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Review

Water-immiscible dissolved oxygen carriers in combination with Pluronic F 68 in bioreactors

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The supply and availability of dissolved oxygen (DO) in aerobic bioprocesses is often a limiting factor for the scaling up, improvement and general performance of these bioprocesses. The use of different DO carriers, particularly the use of perfluorocarbons as oxygen carriers, is discussed in this review. It also highlights interactions of microbial cultures with the surfactant, Pluronic F 68. Although oxygen carriers have been used extensively in the medical field, this review only focuses on their use in microbial bioprocess used for the production of high-value bioproducts. The use of water-immiscible compounds in combination with Pluronic F 68 in bioprocesses is discussed with the intention of analysing their combined effect where bioreactor and biomass performance is affected by DO limitations, nutrient starvation, high concentrations of trace element ions, oxidative stress and cell death from mechanical stress.

Key words: Dissolved oxygen, oxygen carriers, perfluorocarbon, Pluronic F 68, surfactant.

DISSOLVED OXYGEN TRANSPORT LIMITATIONS IN BIOREACTORS

In aerobic bioprocesses, dissolved oxygen (DO) transport from the gas phase into the liquid medium is one of the critical parameters for effective bioprocess operations. In bioreactors such as membrane gradostat reactors (MGRs), where biofilms are attached to membrane surfaces, low shear aeration conditions are employed in order to reduce biofilm sloughing. This particular bioreactor uses biofilms system so that the different parts of the biofilm can experience different nutrient concentrations, such that a gradient could be established. This cannot occur if the biofilm sloughs off. However, under these conditions, the overall DO mass transfer into the immobilised biofilms is restricted and controlled by a liquid film at the gas-liquid-biomass interface (Ju et al., 1991a). This adversely affects overall biofilm- and reactor performance. Furthermore, DO transfer is hampered by extracellular polymeric substances (EPS) produced or stored by microbial biomass during fermentation. In fungal bioprocess systems where high glucose concentrations are used in the nutrient medium, the production of EPS as storage carbohydrates further hampers DO transport because of the availability of excess glucose, necessitating the use of pressurised bioreactor systems. Although the use of pressurised bioreactors might improve DO transport, this is also likely to increase the rate of biofilm metabolism and the generation of carbon dioxide. Furthermore, the possibility of carbon dioxide entrapment in the fermenting biomass will increase because of EPS production associated with high DO transport into the biofilms. EPS production is also reported to be promoted by the high partial pressures of oxygen used during aeration, limited nitrogen availability and low pH conditions that are prevalent in bioreactors used to produce secondary metabolites, such as the MGR system (Ahimou et al., 2007; Ryu and Beuchat, 2004; Damiano and Wang, 1985; Jarman et al., 1978).

As oxygen has a low solubility in aqueous medium, DO transfer becomes an important design aspect for any aerobic bioprocess design. Inefficient DO transfer in-

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fluences the scale-up and economic value of the fermentation processes, as it increases operational costs and reduces recoveries related to the aerobic biosynthesis of high-value bioproducts (Cascaval et al., 2006). Furthermore, respiratory activity of the immobilised microorganism can be inhibited by bioproducts when the biomass is suspended in the product solution. DO cannot be increased by heating the water-based medium, as it has been found that the oxygen-transfer rate (OTR) remained constant between 20 and 55°C (Vogelaar et al., 2000). The presence of dissolved salts in most fermentation media has also resulted in decreased solubility and transfer of DO into microbial cells during the course of bioreactor operations (Anke and Weber, 2006). For example, an increase in the salinity of freshwater from 0 to 8% resulted in the reduction of DO from 200.4 to 192.3 □mol $O_2.l^{-1}$ (Unisense, 2008).

Spargers, agitators and technical-grade oxygen (pressurised ~100% O_2) have been used to improve the availability of DO and the performance of biomass in fermentation broths hampered by DO transfer (Murhammer and Goochee, 1990). However, the use of these devices and aeration sources can result in cell rupture as a result of the mechanical stress caused by mixing and continuous aeration. Conditions that can also limit biomass performance include: hyperoxia resulting from the availability and the use of high partial pressures of oxygen and the generation of reactive oxygen species (ROS), which cause lipid peroxidation and unwanted by-product formation as the biomass tries to protect itself from environmental stressors in the bioreactors.

