5 CONCLUSIONS AND FUTURE WORK

5.1. Conclusions

The research performed during this PhD project involved the application of microfiltration (MF) to remove spores from high solids content Milk Protein Isolate (MPI) solutions up to 16 wt% in laboratory and 25 wt% in pilot trials. MPI feeds were inoculated with *Bacillus mycoides* spores as a safer alternative to *Bacillus cereus* a psychrotrophic spore forming bacteria commonly found in dairy products.

A large amount of material has been published in regards to microfiltration of low solids content dairy feeds such as raw and skimmed milk that contain generally 3.5 wt%. But to the best of my knowledge (at the time of writing) there was no published material available within the literature apart from the two papers produced from this work in regards to the processing of high solids content dairy feeds via microfiltration. This study therefore represents a significant contribution to the field of dairy filtration and goes some way to filling this gap in knowledge.

If possible microfiltration represents an attractive alternative processing technique to pasteurisation for the treatment of dairy products. Whereas pasteurisation (a heat treatment process) only destroys heat-sensitive spoilage and pathogenic bacteria, microfiltration (a size exclusion based technique) is capable of removing all types of microorganisms found within dairy products including thermoduric and spore forming bacteria that can survive pasteurisation. Moreover, microfiltration can be carried out using lower temperatures. Pasteurisation is an energy intensive process that is expensive to perform and can affect the organoleptic properties of milk and other dairy products. Whilst the application of microfiltration for filtering low viscosity diary feeds is relatively well established being used to produce commercially available products such as Cravendale® milk, the filtration of high solids content, high viscosity feeds has not become well established due to low fluxes and poor transmission of solids.

At the start of the project suitable protocols for MPI resolubilisation, *Bacillus mycoides* cell and spore preparation and feed and permeate sample spore content enumeration (*Petrifilm*™ aerobic count plates) were established and the membranes, MPI and spores were fully characterised by SEM, particle size distribution, hydrophobicity, rheology and pure water flux (PWF) measurements.

Initially it was intended that MPI solutions of up to 25 wt% would be filtered but due to issues encountered during MPI resolubilisation and filtration of these highly viscous feeds (4199.1 cP at 20 s^{-1} and 18 °C, ambient temperature), filtration experiments were conducted using MPI solutions up to only 16 wt% during laboratory trials. As stated in section 4.8 MPI solutions of up to 25 wt% were able to be tested during the pilot scale trials as the MPI concentrate was taken directly from the production line before being spray-dried. An ideal MPI resolubilisation protocol for spray-dried MPI was developed using particle size distribution analysis (section 2.4). This protocol involved stirring the solution using an overhead stirrer at 125 rpm for 30 minutes. Unfortunately using this protocol was only possible when small volumes of a low wt% solution (< 5 wt%) were required. When either a large feed volume or a solution of high wt% (> 5 wt%) was required a faster stirrer speed of 700 rpm had to be used to aid resolubilisation. *Bacillus mycoides* cell and spore culture methods developed for *Bacillus mycoides* were adapted from those used by Bowen *et al.,* (2002 a).

Initially during the preliminary filtration experiments involving an inoculated RO water feed carried out on the Danish separation systems (DSS) labunit M10 rig the spore content of collected feed and permeate samples was able to be analysed using a counting chamber. But when inoculated MPI feeds were filtered the squares that make up the counting area on the counting chamber were no longer visible down a light microscope. A solution in the form of *Petrifilm*™ aerobic count plates was researched and used for sample enumeration throughout the remainder of the project. These plates were subjected to a thorough error analysis necessary in order to determine whether changes in the bacterial counts of feed and permeate streams during filtration were significant. The difference in counts produced by these plates and the traditionally used standard spread plate method was determined along with the effect of using 'old' and 'new' *Petrifilm*™ plates and the difference of *Petrifilm*™ plate counts of *Bacillus mycoides* spores with time when stored within both water and MPI.

Understanding the relationships between MPI viscosity with changing temperature and concentration is critical when filtering high solids content dairy feeds. As a result a large amount of rheology measurements were conducted as a function of changing MPI concentration between 1 – 30 wt%, temperature between 18 °C (ambient) – 50 °C (filtration temperature) and time with varying shear rate (1 – 20 s^{-1}).

