Acknowledgements

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Abstract

The purpose of this study was to design and characterise a protein skimmer to improve the quality of water in the CEAS fish tank. Poor water quality prompted the design of a suitable method for removing excess organic material from the tank. Protein skimmers operate based on foam fractionation, an adsorptive separation process which has been found to be effective at separating dilute solutions of surface active materials. The protein skimmer design was based on the desired waste protein removal rates which were then used to determine the gas flow rate, 133 ml min^{-1}, feed rate, 10.32 ml min^{-1}, and skimmer column diameter, 0.25 m, used.

The efficiency of the protein skimmer was determined through experimental work using Cetylpyridinium chloride (CPC) for preliminary experiments and Bovine Serum Albium (BSA) for the crucial experiments. The BSA experiments were operated in continuous mode; feed concentration was varied between 0.1 $mgmL^{-1}$ and 0.5 $mgmL^{-1}$ while maintaining a constant gas flow rate of 130 ml min^{-1}, feed rate of 10.32 ml min^{-1}, feed height above the sparger of 8 cm and a pH of 4.8. Batch mode and reflux were also investigated to aid understanding of the effect of different operating conditions for the protein skimmer.

Performance of the protein skimmer at different initial feed concentrations was characterised by protein enrichment and recovery. Enrichment decreased with increasing feed concentration while the recovery increased; with maximum values of 8.9 and 65% respectively being observed at a feed concentration of 0.1 mg mL^{-1}.The protein skimmer designed is effective at separating dilute protein solutions. Experimental findings were supported by literature and fulfilled the objective of the study. A 65% recovery factor can effectively improve water quality to maintain safe levels of protein in the fish culture water. Starting concentrations as high as 0.4 mg/mL, the upper concentration limit for organics in the tank could be reduced to 0.14 mg/mL in the residual tank water when the experimentally determined recovery factor was applied.

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Nomenclature

γ	Surface tension
R_1	Gas constant
T	Absolute temperature (K)
Γ	Surface excess (g m^{-2})
a	Activity of the surfactant
b	Langmuir constant
C	Surfactant concentration (g L^{-1})
r	local radius of the curved surface (m)
R	Reflux
$\hat{C}^*_w$	effective concentration in equilibrium
C_w	Bottoms Concentration
C_Q	Concentration of the total overflowing foam on a collapsed gas free basis
$\hat{C}$	Effective concentration of the surfactant in rising stream at any level in foam column
G	Volumetric gas flow rate (m^3 s^{-1})
S	Bubble surface area to volume ratio
U	Interstitial liquid upflow (m s^{-1})
C	Concentration of rising interstitial liquid
d	diameter (mm)
D	Column Diameter (mm)
e	Molar absorptivity (L mol^{-1} cm^{-1})
b	Path length of the cuvette in which the sample is contained (cm)
c	Concentration of the compound in solution (mol L^{-1})
A	Area (m^2)
V	Volume (m^3)
U_g	Superficial gas velocity (mm s^{-1})
M	Mass (g)

Subscripts

s	surfactant
sat	saturated
b	bubble
S.A	surface area
C	cross sectional Area
N	nominal
f,	foam
r	residual feed
i	initial feed
o	overflow.

Non standard abbreviation used

CPC	Cetyl pyridinium chloride
BSA	Bovine Serum Albium
TDS	Total Dissolved Solids
TVS	Total Volatile Solids
FS	Fine Solids
CMC	Critical Micelle Concentration
SDS	Sodium Dodecylsulfate
NTU	Number of Transfer Units
TBSA	Total Bubble Surface Area
BPM	Bubble per Minute

1.0 Introduction

Foam fractionation is a low costs separation method that has a wide range of applications in modern industry. Interest in this research area is growing gradually with increasing understanding of the underlying mechanisms of foam fractionation. The massive array of industrial applications include the purification of biomolecules for drug production in the pharmaceutical industry (Chen *et al*., 2006), which is expected to grow 5.5% annually to a global market worth $929 billion in 2012 (Piribo Limited, 2008) according the 'Global Pharmaceutical Market Forecast to 2012'. Another application is bio-surfactant production which has a global market worth of $ 23 billion (Acmite Market Intelligence, 2008) and has significant importance in food and pharmaceutical industries. These applications outline the importance and significance of the foam fractionation technique which has been researched extensively (Lockwood *et al.*, 1997), (Linke *et al*., 2007) and (Sarkar *et al*., 1987).

Aquaculture is the farming of aquatic plants and animals. Aquaculture is responsible for 38% of global sea food production which is expected to grow to 44% by 2020 (Summerfelt, 2007). The importance of aquaculture in modern industry is growing due to increasing seafood demand and rising environmental awareness for sustainable eco-friendly technologies like foam fractionation. In Aquaculture, foam fractionation is used for waste management both in pond management and recirculating aquaculture systems on a large and small scale.

The problem addressed in this study is the removal organic matter (proteins) from fish culture water in a fish tank using foam fractionation. Fish tanks contain dissolved organic waste from uneaten food and faeces in dilute concentration solutions which can be effectively removed by a protein skimmer to prevent toxic conditions in the fish tank. Protein skimmers operate on a foam fractionation principle. Extensive research carried out to determine effectiveness of foam fractionation as a separation technique in aquaculture, confirms that it can deliver desirable results (Chen *et al*., 1992), (Chen *et al*., 1993), (Timmons *et al*., 1995) and (Nava *et al.*, 2004).

1.1 Scope

The main aim of this project was to design and build a protein skimmer for the school of Chemical Engineering and Analytical Science department fish tank. The desired outcome was to improve the water quality using a chemical process and allow for more fish to be accommodated in the tank.

The removal of organic materials and proteins from fish culture water is a stripping process which requires a setup similar to that shown in Figure 1.

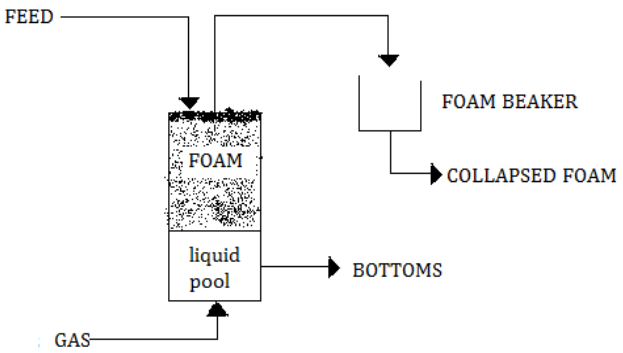


Figure 1: Schematic of continuous foam fractionation column in stripping mode. Adapted from Lemlich (1972)

A review of the theory behind foam fractionation is carried out in Section 2.0 to provide an understanding of the mechanisms and behaviour of surfactants within the foaming column. Surfactant adsorption, foam structure, foam stability and the effects of different operating conditions are some of the factors addressed. This is followed by discussions of the applications of foam fractionation in Section 3.0 to demonstrate its significance as a separation technique in modern industry especially with regard to aquaculture. The pros and cons of the waste removal methods commonly used are also discussed here.

Section 4.0 describes the protein skimmer design constructed for this study which comprised of three glass components assembled to form a closed unit for experimental work. The main experimental techniques reviewed were concentration measurement. The approach used in the experiments is outlined in Section 5.0.

Finally, Sections 6.0 presents the results obtained from both the preliminary work and the main study. The results were compared using enrichment and recovery as performance indicators at different operating conditions to determine effectiveness of the protein skimmer for waste removal.