DO carriers are used to alleviate DO limitations in different bioprocesses. They have been used extensively in the medical field (Goorha et al., 2003; Lowe, 2003; Riess, 2002) with limited applications within membrane-based bioreactors. To alleviate DO limitations, the use of water-immiscible DO carriers becomes necessary to optimise biomass performance. Some of these water-immiscible organic compounds have greater solubilization capacity for oxygen than aqueous media (Junker et al., 1990). These materials include haemoglobin derivatives (polymerised, polymer conjugated, intramolecular cross-linked, recombinant and lipid-based vesicle haemoglobin) and organic oils based on synthetic, highly fluorinated organic compounds named perfluorocarbons (PFCs) (Goorha et al., 2003; Lowe, 2003). The advantage of these PFCs over haemoglobin-based oxygen carriers is that haemoglobin is slowly oxidised by oxygen into methaemoglobin (Adlercreutz and Mattiasson, 1982), thus limiting their potential to be recovered and recycled in highly oxidative processes. The presence of trace element ions can further increase the oxidation of haemoglobin-based DO carriers. The presence of a DO carrier in the liquid phase can have an improved effect on the rate of oxygen transfer in fermentation processes, thus improving the performance of the biomass and bioproduct yield.

WATER-IMMISCIBLE GAS CARRIERS: THEIR APPLICATION AND BENEFITS TO DIFFERENT CELL CULTURES

Gas saturated water-immiscible DO carrier droplets can be used to enhance oxygen supply by liquid-liquid and liquid-biomass contact. Some are heavier than and immiscible in, aqueous medium and can be collected at the base of the vessel for reuse in subsequent fermentations (Richardson et al., 2002; Lowe et al., 1998; Kabalnov et al., 1990). Some DO carriers, such as PFCs, have higher solubilities for carbon dioxide than ordinary aqueous media. Therefore, they can also carry metabolically produced carbon dioxide, removing it from the fermentation broth and thus improving overall biomass and bioreactor performance, especially for continuous bioprocess systems. One such example was the inclusion of 50% (v/v) perfluorodecalin in the fermentation medium using *Streptomyces coelicolor* A3 (2). This resulted in a five-fold increase in the maximum actinorhodin production. The use of water-immiscible DO carriers can also improve the maximum specific growth rates of micro organisms with increasing concentrations (Amaral et al., 2007); while the specific death rate decreases with DO availability. These phenomena clearly suggest that the additional oxygen supplied by inclusion of water-immiscible gas carriers could be readily utilised by aerobic submerged cultures resulting in the improved performance of the fermentation system (Elibol and Mavituna, 1999). Examples of other water-immiscible DO carriers are hexanol (Koide et al., 1985), olive and lard oil (Liu et al., 1994) and soybean and silicone oil (Morao et al., 1999).

The benefits of using gas carriers in microbial culture systems include (1) a reduction in mechanical damage to biomass by eliminating the effects of conventional aeration through sparging or continuous stirring, (2) the provision of a multi-phased interface for effective DO transfer; (3) prolonged survival rates of micro organisms, (4) ease of sterilisation by means of autoclaving or filtering) and (5) ease of recovery and recycling (Lowe et al., 1998). Microbial cultivation in these dispersions is attrac-tive because maximum DO transfer rates in some cases were increased by over 400% when using oxygen carriers (McMillan and Wang, 1988). In the majority of these cases, the emulsions have not been used in volume fractions exceeding 40% (v/v) (Junker et al., 1990), thus limiting fermentation broth viscosity close to that of water. Table 1 lists examples of applications and benefits for different water-immiscible gas carriers in different submerged microbial cultures.

PERFLUOROCARBONS AS DISSOLVED OXYGEN CARRIERS

The use of perfluorinated organic oils as oxygen carriers

Table 1. Application and benefits of water-immiscible oxygen carriers in submerged cultures.