In order to try and determine an optimum protocol for high solids content MPI filtration, experiments were carried out using a variety of filtration rigs, modules and membranes. Specifically two different filtration rigs were tested a DSS labunit M10 and a newly constructed filtration rig that was modified throughout the project resulting in experiments being carried out on three different set-ups. Three different modules were used, a flat sheet stainless steel square module and two stainless steel tubular housings. Lastly, inside these modules two different flat sheet polysulfone polymeric membranes of 0.5 and 1.5 µm pore size (*Alfa Laval*) were evaluated along with five *Membralox*^{TM} α-alumina tubular ceramic membranes (*Pall Filtration*) of 0.8, 1.4, 2.0, 5.0 and 12.0 µm pore diameters.

Experiments were carried out at different CFV's (0.7 – 2.0 m s^{-1}), TMP's (1 and 2 bar) and MPI concentrations (4 – 16 wt%) using both 'sterile' and inoculated MPI feeds through these different membranes.

During filtration experiments samples were taken of the feed and permeate streams and analysed for their solids content by oven drying, their protein content using the Bradford assay and spore content using *Petrifilm*^{TM} Aerobic count plates where appropriate.

Experiments carried out through the tubular ceramic membranes produced the most encouraging results with the majority producing high spore reductions. In general successful filtration outcomes for 5 and 10 wt% feeds were achieved, with the most encouraging result for 10 wt% found using the 12.0 µm membrane and 1.4 m s^{-1} cross flow velocity (CFV). This produced a flux of 123 litres m^2 hr^{-1} (LMH), a 90% protein transmission and a 2.6 log spore reduction. The filtration of 15 wt% MPI solutions proved more challenging. The best set of results for this feed during normal crossflow experiments was also obtained using the 12.0 µm membrane at 1.4 m s^{-1}, producing a flux of 26.6 LMH, 96.5 % protein transmission and a 2.1 log spore reduction. These results indicate that large pore ceramic microfiltration may be a suitable technology to replace or augment pasteurisation for high solids content dairy feeds. This finding showed that the process is mechanistically very different when filtering high solids content feeds as opposed to low solids content feeds as the larger sized membranes produced the most successful filtration results within this work as opposed to the 1.4 µm membrane pore size typically employed for skim milk (low solids) microfiltration.

The effect of backwashing using different durations and frequencies during MPI filtration was also established. Backwashing parameters of 10 seconds every 5 minutes at 1 bar were found to be the most effective during filtration experiments. The most encouraging result during 15 wt% filtration was found using the 2.0 µm membrane and a CFV of 1.4 m s^{-1} producing a 13.0 LMH equating to a 98.5% increase in permeate flux and a 12.6 % increase in solid transmissions equating to a 113.5% increase at steady state after 40 minutes of filtration compared to filtration in the absence of backwashing. This was achieved whilst maintaining high spore reductions between 1.9 – 3.0 log order reductions compared to 2.7 – 2.9 log orders without backwashing.

The relative contributions of concentration polarisation, reversible and irreversible fouling resistances to permeate flux decline were calculated during each filtration experiment. This allowed qualitative analysis of the types of fouling mechanisms occurring when different membrane pore sizes and process conditions were tested.

Lastly, an optimum cleaning regime for MPI fouled tubular ceramic *Membralox*^{TM} membranes was investigated involving testing of various combinations of both hydraulic and chemical cleaning methods. A long rinsing backflush at 1 bar and acid and alkali steps without backwashing were found to be most effective producing a 99.6% recovery in pure water flux.