1.2 Objectives

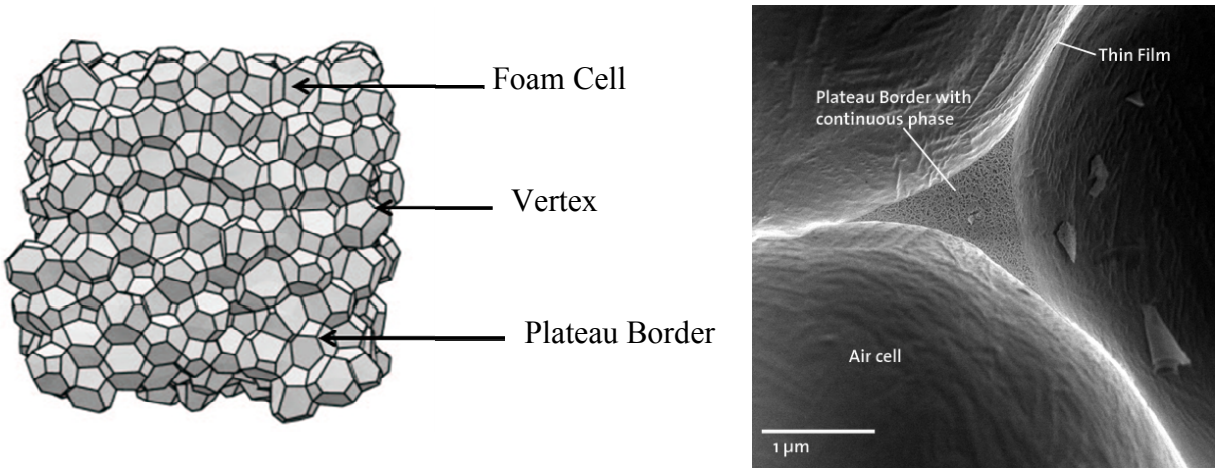
- Design new protein skimmer to be incorporated into fish tank configuration to improve water quality.
- Build the protein skimmer.
- Determine the optimum operating conditions for maximum recovery of organic waste using the protein skimmer through experimental work. The variables that to be investigated included concentration, gas flowrate, feed flowrate, pH and reflux.
- Determine the efficiency of the column and its ability to improve water quality by calculating performance factors for the results obtained like enrichment and recovery.
- Compare the efficiencies of the protein skimmer and the current waste removal system to determine if foam fractionation would improve water quality.
- Improve water quality using protein skimmer

2.0 Background Information

This section reviews foam theory and the mechanisms that contribute to the effectiveness of foam fractionation as a separation method.

2.1 Foam Structure

Foam is a colloidal system which consists of a gas phase dispersed in a continuous matrix. Liquid foam is created by introducing pressurised gas into a liquid containing surfactant molecules like proteins. Air bubbles within the foam are separated by bulk liquid films also known as lamellae, which experience gravitational drainage. Drainage also occurs within plateau borders which are formed at a junction of the lamellae of three neighbouring bubbles within the foam as shown in Figure 2 (b).



(a) (b)

Figure 2: (a) Schematic of foam structure (Reinelt, 2003); (b) Cryo-SEM Picture of a Plateau Border (SFIT, 2008).

Liquid hold up is the liquid volume within the total foam volume and determines the thickness of both the plateau borders and lamellae hence the type of foam formed. Dry foam structures contain small volumes of liquid with thin lamellae and polyhedral bubbles as shown in Figure 2 (a). Dry foam has a rigid structure which is more stable than the fluid structure of wet foam with spherical bubbles. Due to a higher liquid hold-up, wet foam

contains thicker liquid films which causes loss in rigidity. Wet foam is therefore more likely to collapse than dry foams (Weaire, 1999).

The stability of the foam structure is affected by several factors namely; drainage, bubble coalescence, coarsening, bulk liquid rheology, interfacial rheology and the presence of surfactants in the continuous phase. Figure 3 illustrates the mechanisms that occur within the foam fractionation column as foaming takes place.

Proteins increase the stability of foam and are preferentially adsorbed at the gas-liquid interface in foams. Protein skimmers operate based on this principle and are able to separate surfactant material from very low concentration solutions. These processes are discussed in further detail in Section 2.0.

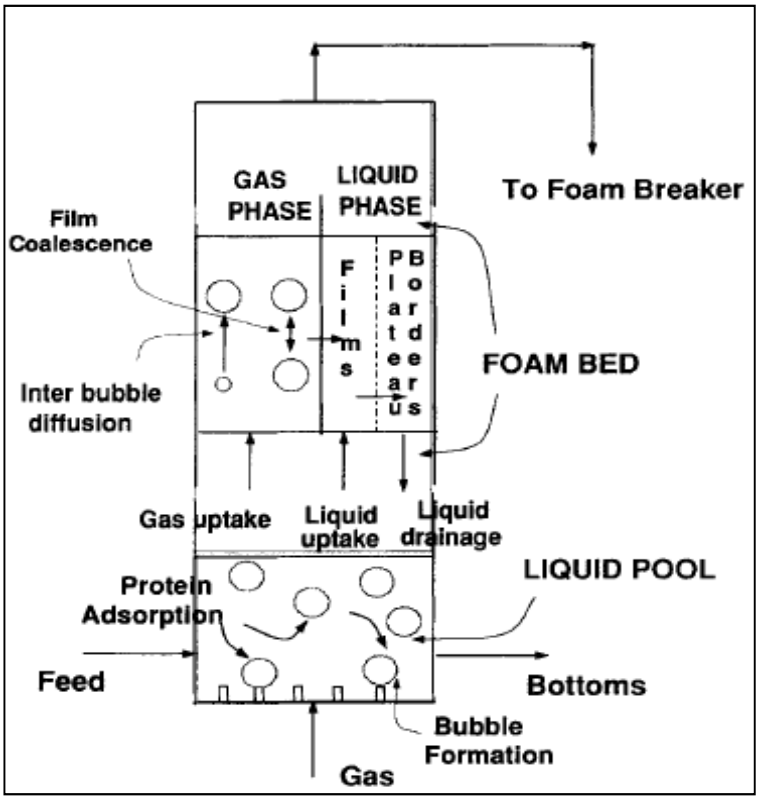


Figure 3: Schematic of mechanisms within foam column (Uraizee and Narsimhan, 1996).

2.2 Foam fractionation Theory

Foam fractionation is a technique used for the partial separation of surface active (surfactants) dissolved in a liquid. Separation is achieved by sparging gas through a liquid containing surfactant molecules. The surfactant molecules are adsorbed onto the surface of the rising gas bubbles resulting in the formation of pneumatic foam. The foam enriched with surfactant material is removed when it reaches the surface of the liquid (Lemlich, 1972). The surfactant concentration is higher in the foam than in the bulk liquid due to drainage.

Surfactants are amphiphillic molecules with a molecular structure comprising of both polar, hydrophilic regions and non-polar, hydrophobic regions. The gas-liquid interface presented by the gas bubbles in the continuous liquid phase is their ideal environment. The non-polar regions prefer the gas phase and the polar regions prefer the liquid phase.

Figure 4 illustrates the adsorption mechanism mentioned above. The hydrophilic heads can clearly be seen on the surface of the air bubble while the hydrophobic tails insert themselves into the air bubble forming a stable surfactant-gas molecule. The process continues as more air is introduced into the system until the liquid surface becomes saturated.

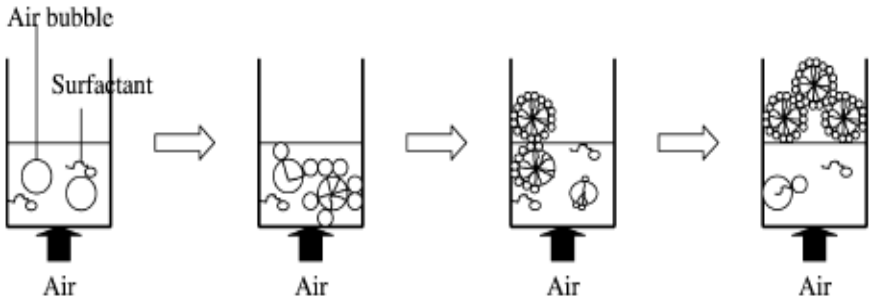


Figure 4: Foam Fractionation principle (Boonyasuwat *et al*., 2005).