Process	Oxygen carrier Surfactant	Micro organism	Consequence	Reference
Dihydroxyacetone production	0.85 mM oxyhaemoglobin	*Gluconobacter oxydans*	> 4.0 mM dihydroxyacetone production	(Adlercreutz and Mattiasson, 1982)
Dihydroxyacetone production	32.4% (v/v) FC-72	*Gluconobacter oxydans*	4 - 5 x dihydroxyacetone production	(Adlercreutz and Mattiasson, 1982)
Dihydroxyacetone production	20-80 mM*p*-benzoquinone	*Gluconobacter oxydans*	70% increase in dihydroxyacetone production	(Adlercreutz and Mattiasson, 1984)
IgG production	Fluorinert FC-40	*Mouse-mouse hybridoma, 4C10B6*	High cell density attained	(Hamamoto et al., 1987)
Escherichia coli and *S. cerevisiae* culture	20% Fluosol-DA (F-DA) Perfluorodecalin	*E. coli HB101* *Saccharomyces cerevisiae*	*E. coli* and *S. cerevisiae* inhibited by F-DA *E. coli* not affected by 15 to 30% PFC	(Chandler et al., 1987)
Hybridoma cell culture	perfluoromethyldecalin	*Mouse-mouse hybridoma, cell line* (#824)	Increased oxygen transfer Increased cell density when compared to batch culture	(Cho and Wang, 1988)
Penicillin fermentation	0. 5, 2, 5% *n*-hexadecane	*Penicillium chrysosgenum, Wis.* 54 to 1255	Increased cell growth Increased penicillin production	(Ho et al., 1990)
Aerobacter aerogenes culture	54.9 $mg.l^{-1}$ n-dodecane and 118 $mg.l^{-1}$ F66E 12.5 mg/ml Pluronic F 68	*A. aerogenes*	3.5 x K_La achieved	(Rols et al., 1990)
E. coli culture	15% (v/v) $FlurO_2$	*E. coli K-12*	2.55 x enhance in biomass concentration	(Ju et al., 1991a)
Protoplast culture	Perfluorodecalin 0.01% (w/v) Pluronic F 68	*Petunia hybrida*	52% plating efficiency than control	(Anthony et al., 1994)
E. coli culture	50% (v/v) Foralkyl 10 mg/ml Pluronic F 68	*Escherichia coli*	0.17 g $O_2.l^{-1}h^{-1}$ supply and 0.23 g $CO_2.l^{-1}h^{-1}$ extraction	(Martin et al., 1995)
C. acetobutylicum culture	18.5% Forane F66E	*Clostridium acetobutylicum*	9% extraction of total CO_2 produced	(Percheron et al., 1995)
Anthraquinone production	0.11% (w/v)n-hexadecane	*Morinda citrifolia*	2 x Anthraquinone production than control	(Bassetti and Tramper, 1995)
E. coli culture	50% (v/v) Foralkyl 10 mg/ml Pluronic F 68	*Escherichia coli*	0.17 g $O_2.l^{-1}h^{-1}$ supply and 0.23 g $CO_2.l^{-1}h^{-1}$ extraction	(Martin et al., 1995)
C. acetobutylicum culture	18.5% Forane F66E	*Clostridium acetobutylicum*	9% extraction of total CO2 produced	(Percheron et al., 1995)
Anthraquinone production	0.11% (w/v)n-hexadecane	*Morinda citrifolia*	2 x Anthraquinone production than control	(Bassetti and Tramper, 1995)

was initially investigated using mice. The oils were shown to assist the animals with liquid breathing (Clark and Gollan, 1966). Over the years, they have also been shown to be effective as DO carriers in bioprocesses using different microorganisms. Recently, Amaral et al. (2007) demonstrated the effects of concentrations of 10 to 20% (v/v) perfluorodecalin in *Yarrowia lipolytica* under agitation conditions. The result was an increased glucose consumption and specific growth rate for the culture. As PFCs are non-corrosive, odourless and colourless (Lowe, 2002), they were also shown to be suitable for the development of synthetic blood (Inayat et al., 2006; Goorha et al., 2003; Lowe, 2003; Riess, 2002). Other examples of PFC-emulsion applications are listed in Table 1.