At the start of the project suitable targets were set for spore rejection, permeate flux and solid transmission during the filtration of a 15 wt% MPI feed, by consideration of values achieved within industry and those reported within the literature. As stated within the 'project aims and objectives' section of the introduction; spore rejections of 2.08 ± 0.12 and 2.15 ± 0.10 $\log_{10}$ cfu ml^{-1} have been reported by Fritsch and Moraru, (2008), 3.79 $\log_{10}$ cfu ml^{-1} by Elwell and Barbano (2006), 4.5 $\log_{10}$ cfu ml^{-1} by Tomasula *et al.,* (2011) and 2.28 ± 0.17 $\log_{10}$ units by Madec *et al.,* (1992). All these rejections were found using a feed of raw skimmed milk and a 1.4 µm membrane. A similar minimum reduction target of ~2 $\log_{10}$ orders cfu ml^{-1} was set for 15 wt% MPI filtration during this project. This target was met for all experiments carried out through the 0.8, 2.0 and 12.0 µm membranes that were subjected to the largest amount of testing. With regards to permeate flux and solids transmission in industry steady state permeate fluxes of 500 litres m^{-2} hr^{-1} (LMH) and solids transmissions of > 99% are typically obtained during the filtration of skimmed milk over a 10 hour period (Saboya

and Maubois, 2000). Considering the difference in solids content between skimmed milk (3 - 4 wt%) and the MPI (up to 15 wt%) a realistic target of > 50 LMH for steady state permeate flux and > 80% for solids transmission were set. The permeate flux target was met for 5 wt% through both the 2.0 and 5.0 µm membranes at 1.4 m s^{-1} CFV and for 10 wt% through the 5.0 µm membrane at 1.4 m s^{-1} CFV. Both permeate flux and solid transmission targets were met during filtration of 10 wt% through the 12.0 µm at 1.4 m s^{-1}. Unfortunately, neither of these targets were met during the filtration of 15 wt% MPI. The closest values of 26.6 LMH and 68.7% were obtained through the 12.0 µm membrane at 1.4 m s^{-1} CFV.

5.2. Future work

Ideally, the feed stream used in this project would have been liquid milk (prior to being pasteurised) or MPI concentrate solution (taken directly off the production line at Kerry Foods). This would have removed the issue of powder resolubilisation and the need for spore inoculation (as bacteria are already present). However, due to safety issues such as the presence of harmful microorganisms (see section 1.8), and practicality in terms of transportation and storage of liquid dairy products, a spray dried MPI powder prepared using pasteurised skim milk was used. As detailed in section 4.8 pilot plant trials were conducted using MPI concentrate feed solutions of 10, 17 and 25 wt%. These studies gave promising results in terms of solids transmissions and spore reductions, but as stated previously there were limitations to this work (see section 4.8). It would be interesting to carry out further optimisation experiments using this feed type with the large pore size tubular ceramic *Membralox*™ membranes of 2.0 and 12.0 µm.

Despite various modifications being made to the filtration system during the course of the project aimed at improving the CFV able to be reached during filtration of high solids MPI, the maximum CFV value able to be reached was 2.0 m s^{-1}. This value is low compared to other microfiltration experiments carried out using dairy feeds that often use up to 8 m s^{-1}. In order to increase this value either the general pipework within the filtration rig would have to be replaced with pipework of a larger diameter and / or a bigger pump should be incorporated into the system that would lead to the pressure within the feed loop to be increased. This was too large a task to be carried out during this project but in order to try and improve the permeate flux values and solid

transmission produced during experimentation using this rig this would have to be carried out.

During this work experiments have been conducted using the MPI in its manufactured state but investigations into MPI feed pH modification through the addition of either hydrochloric acid (HCl) to decrease the pH or sodium hydroxide (NaOH) to increase the pH prior to filtration may improve membrane performance and should be investigated. According to Mukhopadhyay *et al.,* (2010) who filtered liquid egg white through a 1.4 µm *Membralox*^{TM} tubular ceramic membrane found that maximum permeate flux occurs at or near the isoelectric point of the feed, which for protein is pH 6. At pH 6 the overall charges of the protein-membrane are neutral. As the protein is positively charged it adsorbs onto the negatively charged membrane surface causing an increase in flux.

This present research work has focused on the effect that variations in membrane pore size, process conditions and MPI concentration have on the steady state permeate flux, solids and protein content and spore rejection. Although the relative contribution that each type of fouling resistance has on the total resistance was calculated and used to give qualitative conclusions on the type of fouling mechanisms occurring no modelling of the fouling process has been conducted. In order to further increase the steady state permeate flux and solid transmission values into commercially viable regions for high solids content MPI solutions, it may be necessary to model the filtration process to determine what type of fouling is dominant and so determine what methods would aid the filtration. Madaeni *et at* (2011) modelled the different mechanisms responsible for fouling during the filtration of raw whole and skim milk through a flat sheet 0.22 µm polyvinylidenefluoride (PVDF) membrane and used SEM images to confirm their conclusions. They reported during whole milk filtration that at the start standard blocking dominated but over time (180 minutes) bacteria, casein and fat particles filled the pores and formed a cake layer. Whereas, during skim milk filtration three fouling mechanisms occurred standard blocking, intermediate blocking and cake deposition.