2.2.1 Adsorption

The extent of adsorption of the surfactant onto the bubble surface is measured by the surface excess. This is the concentration of the surfactant at the liquid surface per unit area with units of g m^{-2}**.** The saturated surface excess is termed the Critical Micelle Concentration (CMC). The CMC is the concentration at which the surfactant molecules form a saturated monolayer at the surface of the liquid. At a concentration above the CMC, surfactant molecules start to aggregate and form micelles as shown in Figure 5.

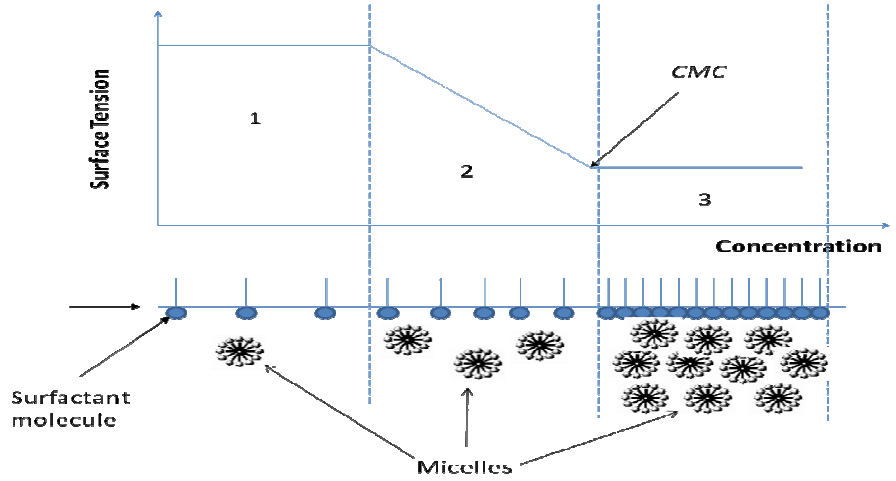


Figure 5: Schematic showing the effect of Concentration on Surface Tension adapted from Birdi (1997)

Gibb's proposed an adsorption equation to calculate Γ under equilibrium conditions, Equation (1). However, this equation is rarely used as surface activity (a) is difficult to measure accurately.

$$d\gamma = -RT \sum \Gamma \, d \ln a \qquad (1)$$

Where; γ is the surface tension, R is the gas constant, T is the absolute temperature, Γ is the surface excess and a is the activity of the surfactant.

At concentrations below the CMC, Equation (2) can be used to calculate the surface excess of non-ionic surfactant solutions. Equation (2) was derived under the assumption that the activity of the surfactant is constant and is equal to the liquid concentration.

$$\Gamma_S = -\frac{1}{R_1 T}\frac{d\gamma}{d \ln C_S} \qquad (2)$$

Subscript S denotes the surfactant and the other parameter are as defined for Equation (1).

Surface excess is directly proportional to the concentration therefore as the bulk concentration increases; the surface excess also increases until the CMC is reached. The surface tension decreases with increasing concentration of the surfactant solution. At the CMC, the surface tension reaches a constant minimum value.

Therefore, the surfactant concentration is the limiting factor for both the surface excess and surface tension change above the CMC. This is because of excess surfactant in the solution after the formation of a saturated monolayer of surfactant molecules (Birdi, 1997).

Figure 5 also demonstrates how the surface tension changes with increasing concentration. Section 1 of the figure shows that surface tension remains constant. This is because there is limited surfactant at the surface to influence any change, the solution is still very dilute. In section 2, the surface tension begins to decrease as more surfactant molecules are present at the surface. The surface of the liquid becomes saturated in section 3; no more surfactant can adsorb to the surface so the surface tension reaches a constant minimum value. The concentration at which this happens is known as the CMC. The number of micelles in the solution increase as the concentration is increased.

At higher concentrations, below the CMC, Equation (2) does not apply so the Langmuir Isotherm shown by Equation (3) is used instead (Lemlich, 1972).

$$\Gamma = KC \qquad (3)$$

Where; *K* is the equilibrium constant and C is the surfactant concentration

$$\Gamma = \frac{C\,\Gamma_{sat}}{b + C} \qquad (4)$$

Where; Γ_{sat} is the saturated surface excess at equilibrium, b is a constant and C is the surfactant concentration.

At very high concentrations where C is significantly greater than b, the increase in the surface concentration will become very small and Equation 4 will eventually become $\Gamma = \Gamma_{sat}$. This behaviour is shown more clearly by Figure 6.

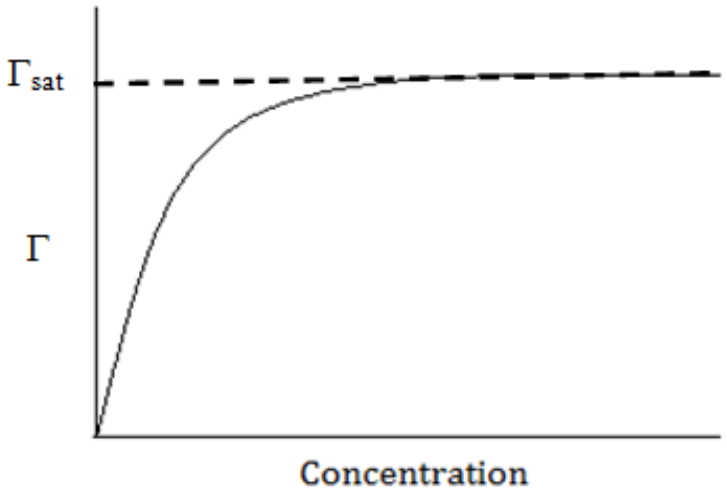


Figure 6: Effect of Bulk liquid concentration on Surface surfactant foam concentration

2.2.2 Foam Stability

Foam stability is affected by mechanisms like drainage, coalescence and disproportionation. Liquid drainage within the foam occurs due to and gravity and capillary suction caused by surface tension gradients. Drainage of the plateau borders due to gravity lowers the pressure within the borders and creates a pressure gradient between the lamella and plateau borders. The pressure gradient causes the suction of the lamella into the plateau borders (Ettelaie, 2003).

Drainage is governed by the Laplace-Young law shown in Equation (5); where γ is the surface tension and r is the local radius of the curved surface. A uniform lamellae thickness is maintained due to surface tension gradients and disjoining pressure.

$$\Delta p = \frac{4\gamma}{r} \qquad (5)$$

Surfactants lower the surface tension at the liquid-gas interface due to high surface activity, which allows for stable foam formation. The Gibbs-Marangoni effect which is governed by the Laplace equation ensures the presence of uniform lamellae thickness using interfacial surface tension gradients within the liquid films (Weaire, 1999). Surface tension gradients at the lamellae surfaces are created as a result of film drainage which causes a local change in surfactant concentration. Uniform thickness is restored through movement of liquid high surface tension regions as shown in Figure 7. Low liquid film drainage and the presence of disjoining pressure significantly reduce lamella thinning rate and rupture (Damodaran, 2005). Disjoining pressure occurs due to repulsive forces namely van der Waals and steric interactions between bubbles covered in surfactant molecules in the foam. These oppositely charged forces stop adjacent bubbles from approaching each other hence maintain a uniform lamellae thickness.

