the difference between polymeric NAPs and monomeric NAPs is the phenomenon of polydispersity. In the case of a solvent such as cyclohexane, it is expected that >99% of the solvent is a single compound, with no important differences between solvent samples. When analyzing polymeric compounds, a distribution of molecular weights would be expected, as polymerization invariably leads to a range of molecular weights. This can be seen visually in Figure 2, as each of the peaks represents a slightly different log P. Although this phenomenon is to be expected, this work characterizes the polydisperse character through use of HPLC. The phenomenon of polydispersity has also been shown to be important to biocompatibility, as removing the low log P fraction of a polydisperse polymer sample improves biocompatibility. This explains the volume dependency seen in Figure 5 and 6–the degree of toxicity is likely proportional to the amount of low log P polymer. Therefore, these results suggest that polymeric samples do not adhere strictly to the critical log P model as a result of the presence of a very low molecular weight fraction. Better understanding of this low molecular weight fraction could lead to further insights into polymeric NAP selection, and will be the object of future studies.

The simple washing procedure implemented in this study was effective at shifting PPG log *P* values as seen in Table II, and the

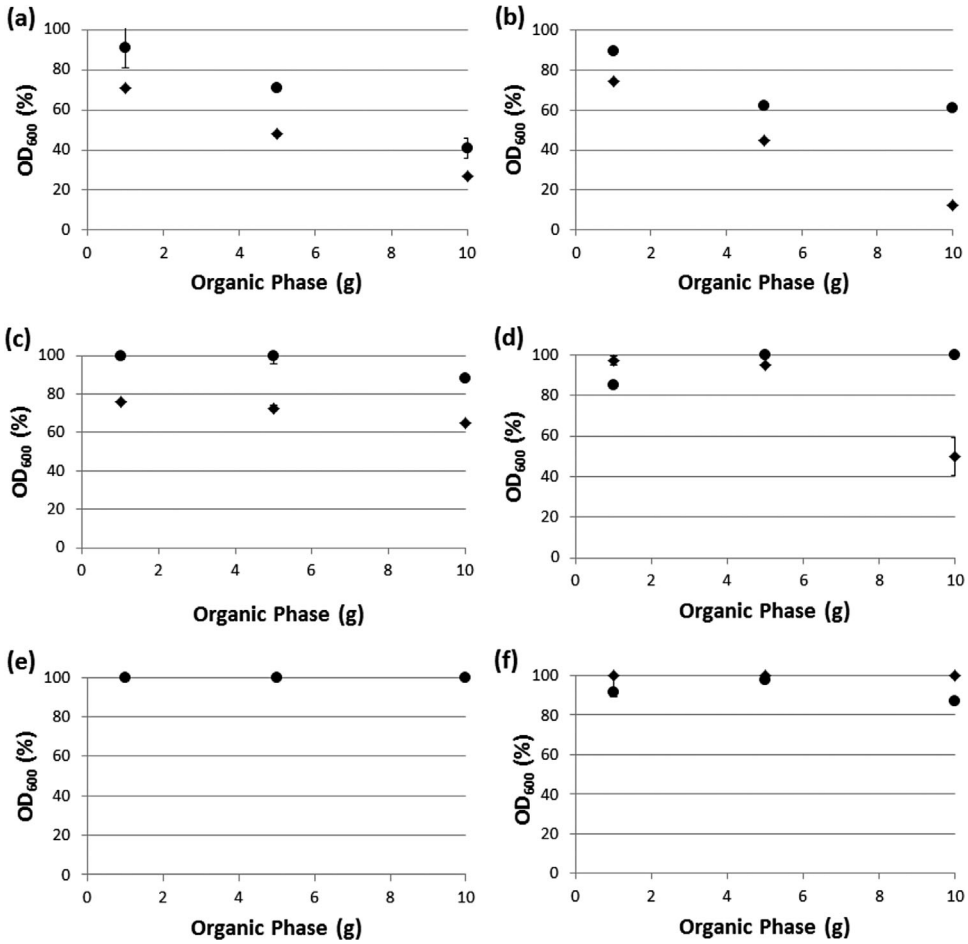

Figure 6. Cell growth after 24 h in washed versus unwashed PPG. (**a**) *S. cerevisiae* with PPG 425 (**b**) *P. putida* with PPG 425 (**c**) *S. cerevisiae* with PPG 725 (**d**) *P. putida* with PPG 725 (**e**) *S. cerevesiae* with PPG 1,000 (**f**) *P. putida* with PPG 1,000. (●) washed polymer (◆) unwashed polymer. Error bars have been given showing 1 standard deviation.

improving polymer NAP biocompatibility as seen in Figure 5. By examining the chromatograms of the washed polymers (Figure 2b), it is clear that this method is particularly useful for removing the low log P fraction from polymer samples. This also provides a relative increase in high log P fractions, as seen in the region around log P 5.00. These shifts in average log *P* values are substantiated by the gravimetric analysis that was performed, as shown in Table II. Significant weight is lost when washing low molecular weight samples (e.g., 20% loss in PPG 425), while no change is seen in high molecular weight samples (e.g., PPG 2,000, and 4,000). This further implies that a low log P polymer fraction is lost upon washing, and that this loss is more substantial in low MW polymer samples. This also suggests that washing polymers is a potential method for limiting the toxicity of low molecular weight polymer samples in TPPB applications. By removing the very low log P fraction in a polymer sample it may be possible to improve the biocompatibility of low MW polymers, while retaining much of the gains in solute uptake (Bacon et al., 2014). Future work will aim to refine the method of washing polymer samples, and use it to examine the contents of the organic and aqueous phase after washing various polymers in order to add greater understanding to the models of polymer selection in TPPB systems.This work reaffirms that toxicity of polymers decreases as the molecular weight increases (Barton and Daugulis, 1992). The MW of PPG at which biocompatibility was achieved for *S. cerevisiae* and *P. putida* was approximately 1,000, with all samples exceeding 1,000 being entirely biocompatible. The toxicity of low molecular weight polymers is connected to the log P of the polymer samples. Washing polymers by rinsing with water lead to an increase in log P and a decrease in toxicity. Although the biocompatibility results do not conform exactly to the critical log P model, there is a clear trend that low log P compounds tend to be more toxic than high log P compounds. Washing polymer was also shown to be an effective technique to reduce toxicity. This method will be more fully explored in future work, along with a more in-depth analysis of the low log P fraction of polymer samples, and further validation of this revised critical log P biocompatibility model using other polymer and other organisms.

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