Due to the limited supply of tubular ceramic *Membralox*^{TM} membranes having only one in each membrane pore size tested, it was not possible to carry out dissections of the membranes for testing at different stages of the filtration cycle such as before and after fouling, after rinsing and cleaning. If more than one pore size of each membrane were

available it would be interesting to carry out analytical techniques on sections of the membranes. These analytical techniques could include Scanning Electron Microscopy (SEM) imaging, Atomic Force Microscopy (AFM) measurements, Fourier Transform Infrared (FTIR) spectroscopy and contact angle measurements. SEM images would allow the morphology of the membrane surface to be visualised at different points of the operational cycle. These images would allow the effectiveness of different fouling and cleaning process conditions to be qualitatively analysed. AFM force measurements could be used to probe the membrane surface topography and interactions and provide quantitative information on the effectiveness of different fouling and cleaning process conditions. Lastly, FTIR analysis would allow the types of chemical bonds and functional groups present on the membrane surface to be determined and contact angle measurement would provide a measure of the hydrophobic / hydrophilic nature of the membrane surface.

It could also prove useful to carry out a combination of Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and High performance liquid chromatography (HPLC) on collected feed and permeate samples in addition to measurements of the solid and protein content. This could be used in order to quantify the relative contributions of each amino acid to the solid and protein content values. This data could be collected as a function of filtration time to determine which amino acids are transmitted through the membrane, which amino acids are responsible for membrane fouling and whether this changes as filtration time progresses.

It would also be of interest to determine the long-term effect of multiple fouling and cleaning cycles on the *Membralox*^{TM} ceramic membranes performance. A large amount of work of this nature has been conducted within our research group using different feeds and membranes. Evans (2008) carried out 18 fouling (black tea liquor) and 17 cleaning cycles (sodium hydroxide) on a single 30kDa flat sheet Fluoropolymer membrane and found that both the steady state permeate flux and solids transmission varied insignificantly. Weis (2004) found that flat sheet polyethersulfone and polysulfone membranes fouled with spent sulphite liquor and cleaned with sodium hydroxide showed a steady state permeate flux decline of 45% and 85% respectively after multiple operational cycles. In addition when cleaning with *Ultrasil 11* this difference in flux was found to be even higher.

In the long term once the filtration process for high solids content MPI has been optimised it would be interesting to carry out further pilot plant trails under these conditions to see the effect if any, of scale-up. This would have to be determined if the process were to be used commercially to augment or replace pasteurisation. If achieved this technique could be used to reduce the bacterial load of all types of dairy products that at present are processed via heat treatment.

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APPENDICES

1. Filtration flux equations

System performance is usually defined in terms of permeate flux (J_v) which can be determined using equation 31. Where V is the volume of permeate collected, in a given time period t, through a known membrane area A_M.

$$J_V = \Delta V \Big/ \Delta t \times A_M \tag{31}$$

Permeate flux can be expressed either as a flow rate having units of litres m^{-2} hr^{-1} (LMH) or as a velocity having units of m s^{-1}.

The first step that needs to be carried out when conducting membrane filtration experiments is the measurement of permeate flux with varying transmembrane pressure using a feed consisting of RO water. This measurement can help determine if the equipment is set up properly by comparing water fluxes with expected values and to calculate the membrane resistance $R_{m.}$

$$R_M = \Delta P \Big/ \mu_p \times J_V \tag{32}$$

Where J_v is the permeate flux (m s^{-1}), μ_p is the permeate fluids viscosity (Pa.s) and ΔP is the transmembrane pressure (Pa).