PFCs are chemically inert compounds consisting of fluorine-substituted hydrocarbons in which most or all of the hydrogen atoms have been replaced by fluorine. A

Table 1. (continued)

Process	Oxygen carrier Surfactant	Micro organism	Consequence	Reference
Actinorhodin production	10% (v/v) Perfluorodecalin 4% (w/v) Pluronic F 68	*S. coelicolor* A3(2)	2 x increase in actinorhodin production compared to 10% PFC (/v) without Pluronic	(Elibol and Mavituna, 1996)
Mycobacteria parafortuitum culture	26% (v/v) FC-40 18.2% (v/v) FC-40	*M. parafortuitum*	1.8 x KLa achieved 1.2 x KLa achieved	(Cesario et al., 1996)
Post thaw culture	Perfluorodecalin 0.01% (w/v) Pluronic F 68	*Oryza sativa cv.*	21% increase in post thaw viability	(Anthony et al., 1997)
Actinorhodin production	50% (v/v) Perfluorodecalin	*Streptomyces coelicolor* A3(2)	5 x increase actinorhodin production	(Elibol and Mavituna, 1997)
Hybridoma antibody production	Natural bovine haemoglobin Erythrogen™-1 Formula-1™ Polyethylene glycol cross-linked haemoglobin perfluorocarbon	*Hybridoma 3C11*	No antibody production increase for natural bovine haemoglobin 104% antibody production increase using Erythrogen™-1 20% antibody production increase using glycol cross-linked haemoglobin 78% antibody production increase using perfluorocarbon	(Shi et al., 1998)
S. cerevisiae culture	Perfluorodecalin 4% (w/v) Pluronic F 68	*Saccharomyces cerevisiae*	Significant increases in K_La values	(Elibol, 1999)
Actinorhodin production	50% (v/v) Perfluorodecalin	*S. coelicolor A3(2)*	5 x increase in actinorhodin production	(Elibol and Mavituna, 1999)
Lactate production	Fluorinert FC-40	*Spodoptera frugiperda sf9*	Increased cell density, growth yield and lactate yield	(Gotoh et al., 2001a)
Virus infected *S. frugiperda* culture	Fluorinert FC-40	*S. frugiperda; sf9*	Cell density achieved was higher than that in surface aeration Recombinant protein yield increased 1.6 x K_La achieved	(Gotoh et al., 2001b)
Actinorhodin production	10% Perfluorodecalin	*S. coelicolor* A3(2)	3.0 x K_La achieved	(Elibol, 2001)
Aspergillus terreus culture	n-dodecane	*Aspergillus terreus ATCC* 20542	1.4 x increase in lovastatin production	(Lai et al., 2002)
Egg hatching after storage	Fluorinert-77	*Oncorhynchus mykiss*	> 75.1% hatching/ compared to 14.3 % in control	(Richardson et al., 2002)
Ropionibacterium shermanii culture	5 -20% (v/v) n-dodecane	*P. shermanii*	3.5 - 5 x KLa achieved	(Cascaval et al., 2006)
S. cerevisiae culture	5 -20% (v/v) n-dodecane	*Saccharomyces cerevisiae*	3.5 - 5 x KLa achieved	(Cascaval et al., 2006)
A. chroococcum culture	5%(v/v) perfluorodecalin	*Azotobacter chroococcum ACB 121*	> 5 x increase in cell concentration 3.4 x nitrogenase activity 4.5 x increase in nitrogen content	(Bakulin et al., 2007)

progressive substitution of fluorine for hydrogen led to an increase in molecular mass, resulting in liquids that are much heavier than other hydrocarbon oils such as mineral oil. The oils typically have specific gravities, approximately twice that of water (Lowe et al., 1998). They are stable, non-toxic and can store and release oxygen at a greater rate (Goorha et al., 2003; Lowe, 2003; Richardson et al., 2002; Riess, 2002). Oxygen solubility in these oils is related to the molecular volume of the dissolving gas and decreased in the following

Table 2. Characteristics of PFC liquids compared to those of water at standard pressure and temperature (Lowe et al., 1998; Ju et al., 1991a).