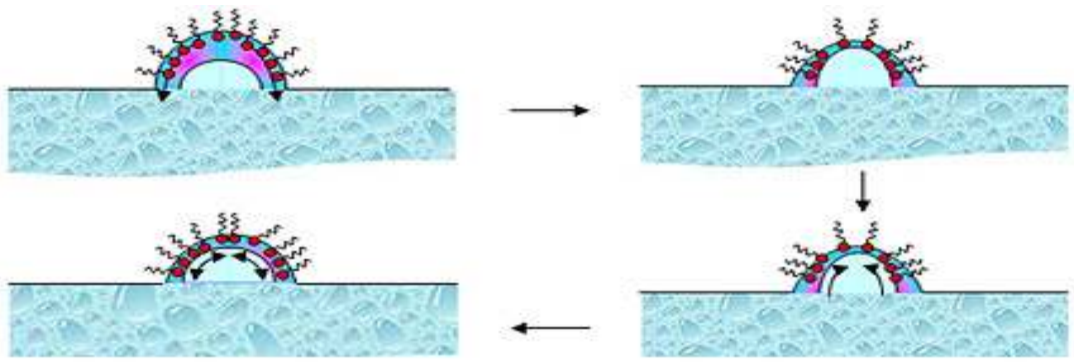


Figure 7: Marangoni Effect (BASF/CIBA, 2000)

2.2.3 Column Operation

Design of the column requires an in-depth look at the factors that will influence performance of the process. The factors that affect the performance of a protein skimmer are mode of operation for the column, operating conditions and the column design.

2.2.3.1 Modes of Column Operation

Column operation has been widely investigated in the literature. The column can be operated in two modes, simple mode or stripping/enrichment mode. Simple mode operation is simple batchwise or simple continuous operation. Batchwise foam fractionation is the simplest mode of operation whereby the all the feed is added prior to running the foaming process. The surfactant concentration and volume of the liquid pool varies with time as the foaming proceeds. Neely (2001) developed a mathematical model for simple batchwise foam fractionation based on an analogy with distillation proposed by Lemlich (1972). However, the model is considered to be highly empirical due to the methods used to calculate the rates of rising and falling films (Neely, 2001).

Continuous foam fractionation involves having a continuous feed supply to the column throughout the foaming process. The feed is supplied at the same rate as it's removed by a pump to maintain a constant concentration and volume of the feed solution. The operation of a continuous column can be modified to operate in enrichment or stripping mode.

Maruyama *et al*. (2006) investigated the single stage continuous enrichment of foam in the axial direction at different heights. The model developed gave consistent results between experimental and theoretical calculated values. However, the model can only be confidently applied if the same conditions and surfactant used are replicated.

Further studies were conducted by Stevenson *et al* (2007) who investigated continuous foam fractionation with reflux and developed a model for predicting the flow rates in rising foam. This model can be used to determine performance of simple batch and continuous operation of foam fractionation. Although an enrichment system was studied, the model can be applied to both stripping and combined operation modes simply by altering the appropriate mass balances (Stevenson *et al.,* 2007).

Multistage fractionation has also been widely investigated as continuous foaming process. Multistage foam fractionation is used to achieve enrichment in more than a single stage. Boonyasuwat *et al* (2005) demonstrated that multistage enrichment achieved higher enrichment than single stage fractionation in terms of the enrichment ratio and also exhibited a short residence time for surfactant removal.

Their previous study showed that recovery was greater with a cationic surfactant CPC than with an anionic surfactant sodium dodecylsulfate (SDS).

Darton *et al*. (2004) also investigated multistage foam fractionation and were able to model and develop apparatus that was used to successfully separate surfactants from water. Experiments were conducted with different commercially available manufactured surfactants Triton X-100 a non ionic surfactant and CPC surfactant. They found that surfactants could be used to remove non surface active molecules from solutions if an affinity between the surfactant and the solute was present.

2.2.3.2 Theoretical Column Design

Foam fractionation is analogous to distillation therefore the foam column can be defined using operating lines and number of transfer units. The vapour in distillation columns corresponds to the bubbles rising in the foam column. Entrainment in distillation corresponds to the interstitial liquid carried up by the moving bubbles which is denoted by U. The downflow in distillation corresponds the liquid draining downwards through the foam due to gravity which is denoted by L.

According to Lemlich (1972) the number of transfer units for in the foam column can be determined using Equation (6).

$$\mathrm{NTU} = \int_{\widehat{C_w^*}}^{C_Q} \frac{d\hat{C}}{\widehat{C^*} - \hat{C}} \qquad (6)$$

Where $\hat{C}^*_w$ is the effective concentration of $\hat{C}$ in equilibrium with C_w, which is the bottoms concentration; C_Q is the concentration of the total overflowing foam on a collapsed gas free basis and $\hat{C}$ is the effective concentration of the surfactant in the rising stream at any level in the foam column.

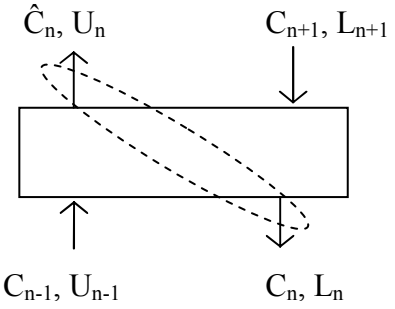


Figure 8: Material balance at an Equilibrium stage

The number of transfer units can also be determined graphically by carrying out stage wise calculations using a McCabe Thiele plot as shown in Figure 9.

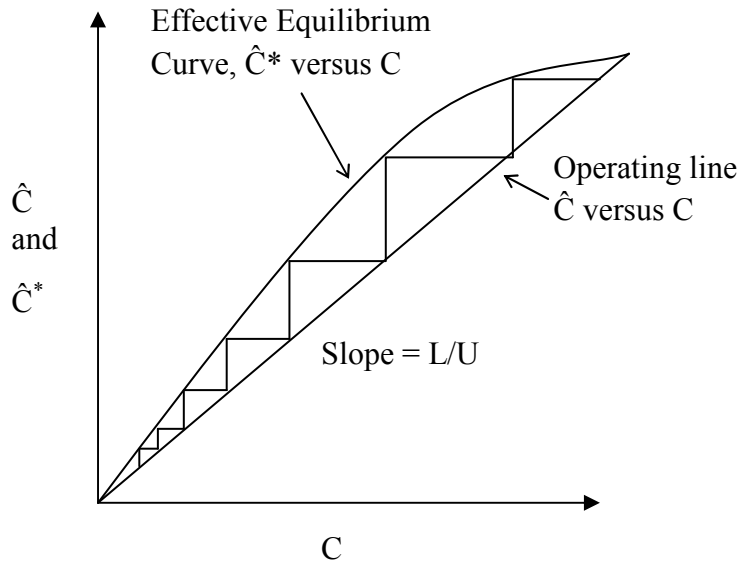


Figure 9: McCabe Thiele plot to determine number of theoretical stages needed in the foam column to achieve the desired enrichment. Adapted from Lemlich (1972)

The number of transfer units is a function of column height. Increasing the number of stages in the foam fractionation column increases the separation efficiency because of increased surface area for adsorption and extended residence time for gas-liquid contact. This causes the enrichment ratio and removal fraction to increase as shown by several studies (Boonyasuwat *et al*., 2005), (Goldberg and Eliezer, 1972) and (Du *et al*., 2000).

The system under investigation is a single stage system so the column is will be operated in simple mode. A mass balance over stage n is illustrated in Figure 8. The mass balance carried out on the foam fractionation column is used to determine the equation of the operating line.

From mass balance calculations, an equilibrium operating line was derived and is shown by Equation (7) (Lemlich, 1972).

$$\hat{C} = C + \frac{GS\Gamma}{U} \qquad (7)$$

Where; $\hat{C}$ is the effective concentration of the surfactant in the rising stream at any level in the foam column, C is the concentration of rising interstitial liquid, G is the volumetric gas flow rate, S is the bubble surface area to volume ratio, Γ is the surface excess and U is the interstitial liquid upflow.

2.2.3.3 Operating Conditions

The optimum conditions for the operation of foam fractionation columns have been widely investigated and several studies have highlighted trends obtained when different parameters are varied. The theory behind these trends is discussed in the subsequent section. Continuous foam fractionation systems were the main focus due to their relevance to the current investigation. Optimum conditions were determined using the enrichment ratio and removal fraction as performance indicators which are defined Equations (21) and (22) respectively.

Figure 10 shows the different variables that can be varied to obtain optimum conditions within a continuous foam fractionation set up and was used as a point of reference for all operating conditions discussed in this section.