The membrane resistance can be calculated in one of two ways either from the gradient of a flux data plot or from individual flux data points. Rearranging equation 32 gives: -

$$J_V = \left(1 \Big/ R_M \times \mu_p \right) \Delta P \tag{33}$$

Using equation 33 a plot of J_v against ΔP gives a linear plot with the gradient 1/ R_M μ_p, by knowing the fluid viscosity the resistance of the membrane (R_M) can be calculated using equation 34.

$$R_M = 1 \Big/ \mu_p \times gradient \tag{34}$$

All resistances such as the membrane resistance R_M are calculated with units of m^{-1}.

The total hydraulic resistance R_{TOT} for a filtration experiment was calculated using equation 35. Where J, is the final recorded permeate flux (LMH), ΔP is the transmembrane pressure (bar) at which the experiment was performed and η is the viscosity of the permeate solution collected at the end of the filtration calculated from the total solids transmission measured (as described in section 2.10.1 using equation 15) for the experiment and the equation of the plot shown in Figure 53.

$$R_{TOT} = \frac{\Delta P}{J \times \eta} \tag{35}$$

2. SEM sample preparation protocol

2.1. *Bacillus mycoides* cells

Bacillus mycoides cells were prepared following the method described in section 2.2.1. Two glass coverslips were placed in two wells and covered in 2.5 ml of TS culture this was then placed inside a static incubator at 25 ºC for 6 hours, in order for the bacterial cells to adhere to the coverslip surface.

Prefixation: Each coverslip was then dipped in 0.1 M SCB and then immersed into a solution made up of GDA (2.5 ml, 25%), SCB (12.5 ml, 0.2 M) and distilled water (35 ml) inside a small vial which was then sealed and left inside a fridge overnight.

Rinse 1: Each coverslip was rinsed in buffer- x3 changes left for 5 minutes in each

Postfixation: Both coverslips were left in a solution of Osmium tetroxide (1%) and SCB (0.1 M, pH 7.3) for two hours at room temperature.

Rinse 2: Each coverslip was rinsed in distilled water- x3 changes left for 5 minutes in each

Freeze-drying: One of the coverslips was then frozen and left overnight in the freeze drier

Dehydration: The remaining coverslip was placed in a series of acetone solutions; 30%, 50%, 70%, 80%, 90%, 95% x2 changes in each over 15 minutes, then into 100% dry acetone x4 changes over 30 minutes. The coverslip was transferred into 100% HMDS x2 changes over 20 minutes, after which time as much of the HMDS as possible was pipetted off. The coverslip was then left partially covered inside the fume cupboard in order for the remaining HMDS to evaporate off. Once evaporated the coverslip was placed in silica and left in a sealed container overnight.

2.2. *Bacillus mycoides* spores

Bacillus mycoides spores were prepared following the protocol described in section 2.2.1. During harvesting 10 ml of the solution was left untouched and 10 ml was

removed after each centrifuging and washing step so that 10ml spore culture samples were prepared, one before it had been centrifuged, one after centrifuging and one after each of the three washing steps.

Prefixation: 2.5% (1 ml) glutaraldehyde solution was added to each sample inside a universal bottle which was sealed with paraffin film and left overnight at room temperature.

Rinse 1: Each solution was used to fill two small eppendorf's and centrifuged the supernatant was discarded and replaced with distilled water (cell culture grade) - x3 changes 5 minutes each.

Postfixation: Osmium tetroxide (1%) was added to each solution and left for 1.5 hours at room temperature

Rinse 2: Each eppendorf was centrifuged replacing the supernatant with distilled water (cell culture grade) - x3 changes 5 minutes each

Freeze-drying: All samples were then frozen and left overnight inside the freeze drier.

The accelerating voltage, magnification, spot size and z value used were 15 kV, either x10,000, 30,000 or x45,000, 26 and 15 these values are also shown on the photographs themselves displayed in Figure 21. The sample that had undergone three washing steps had a second set of SEM images taken in order to determine spore size, these were taken using an accelerating voltage of 15 kV, a magnification of x5,000, a spot size of 20 and a z-value of 23.

2.3. Spray dried Ultranor^{TM} 9075 Milk Protein Isolate powder and solution

A 20 wt% MPI solution was prepared following the method described in section 2.1.1.