Liquid	Oxygen	Carbon dioxide	Density[c]	Boiling point[d]	Molecular weight[e]
Water	2.2[a]	57[a]	1.0	100	18
FC-40	37[b]	142[b]	1.87	155	650
FC-43	36[b]	140[b]	1.88	174	670
FC-77	56[b]	214[b]	1.78	97	415
FC-84	59[b]	224[b]	1.73	80	388
Bis-(Perfluorobutyl) ethene	44.0[a]	203[a]	1.41	60	464
Perfluorobutyl tetrahydrofuran	51[b]	209[b]	1.77	102	416
Perfluorodecalin	35.5[a]	125[a]	1.92	142	462
Bis-Perfluorohexyl ethene	37.9[a]	159[a]	1.77	195	664
Perfluoro-n-hexane	65[b]	248[b]	1.68	59	340
Perfluorooctyl bromide	44.0[a]	185[a]	1.93	142	499
Perfluorotributylamine	35.2[a]	123[a]	1.85	155	671
Perfluorotripentylamine	31[b]	117[b]	1.94	215	821
Perfluorotripropylamine	39.6[a]	146[a]	1.82	130	521

[a]Gas dissolving capacity in mM at 25°C; [b]Gas dissolving capacity in ml gas/100 ml PFC at 37°C; [c]Density in $g.cm^{-3}$; [d]Boiling point in °C; [e]Molecular weight in g/mol.
FC 40/43/77/84-are Fluorinert electronic liquids, products of 3M Company.

order: $CO_2 > O_2 > N_2$ (Lowe et al., 1998), making them suitable for oxygen delivery and metabolic CO_2 removal from fermentation systems.

PFCs have the following advantages: 1) they do not react chemically with oxygen or other gasses; 2) oxygen solubility is not subject to the effects of pH; 3) they are not susceptible to dissolved salts in the fermentation medium and 4) they facilitate an effortless transfer of oxygen. Oxygen solubility here is inversely proportional to the molecular weight and directly proportional to the number of fluorine atoms in the oils (Goorha et al., 2003). When comparing traditional bioprocesses with PFC-supplemented bioprocesses, it can be seen that the OTR is enhanced without the need for supplementary energy consumption for mixing. However, the use of these oxygen carriers needs to be further analysed to determine their compatibility with microbial strains that are chosen for a defined bioprocess (Cascaval et al., 2006). The characteristics of commonly used PFC liquids at standard pressure (1 atm.) and temperature (25°C) are listed in Table 2.

There are several disadvantages relating to the application of pure PFCs in cell cultures, such as: 1) a reduction in biomass generated and 2) increased lag phases in the fermentation (Amaral et al., 2007). In order to use PFCs as oxygen carriers, their effects on the physiological state and performance of microorganisms need to be determined. The consensus is that PFCs have different effects on prokaryotic and eukaryotic cells. PFCs in their emulsion form have no apparent effect on prokaryotic cells, whilst some eukaryotic cells show ultrastructural changes after treatment with pure PFCs (Elibol, 2001; Chandler et al., 1987). Problems may also occur in batch PFC-mediated aeration cultures, where ventilation is only carried out through PFCs, as volatile organic compounds (VOCs) can be produced and thus hamper the metabolic activity of micro organisms (Gotoh et al., 2001b). VOCs are organic chemical compounds, mostly carbon-based molecules such as aldehydes and ketones, with high pressures, which enable them to rapidly vaporise and enter the atmosphere. PFCs cannot dissolve electrolytes and other organic compounds, except for fluorine-substituted compounds. The VOCs can, therefore, accumulate in the medium without being removed, especially in batch cultures (Gotoh et al., 2001b).

PREPARATION OF PERFLUOROCARBON EMULSIONS

Being virtually insoluble in water, PFCs are usually formulated as submicron emulsions. PFC emulsions can be prepared using different types and concentrations of surfactants as nano- or micro-emulsions. Nano-emulsions (droplets covering the size range of 100 to 600 nm) are preferable, as they have increased stability (Bouchemal et al., 2004). The formulation of an emulsion requires a surfactant capable of ensuring adequate dispersion, homogeneity, reproducibility, stability and biocompatibility (Riess, 2002).

The droplet size of the dispersed PFC phase can be controlled with the concentration of the surfactant. After preparation, the initial oil-particle size decreases with increasing surfactant concentration. PFC emulsions containing 10% (w/w) PFC were determined to be stable at surfactant concentrations as low as 0.5% (w/w) (Magdassi and Siman-Tov, 1990). In the preparation of PFC

Table 3. Hydrophilic-lipophilic balance of perfluorochemicals and surfactant (Weers, 1993).