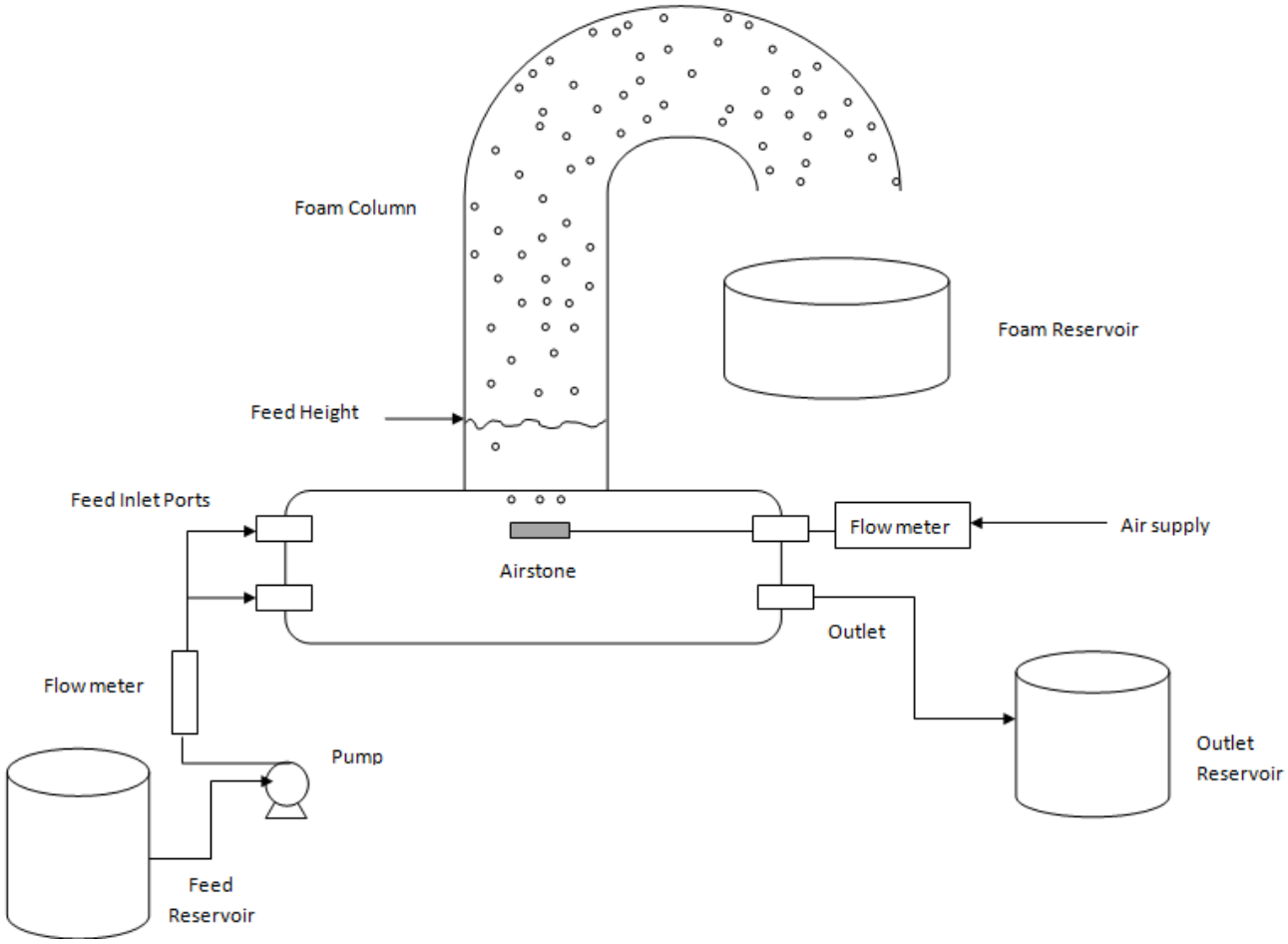


Figure 10: Schematic of a continuous Foam Fractionation set up

Effect of gas flow rate and bubble size

Gas is usually supplied through a sparger or airstone to produce bubbles in the liquid phase. As superficial gas velocity increases, the foam production rate and liquid entrainment increases but surfactant concentration in the foam decreases. Movement of air bubbles through the liquid at a faster rate reduces the residence time for surfactant adsorption to the bubble surface. Less drainage occurs at high gas flow rates due to limited foam residence time within the column resulting in the production of wetter less concentrated foam. Both these factors contribute to a lower enrichment ratio and surfactant removal fraction as revealed by the studies conducted by Ahmad (1975) and Uraizee and Narsimhan (1996).

Although lower gas flow rates produce dry and very surfactant enriched foams with a small liquid hold up, coalescence and coarsening become more significant at these conditions. Coalescence occurs due to differences in Laplace pressure, which lowers the bubble radius causing increased bubble pressure. Diffusion of gas between adjacent bubbles occurs from small to larger bubbles. The smaller bubbles shrink and disappear and larger ones may burst due to overwhelming pressure within the gas bubble.

Foam height is closely related to the liquid hold up within the foaming column. Increasing the foam height causes the liquid holdup in the foam to decrease and significantly increases the enrichment ratio and removal fraction. Increased residence time for bubbles in the foam column also allows for better drainage giving an enriched dry foam (Ahmad, 1975).
The bubble size generated is also affected by the gas flow rate, devices used for introduction of gas into the system and the feed concentration.
Gas flow rate affects the bubble size as a result of 'formation pressure' which causes the bubbles to vary slightly in size especially at high gas flow rates.
The mean bubble diameter (d_m) is a good estimation of the bubble size (d_i) and is calculated based on Equation (8); which was proposed by Winterson (1994).

$$d_m = \frac{\sum_{i=1}^{n} d_i^3}{\sum_{i=1}^{n} d_i^2} \qquad (8)$$

Using Equation (9), the bubble diameter (d_b) for any given system can be estimated from the column diameter (D) (Winterson, 1994).

$$d_b = 0.04D \quad (9)$$

The appropriate diffuser for gas delivery can be chosen based on this bubble diameter estimated using Equation (9). In aquaculture, air pumps and venturi devices are used for air introduction into foaming columns. A sparger or an airstone is attached to the air pump to aid formation of uniform bubbles. Air stones are porous stones which act as diffusers when air is pumped through then and produce coarse, medium or fine bubbles. The effect of the air stones on bubble size will decrease with an increase in gas flow rate until the threshold where the bubble size becomes independent if the gas flowrate (Chen *et al*., 1992)**.**

Bubble size decreases with increasing feed concentration. Surfactant material adsorbed onto the bubble surface lowers the surface tension at the gas-liquid interface. The surfactant molecules attached to the sparger create a surface tension gradient at the liquid-solid interface and causes premature detachment of bubbles from the air holes and small bubbles are formed as a result. The bubbles detach earlier to move towards high surface tension regions.

For foam fractionation, a large number of smaller bubbles are preferred as they create a large available surface area for surfactant adsorption. Small bubbles also rise more slowly which increases the residence time of the bubbles within the liquid reservoir. The bubble surfaces become saturated with surfactant material which increases the enrichment ratio and the removal fraction (Chen *et al*., 1992).

Larger bubble sizes have two opposing effects on the enrichment. Larger bubbles provide a smaller surface are for adsorption which means the enrichment would be lower compared to smaller bubble surface area within the same total volume.

However because larger bubbles have a faster draining rate and less liquid hold up hence enrichment of the surfactant increases. Uraizee and Narsimhan (1996) investigated these two factors and found that enrichment increased with increased bubble size within the bubble range tested (0.762, 1.006, 1.524 μ m).