Freeze-drying: The MPI powder and 20 wt% solution were freeze-dried and left inside a freeze-drier overnight.

The two *Bacillus mycoides* cell samples and the MPI powder and resolubilised solution samples were examined and photographs taken using an accelerating voltage of 15 kV, a spot size of 35 and a z value of 20.

2.4. SEM preparation

All samples during SEM preparation once freeze-dried were stuck onto carbon double sided tape which itself was stuck onto a small circular aluminium disk, known as a specimen stub. They were then placed into a vacuum evaporator to remove any remaining moisture and air from the surface of the samples and finally sputter coated with a thin layer of gold in order to make the samples conductive.

3. Light microscope calibration for spore size measurement

A new calibration had to be carried out for each magnification used for example, using a x40 microscope magnification with a x10 eyepiece magnification = x400 magnification, the following calibration was found:

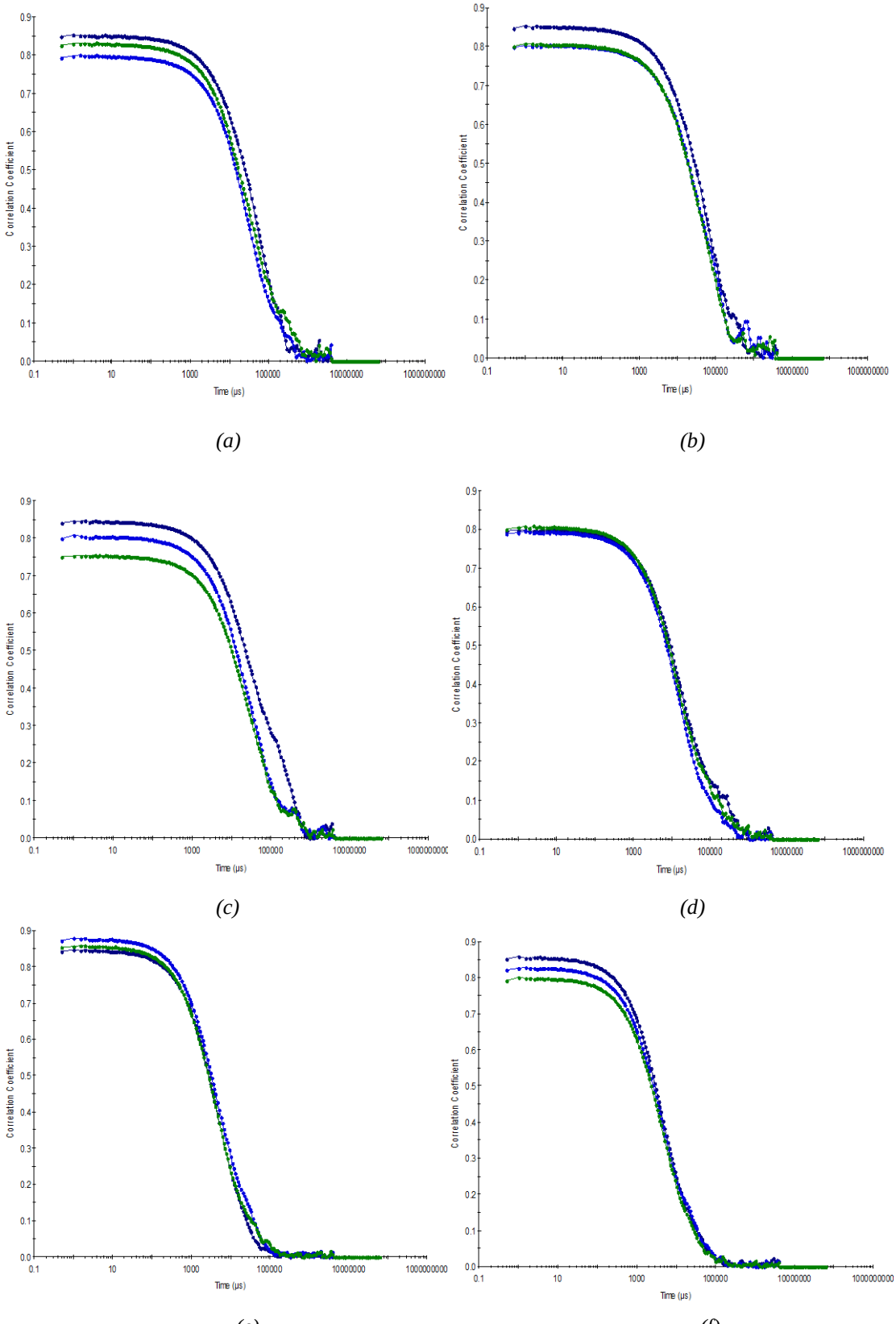
0.25 mm (on stage micrometer slide) = 98 GU (on eyepiece)
1 GU = 0.00255 mm = 2.55 µm