Perfluorochemical	HLB value	Surfactant	HLB value
Perfluorodecalin	9.5	Lecithin	8.0
Perfluorooctyl bromide	6.0	Potassium oleate	18.0
Perfluorotributylamine	10.3	Pluronic F 68	24.0 - 29.0

HLB value = 3 to 6 (water-in oil surfactant); HLB value = 8 to 15 (oil-in-water surfactant); HLB value = > 15 (solubiliser).

Table 4. Surfactants most commonly used in PFC-based emulsions (Floyd, 1999).

Surfactants and/or their combinations	Range/ratio
Glycerol/propylene glycol	30 - 70% (w/w individually)
Egg lecithin	1 - 3% (w/w)
Pluronic F 68, 88, 108	1.5 - 10% (w/v)
Polysorbate 80	0.4% (w/w)

emulsions, dispersions with surfactant-PFC ratios of < 2% were determined to be surfactant limited, whereas those with a ratio of > 5% were energy-input limited (De Vleeschauwer and Van der Meeren, 1998). Although, several disadvantages of supplying pure PFCs in cell cultures are evident, the use of PFC emulsions with lower oil concentrations outweigh any disadvantages determined when cells are cultured in pure PFC dispersions (Ju et al., 1991b).

Emulsions are usually prepared with a mixture of surfactants with different hydrophobicity. The concept of hydrophilic-lipophilic balance (HLB) was introduced as an indication of the relative strength of the hydrophilic-lipophilic portions of the surfactant molecule and can be characterised by the relative affinity of surfactants for the aqueous and organic parts of the surfactant molecule (Griffin, 1949). The HLB arbitrary scale range is 1 to 30. As a result, surfactants with high HLB values (> 15) tend to stabilise oil-in-water (O/W) emulsions, while surfactants with low HLB values (< 10) tend to stabilise water-in-oil emulsions. As most PFC emulsions used in bioprocesses are O/W emulsions, surfactants with high HLB values are preferred in the preparation of these emulsions (Sajjadi, 2006). Examples of the HLB values of several PFCs and commonly-used surfactants are listed in Table 3, indicating that PF 68 with an HLB value of 24 to 29 is suitable for O/W emulsions.

PLURONIC F 68 AS A SURFACTANT AND A BIOMASS PROTECTOR

Polymeric surfactants have been extensively used in various applications ranging from personal care products to pharmaceutical and industrial use (Plucktaveesak et al., 2000). Pluronic F 68 (Poloxamer 188/ PF 68) is a non-ionic block copolymer of polyethylene glycol and polypropylene glycol (Elibol, 1999), which has been used to emulsify PFCs for use in oxygen-transport fluids (Johnson et al., 1990). This surfactant has been used as a growth-promoting additive to animal and microbial cultures (Lowe et al., 1994). When a PF 68 concentration of 10mg.ml^{-1} was used in Perfluorooctyl bromide (PFOB) emulsions, the emulsion was extremely stable and remained in this state for more than 7 days. This was advantageous for cultures, as the emulsions were homogeneous, but led to great difficulty in PFOB recovery. The total recovery percentage was low (78%) after centrifugation. When a PF 68 concentration of 5 mg.l^{-1} was used, destabilisation occurred rather rapidly, but the recovery of PFOB was easier, resulting in a 91% recovery rate (Martin et al., 1995).

The mass transfer characteristics of PF 68 were investtigated in a MYGP (malt extract-yeast extract-glucose-peptone) medium using Saccharomyces cerevisiae (NCYC 239) and was found to reduce the volumetric oxygen transfer coefficient ($K_L a$). This was not the case in the presence of PFC oils, where final biomass concentrations were unaffected (Elibol, 1999). Typical usage levels of surfactants in an emulsion system are: 1) 1 to 5% (w/v) for water-in-oil emulsions and 2) 5 to 10% (w/v) for oil-in-water emulsions (Floyd, 1999). Table 4 shows concentration levels for some of the surfactants most commonly used in PFC-based emulsion formulations, as compared to PF 68.

During PFC-based emulsion preparation, the particle size of the oil decreased when a combination of PF 68 and Tween 80 were used as hydrophilic surfactants. It was concluded that PF 68 greatly influenced the particle-size distribution profile in the PFC-based dispersions (Bouchemal et al., 2004). Emulsions prepared with PF 68 have a lower probability of stability loss due to coalescence resulting in homogeneous emulsions with an

Table 5. Examples of different applications of Pluronic F 68.