Effect of Feed Condition

Increasing the feed concentration has a negative effect on the enrichment ratio. Boonyasuwat *et al*. (2005) investigated the effect of feed concentration on enrichment using the surfactant CPC. Increasing the feed concentration resulted in lower enrichment. High surfactant concentrations increase foam production rates and foam stability due to reduced drainage of the liquid films leading to production of wetter foam. Therefore as foam production increases, the enrichment ratio decreases. This can also be explained using the linear Langmuir isotherm expressed in Equation (3). Enrichment is given by the ratio Γ/C so high feed concentration correspond to low enrichment and low feed concentrations correspond to higher enrichment ratios (Lemlich, 1972). Similar trends were observed when the foam separation of proteins was investigated (Uraizee and Narsimhan, 1996).

Increasing the feed flow rate increases the enrichment achieved. Boonyasuwat *et al*. (2005) investigated the effect of feed flow rate on enrichment using CPC. Their work shows that enrichment increases with increasing the feed rate below the critical micelle concentration (CMC). This effect was explained by the presence of turbulence within the solution which creates eddies swirling upwards within the feed. This swirling disrupts the foam and causes coalescence and rupture of the liquid films between bubbles. As a result, larger bubbles are created and more liquid drainage within the foam occurs so enrichment is higher as the foam is drier. The effect of increasing the feed flow rate is limited by flooding limits. At or above the CMC, there was little effect observed on the enrichment.

The degree of enrichment can be altered by changing the feed location which varies with the chosen mode of operation as mentioned in Section 2.2.3.1.
When considering a stripping mode fractionator, the feed is introduced at the top of the column to achieve a counter current flow within the column between the liquid entrained by the rising foam and the down coming liquid feed. This is a desired effect because the surfactant present in the feed will be adsorbed onto the rising bubbles to give a more concentrated foamate. For enrichment fractionators, the feed is introduced within the liquid pool and if a combination of the two is chosen, then the feed is introduced into the middle of the column (Lemlich, 1972).

Alternatively enrichment of foam can be increased with increased feed height above the sparger. Bubble residence time within the liquid is increased so more surfactant can absorb to the bubble surface and enrichment increases until the surface becomes saturated. Above this limit micelles begin to form in the liquid pool and the saturated surface acts as a limiting factor. At very low liquid pool heights, large bubbles are formed and coalescence dominates because the draining rate is very fast. This results in the enrichment being highest at very low liquid heights. Experiments conducted by Uraizee and Narsimhan (1996) showed that the highest protein enrichment was observed at the lowest liquid pool height investigated.

The pH of the feed solution affects the foam stability and enrichment of proteins solutions. Enrichment is expected to be highest at the isoelectric point because of these factors. The isoelectric point of a protein is the pH at which maximum surface adsorption occurs and minimum solubility of the protein is also observed. This is due to reduced electrostatic interactions between protein molecules which cause the surface tension of the solution to increase. Several studies conducted at the isoelectric point using BSA showed maximum enrichment (Hossain and Fenton, 1998) and (Schnepf and Gaden, 1959).

Conflicting trends have been observed regarding the effect of pH on enrichment in foaming experiments. Uraizee and Narsimhan (1996) investigated the effect of pH on foaming systems and found that increasing the pH increased BSA protein enrichment. The pH range investigated was between 3 and 7 and the lowest enrichment was observed at the isoelectric point of pH 4.8. The enrichment was higher at pH 7 than at pH 4.8 because of a change in bubble sizes observed in the column. Larger bubbles were observed at pH 7 and smaller bubbles at pH 4.8. The effect of bubble size on enrichment has been discussed above. A similar trend was observed in another study (Brown *et al*., 1990).

2.3 Concentration Measurement Techniques

The concentration of a solution can be determined using absorption spectroscopy. Lambert discovered the proportional relationship between concentration and absorbance and proposed Equation (10) which is based on experimental results. Known concentrations were tested to find their corresponding absorbance and the results were used to construct a calibration plot. This linear relationship between absorbance and concentration is only valid at low concentrations.

The curve is non linear at high concentrations due to there is high electrostatic interaction of molecules with each other as hydrogen bonding and Van der Waals forces. This causes the structure of the molecules to change leading to non linear behaviour. Another reason may be because the sample itself scatters the incident radiation resulting in only a small amount of light being absorbed so the true reading of the absorbance is not obtained (Ingle, 1988).

$$A = e \cdot b \cdot c \qquad (10)$$

Where; e is the molar absorptivity (L mol^{-1} cm^{-1}), b is path length of the cuvette in which the sample is contained (cm) and **c** is the concentration of the compound in solution (mol L^{-1}).

Protein Concentration

Different methods are available for protein concentration measurement. A comparison of the most commonly used methods; Lowry, BCA and Bradford in Appendix C showed that the Bradford method is the most appropriate method for this study because of the protein concentrations investigated in the study.

The Bradford method of protein measurement involves the use of Coomassie Brilliant Blue G-250 dye to test the protein concentration. The dye exists in two colours, red and blue. When the dye is added to a solution, it binds to proteins and forms a protein-dye complex that has a high extinction coefficient which means it is forms relatively quickly (approximately 2mins) but remains dispersed for a long time (approximately 1 hour). The colour change of the dye from blue to red is an indication that the protein dye complex has been formed (Kruger, 2002). The functional range for the Bradford assay is 100 – 1,500mg/L (Pierce-Biotechnology). This approach is favourable because the absorbance approach can be used and contamination is eliminated as only the dye will absorb light at the chosen wavelength.

3.0 Foam Fractionation Applications

3.1 General Applications

Foam fractionation is widely used in the pharmaceutical industry and food industry. Studies have shown that foam fractionation has been used for enrichment of bio-products (Lockwood *et al*., 1997), purification of enzymes with no loss of catalytic enzyme activity (Linke *et al*., 2007), isolation and purification of protease from human placenta (Sarkar *et al*., 1987), enrichment of Bovine Serum Albium (BSA) (Ahmad, 1975) and is also used for the removal of surface active contaminants from industrial waste and municipal sewage.

3.2 Aquaculture Applications

Aquaculture is one of the leading industries that use foam fractionation for removal of organic wastes. Waste removal can carried both in recirculation aquaculture systems on a large scale and pond management on a smaller scale. Small scale foam fractionation was investigated for this study based on a freshwater fish tank.

A fish tank is a closed entity filled with water in which aquatic organisms and plants are kept. Aquatic organisms thrive in a variety of environments classified according to salinity of water (Alderton, 2005) and temperature. Foam fractionation can be applied to both saltwater and freshwater aquaria. The temperature classification is 3-15^{0}C for cold water species, 15 - 20^{0}C for cool water species and above 20^{0}C for warm water species (Timmons, 2002).

Waste Management

Waste produced by aquatic organisms in fish tanks originates from faeces, uneaten food and decomposition of dead organisms. This waste and has to be removed before it breaks down into more harmful products like ammonia and nitrite which are produced by protein metabolism (Timmons, 2002). Accumulation of these by-products alters the environment in which the fish thrive and can be catastrophic. Timmons (2002) proposed the safe concentration limits for a range of components found in fish culture water needed to maintain good water quality.