Using a x100 microscope magnification with a x10 eyepiece magnification = x1000 magnification, the following calibration was found:

99 GU = 0.1 mm
1 GU = 0.00101 mm = 1.01 µm

Once the calibration had been completed the stage micrometer slide was replaced by a specimen slide that has the sample on and the spore size can then be measured in GU and converted to µm using the calibration conversion shown above.

4. Particle size distribution- Correlation against time graphs for MPI solutions prepared using a slow (optimal) stirrer speed.



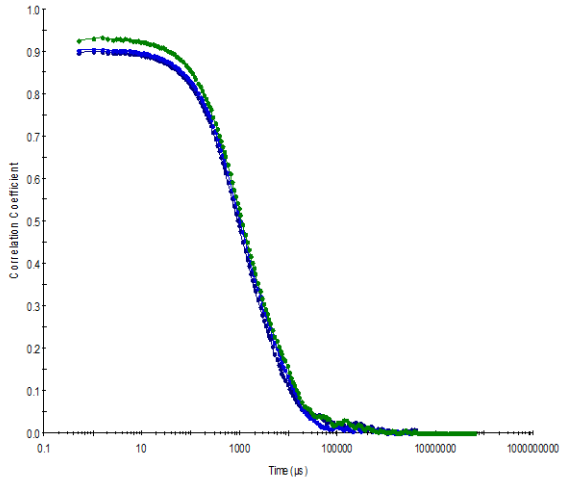
(a) *(b)*

(c) *(d)*

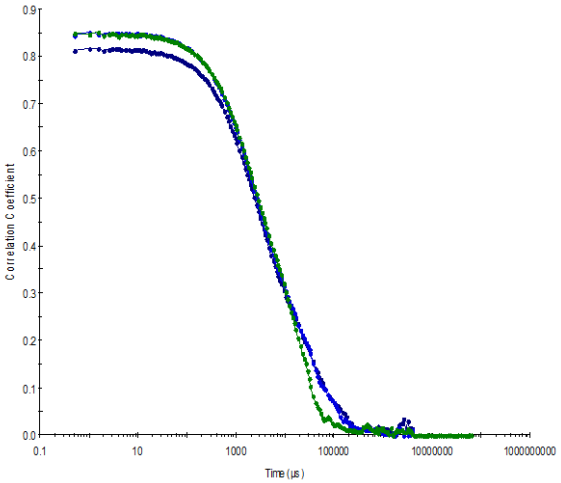
(e) *(f)*

Figure 88. Correlation against time graphs for prepared solutions 1 - 6 (a - f) as described in section 2.4.

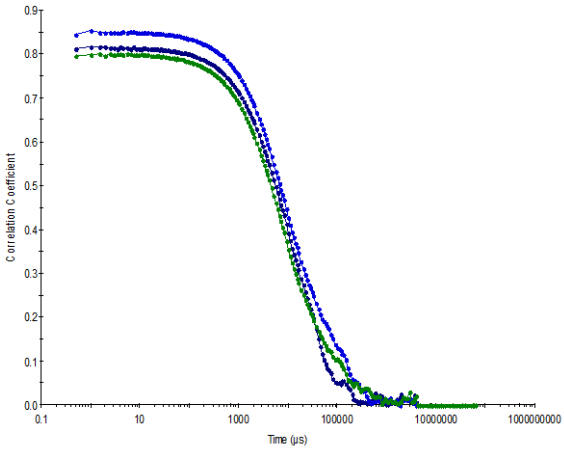
5. Correlation against time and volume statistics data tables, for 5, 10 and 15 wt% MPI solutions prepared at 700 rpm.