Surfactant	Micro organism	Consequence	Reference
0.2% (w/v) Pluronic F 68	*Spodoptera frugiperda* Sf9	Cell protection	(Murhammer and Goochee, 1990)
20% (w/v) Pluronic F 68	*Saccharomyces cerevisiae*	Improved fluorescein diacetate uptake	(King et al., 1991)
0.1 - 1.0% (w/v) Pluronic F 68	*Saccharomyces* cerevisiae (X 2180 1B)	No adverse effect on growth kinetics	(Laouar et al., 1996)
0.01% (w/v) Pluronic F 68	*Tetrahymena thermophila*	Protection against chemical/physical stress	(Hellung-Larsen et al., 2000)

Note: Quantities of reagents are listed at their final concentration in the nutrient medium.

Even distribution of DO. When coalescence and ripening are suppressed, the emulsion might remain stable for years (Mason, 1999).

PF 68 has also been used as a cytoprotectant and growth promoting additive to animal cell and microbial cultures (Lowe et al., 1994). Microbial cell damage arising from gas sparging is considered to be a major obstacle in the operational longevity of large-scale bioprocesses (Wu, 1995), thus requiring the application of protective additives in the culture medium. PF 68 is one of the most recognised and commonly used additives, as it has been shown to have strong protective effects in microbial cultures (Wu, 1995). Several other poloxamers (F88, F108 and L35) have also shown varying degrees of protective effects in agitated and aerated fermentation systems (Wu, 1995).

PF 68 has been determined to protect cells by coating the membranes of micro organisms, thus directly altering the cell membrane and resulting in the reorganisation of membrane lipids. The surfactant affects lipid-lipid and lipid-protein interactions, thus improving the survival rate of micro organisms by inhibiting damaging interactions between the cell membrane, fermentation broth and the air-liquid interface (King et al., 1991; Murhammer and Goochee, 1990). PF 68 was also shown to protect and prolong the survival of low concentrations of cell suspensions during nutrient starvation. Furthermore, the surfacetant prevented death caused by concentrations of Ca^{2+}, prolonging the survival of cells exposed to higher ion concentrations of Ca^{2+}, Na^{+} and K^{+}. It was effective in the postponement of death caused by trace element ions like Zn^{2+}, Fe^{3+} and Cu^{2+} and death caused by shearing forces, while prolonging the survival of cells exposed to hyperthermia (Hellung-Larsen et al., 2000). The surfactant protects cells by regulating the permeability and loss of ions from the affected cells (Laouar et al., 1996). Table 5 summarises examples of microbial cultures and the effects caused by the addition of PF 68 to these cultures, showing that PF 68 has mostly positive effects on microbial cultures. In addition, no quantitative results were reported in literature as to whether PF 68 improves microbial growth when used as an additive to synthetic media.

CONCLUSIONS

As new technologies are developed to continuously produce high-value biopharmaceuticals in order to meet increasing demand, the effective use of water-immiscible DO carriers and additives to improve bioreactor operational efficiency and longevity remains overlooked. This review clearly shows the positive effects of adding DO carriers to different microbial systems.

PFCs have been shown to be effective in carrying and delivering oxygen to biological cells in storage or culture systems. The general consensus was that PFCs, espe-cially those in emulsions, can have different effects on prokaryotic and eukaryotic cells, but their use in many instances showed that they increased the overall biomass performance and in some systems, they were shown to increase the yields of commercially important cellular products such as antibiotics. However, their application in bioprocessing systems needs to be evaluated in order to avoid harmful effects. Therefore, determining adequate concentration levels for a predetermined bioprocesses is important.

The promising capabilities of PFC and PF 68-based emulsions for providing culturing conditions suitable for a general improvement in microbial biomass performance and extended product formation have been illustrated. PF 68's ability to improve the functioning of individual cells in fermentation systems, while protecting the cells against trace element toxicity, shearing effects, hypothermia and product inhibition, will provide for increased biofilm and bioreactor performance in fixed-film bioreactors, thus improving the economic viability of these continuous systems.

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