Table 1: Table showing Water Quality Criteria for Aquaculture (Timmons, 2002)

Parameter	**Concentration (mg/L)**
Alkalinity	50-300
Aluminium	<0.01
Ammonia	<0.0125 (salmonids)
Ammonia (TAN) cool-water fish	<1.0
Ammonia (TAN) warm-water fish	<3.0
Arsenic	<0.05
Barium	<5
Cadmium Alkalinity < 1000 mg/L Alkalinity > 1000 mg/L	 <0.0005 <0.005
Calcium	4-160
Carbon Dioxide Tolerant species (tilapia) Sensitive species (salmonids)	 <60 <20
Chlorine	<0.003
Copper Alkalinity < 1000 mg/L Alkalinity > 1000 mg/L	 <0.006 <0.03
Total hardness ($CaCO_3$)	>100
Hydrogen cyanide	<0.005
Hydrogen sulphide	<0.002
Iron	<0.15
Lead	<0.02
Magnesium	<15
Manganese	<0.01
Mercury	<0.02
Nitrogen	<110% total gas pressure <103%as nitrogen gas
Nitrite	<1,0.1 in soft water
Nitrate	0-400 or higher
Nickel	<0.1
Dissolved Oxygen (DO)	>5 >90mm Hg partial pressure
Ozone	<0.005
PCB's	<0.002
pH	6.5-8.5
Phosphorous	0.01-3.0
Potassium	<5
Salinity	Depends on salt or fresh species
Selenium	<0.01
Silver	<0.003
Sodium	<75
Sulfate	<50
Total Gas Pressure (TGP)	<105% (species dependant)
Sulfur	<1
Total Dissolved Solids (TDS)	<400 (site specific and species specific; use as rough guideline)
Total Suspended Solids (TSS)	<80
Uranium	<0.1
Vanadium	<0.1
Zinc	<0.005

Waste Removal Methods

Different removal methods and mechanisms can be exploited to separate suspended solids from fish culture water. Biological and mechanical techniques that are used include gravity separation, filtration, flotation and foam fractionation (Timmons, 2002).

Gravitation and filtration are the most practical and more commonly used methods based on the principle of sedimentation and settling velocities. The matter accumulates at the bottom of the tank and filtration is used to remove it using suitable filters. Granular and porous media like rapid sand filters, pressure sand filters, floating bead filters, screen filters and porous filters can also be used. These methods are restricted to the removal of larger particles and are not effective when applied for removal of small particles with a diameter of less than 30µm. The minute particles have very low settling velocities and cannot be removed using sedimentation techniques. Protein skimmers can remove both surface active molecules and minute particulates of less than 30µm from very dilute solutions like culture water (Timmons, 2002).

3.2.1 Protein skimmers

A protein skimmer is a filtering device that is used to remove organic compounds from fish culture water and operates based on the principles of foam fractionation as discussed in Section 2.2.

High enrichment efficiency from dilute solutions of dissolved organics and fine particles can be obtained using protein skimmers. Chen *et al* (1993) found that 90% of total particle mass in recirculating aquaculture systems is less than 30µm. A similar assumption can be made for the fish tanks. The ability of protein skimmers to remove small particles as well as dissolved organics makes their use an attractive method for waste removal.

Different types of protein skimmers all operate based on the same primary principle. The differences arise from the type of diffuser used; airstones, sintered glass or venturi devices. The flow within the skimmer may also differ depending on whether counter-current or co-current flow of air and water in the system is selected. Commercially available protein skimmers like the Aqua Remora Protein Skimmer which comes with a Rio 800 Pump and Deltec AP702 Protein Skimmer are all relatively expensive.

Several studies have been undertaken investigating foam fractionation with a focus on aquaculture, especially in recirculating aquaculture systems. One of the more detailed studies was carried out by Chen *et al*. (1993) who investigated fish culture water using a foam fractionation column. The study involved the analysis of water from three different recirculating systems with different protein types and levels depending on the type of fish kept in the tank.

Experiments were conducted in batch mode using a short column and the in continuous mode using a longer column. The longer column was used to establish equilibrium so that the saturation constant (K) in Equation (3) could be determined from the equilibrium data. The Langmuir Isotherm shown in Equation (3) is a linear relationship between the surface concentration and the bulk liquid concentration and the saturation constant is given by the gradient of the data. The surface concentration was found experimentally and plotted against a range of bulk liquid concentration. A gradient of 8.7×10^{-5} was established as the saturation constant.

Protein removal was investigated by monitoring concentration change during the foam fractionation. Results showed a limited protein removal rate of 11% of proteins detected in the fish culture water. This value is quite low and may be due to several reasons.

Chen *et al* (1993) suggest that some of the proteins detected were not surface active so they could not be removed. According to Lemlich (1972), this can occur when proteins are exposed to an air-water interface which causes the hydrophobic and hydrophilic bonding within the protein molecule to become stressed. This stress may be significant enough to alter the structure of the protein causing it to become denatured and lose surface activity.

The pH can also change the surface activity of the protein leading to loss of surface activity as we move further away from the isoelectric point. The pH was varied within a pH range of 5.3 -8.6 and only a small effect on enrichment was observed.

Another limitation to the work of Chen *et al* (1993) experienced was the short duration of the experiment. The experiment was stopped after 30 minutes because the system stopped foaming. The concentration in the fish dropped below the CMC (100mg/L) of the fish culture water. This shows that foam fractionation is only effective when applied to surfactant concentrations above the CMC otherwise low separation is achieved and water quality is not effectively improved.

4.0 Protein Skimmer Design

4.1 Equipment Design

The following criteria were used to design the protein skimmer used in this study.

- Easily integrated into the current fish tank design.
- Incorporate a foam breaking mechanism.
- Incorporate a air supply system
- Changeable so both continuous and batch experiments could be conducted.
- Allow for collection and sampling of foam, overflow and the bulk liquid feed inside the column.

The fish tank design shown in Figure 11 is constructed from commercially available sections of QVF glassware. The different glass components are fitted together with stainless steel couplings and flanges. The planned modification to the current design was the replacement of the pipe section highlighted in Figure 11, with the foaming apparatus designed in this study. The design of the protein skimmer was developed such that it could be fitted to existing tank.

It was decided that the protein skimmer components would be made of QVF glassware for three main reasons. Glass was chosen to match the aesthetics of the current installation. Surfactant tends to stick to the surfaces especially plastic so glass was chosen as the chosen material of construction to minimise error in mass balance measurements. The use of glass also allows the user to observe the foaming process as it occurs and carry out bubble size and bubble shape measurements as well as other relevant observations.

Measurements carried out prior to design development showed that the maximum allowable height and length of the apparatus were 380mm and 200mm respectively. This height is the maximum distance above the pipe section within which the apparatus can fit and the specified length is the exact length of the pipe section to be replaced.

4.1.1 Protein Skimmer Design Calculations

4.1.1.1 Foaming Potential of fish tank water

The column dimensions were estimated from the desired waste removal rates. The maximum concentrations of organic waste which is mainly protein to be removed from the 50 litre freshwater fish tank were estimated using the feed mass.

The total mass of feed per day was weighed at 0.2273g ± 0.001g. Only 48% of the feed is protein. Protein mass was calculated using Equation (11) and then used to calculate the protein concentration using Equation (12). The fish tank has a volume of 50 litres. Protein concentration in the tank was calculated using the equation below.

$$Protein\ Mass\ (g) = 0.48\ \times Feed\ Mass\ (g) \quad \textbf{(11)}$$

$$Protein\ Concnetration\ \left(\frac{g}{L}\right) = \frac{Mass\ of\ protein(g)}{Water\ Volume\ in\ tank(L)} \quad \textbf{(12)}$$

Several assumptions based on literature were made to aid the estimation of waste production in the fish tank. It was assumed that 25% of the feed is generated as suspended solids (Timmons, 2002) and 3% of the suspended solids were fine solids with a diameter < 30µm (Timmons, 2002). The values shown in Table 2 were calculated based on the assumptions listed.

A total of 30 fish was counted in the tank, 17 were tiger barbs and 13 were neon tetras. The average weight of each fish type was determined from literature. Estimations of liquid waste are shown in Appendix B were used together with the solid waste estimates shown in Table 2. These values were used to determine the optimum gas velocity, column diameter and bubble size needed to remove excess organic matter. Table 3 shows the limits proposed by Timmons (2002) which were used as a benchmark for the calculations.