(a)



(b)



(c)

Figure 89. Correlation against time graphs for 5, 10 and 15 wt% MPI solutions prepared using the faster stirrer speed of 700 rpm.

Table 48. Volume statistics data for 5 wt% MPI resolubilised at 700 rpm.

Size (d. nm)	Mean volume (%)	Standard deviation volume %
37.8	0.2	0.2
43.8	0.8	1.2
50.7	1.3	1.8
58.8	0.9	1.2
68.1	0.1	0.2
142	0.6	0.8
164	1.6	2.3
190	2.8	4.0
220	4.9	3.6
255	11.2	4.6
295	17.0	14.2
342	16.7	17.0
396	9.9	10.8
459	1.9	2.2
615	0.5	0.8
712	1.8	2.6
825	3.5	4.9
955	4.9	6.9
1110	5.5	7.8
1280	5.1	7.2
1480	3.8	5.3
1720	2.0	2.9
1990	0.6	0.8
5560	2.4	3.3

Table 49. Volume statistics data for 10 wt% MPI resolubilised at 700 rpm.

Size (d. nm)	Mean volume (%)	Standard deviation volume %
295	2.6	1.9
342	16.3	4.7
398	35.7	1.9
459	33.1	4.0
531	11.8	3.6
615	0.5	0.9

Table 50. Volume statistics data for 15 wt% MPI resolubilised at 700 rpm.

Size (d. nm)	Mean volume (%)	Standard deviation volume %
220	0.2	0.4
255	5.8	10.1
295	16.2	21.8
342	21.6	18.7
396	17.9	21.2
459	12.0	10.4
531	12.3	21.2
615	10.6	18.3
712	3.3	5.8

6. Mass balance error analysis

The error in the weights given by the mass balance that was used during total solids determination of samples collected during filtration runs was found during the drying protocol development experiment detailed within section 2.10.5. This was achieved simply by placing each of the six containers used within the experiment onto the balance three times. From these weights the standard deviation and error were calculated and found to be no greater than 0.0005 and 0.0003 respectively, as can be seen below in Table 51.

Table 51. Mass balance error data

	0.5 wt%	0.75 wt%	1.0 wt%	1.25 wt%	1.5 wt%	Feed
Weight 1 (g)	51.2288	48.4018	48.4018	48.4018	48.4018	0.6865
Weight 2 (g)	51.2287	48.4021	48.4021	48.4021	48.4021	0.6859
Weight 3 (g)	51.229	48.4019	48.4019	48.4019	48.4019	0.6868
SD	0.00015	0.00015	0.00015	0.00015	0.00015	0.00046
Standard error	8.819×10^{-5}	8.819×10^{-5}	8.819×10^{-5}	8.819×10^{-5}	8.819×10^{-5}	0.00026

7. Osmotic pressure effect calculation

$$\pi = \frac{CRT}{M} \tag{36}$$

Osmotic pressure for a typical 15 wt% feed stream was calculated using equation 36. Where C is the feed concentration (g litre^{-1}), R is the universal gas constant (litre bar K^{-1} mol^{-1}), T is the temperature (K) and M is the molecular weight of the solute (g mol^{-1}). In order to calculate the molecular weight of MPI the assumption was made that it had the molecular weight of glutamic acid, which is the most abundant amino acid in MPI according to Table 7.

$$\pi = \frac{(0.0001552 \times 0.08314 \times 323)}{147.05} = 2.83 \times 10^{-5} bar$$

Osmotic pressure for the respective permeate stream calculated using equation 36.

$$\pi = \frac{(0.000003254 \times 0.08314 \times 323)}{147.05} = 5.94 \times 10^{-7} bar$$

Osmotic pressure difference across the membrane was calculated by subtracting permeate osmotic pressure (bar) from the feed osmotic pressure. For this system this worked out to be a difference of 2.77 x 10^{-5} bar.

During filtration experiments a TMP of either 0.5, 1 or 2 bar has been employed during MPI filtration with the majority of experiments being carried out at 2 bar. As the osmotic pressure difference is so small in comparison to even the lowest TMP of 0.5 bar used it was considered to have a negligible effect during this work.