Table 2: Estimations of Solid Waste generated in tank

Number of fish in tank: 30 Weight of fish : 0.26g per fish Fish Tank Volume : 50 Litres		
	Mass (mg/day)	Concentration (mg/L)
Feed (48% protein)	227.3	2.18208
Total Volatile solids (TVS)	2727.6	54.552
Protein to be removed	109.104	2.18208
Total Suspended Solids generated (TSS)	56.825	1.1365
Fine solids generated daily (FS)	1. 0475	0.034095
TVS concentration fraction of limits (%)	13.638	

Table 3: Concentration limits Timmons (2002)

Solids Type	Concentration (mg/L)
TVS	400
TSS	80
FS	2.4

Total Volatile solids (TVS) account for uneaten food and faeces and other organic waste. The TVS concentration is the basis of the protein skimmer design as it determines when the system will foam and is also used to estimate protein skimmer size, the air flow rate used and bubble size used for foam fractionation.

The TVS concentration was estimated from the protein concentration calculated using Equation (12). The relation developed by Chen *et al* (1993) states that 4% of total volatile solids (TVS) in fish culture water are protein concentration. Therefore the TVS concentration was found by dividing the protein concentration by 4%.

Studies by Chen et al (1993) showed that the CMC of fish culture water was 100 mg/L and the system stopped foaming below this concentration. It was estimated that the concentration of volatile solids in the tank was 54mg/L after one day of feeding as shown in Figure 2. Therefore for completely new and clean tank water, it would take two days of feeding for the water to start foaming.

4.1.1.2 Gas Flow rate and column diameter estimation

The gas flow rate was estimated based on maximum protein removal from the fish tank. An assumption was made that all protein in the feed would be removed by the protein skimmer. The results of the calculations carried out are shown in Table 5.

A gas flow rate of 133 ml min^{-1} was estimated for maximum removal of protein. A bubble diameter of 2mm was estimated and used to calculate the total bubble surface area and volume using Equations (18) and (15) respectively. The protein skimmer diameter of 25 mm which was used to calculate the pipe dimensions. The surface excess was estimated using the correlation Γ = 8.7 x 10-5 C (Chen *et al*., 1993). The gas flow rate was found through iteration to determine the gas flow rate need to remove all protein present in the feed. The equations used for all calculations are shown in Table 4.

Table 4: Equations used to estimate gas flow rate.

Parameter	Equation	Units	Equation
Surface Area ($A_{S.A}$)	$A_{S.A} = \pi d^2$	m^2	(13)
Cross sectional Area (A_C)	$A_C = \pi d^2$	m^2	(14)
Volume	$V_{bubble} = \frac{\pi d^3}{6}$ $V_{pipe} = A_{C,pipe} * Height_{pipe}$	m^3	(15)
Superficial gas Velocity (U_g)	$U_g = \frac{Q_{air}}{A_{C,pipe}}$	m $minute^{-1}$	(16)
Bubbles per minute (BPM)	$BPM = \frac{Q_{air}}{V_{bubble}}$	Bubbles $minute^{-1}$	(17)
Total bubble surface area (TBSA)	$A_{S.A,bubble} * BPM$	m^2	(18)
Residence time	$\frac{A_{C,pipe} * Height_{pipe}}{Q_{air}}$	minutes	(19)
Protein removed	$Protein = \Gamma * TBSA$	g	(20)

Table 5: Column diameter and gas flow rate estimation using protein removal rate.

Bubble Dimensions	
Diameter (m)	0.002
Bubble Surface Area (m^2)	1.25664E-05
Bubble Volume (m^3)	4.18879E-09
Pipe dimensions	
Diameter (m)	0.025
Height of column (m)	0.175
Surface Area (m2)	0.001963495
Cross sectional Area (m^2)	0.000490874
Volume (m^3)	8.59029E-05
Gas flowrate (m^3 min^{-1})	0.000133035
Gas flowrate (mL min^{-1})	133
Superficial Velocity (m min^{-1})	0.271017357
Bubbles per minute	31760
Total Bubble Volume (m^3) per min	0.000133035
Total bubble surface area (m^2) per min	0.4
Residence time (minutes)	1
Daily Feed (g)	0.2273
Protein in feed (g) - 48%	0.109104
Volume of Water (m^3)	0.05
Protein Concentration (g m^3) for a day	2.18208
Surface Excess (g m^{-2})	0.0001898
Protein mass removed (g) per min	7.57667E-05
Protein mass removed daily (g)	0.109104
Protein initially in feed removed (%)	100%

Current Fish Tank Installation

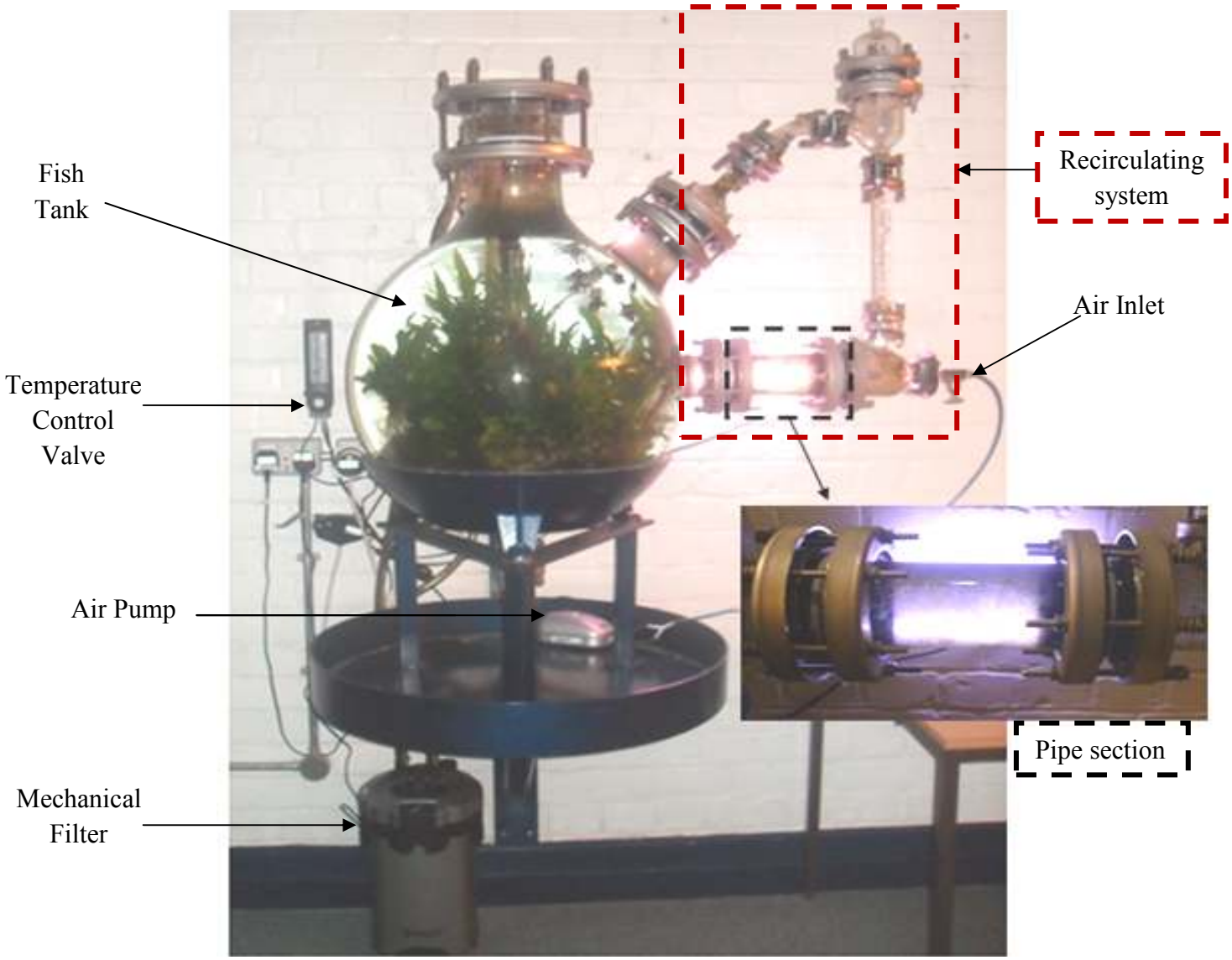


Figure 11: Current Fish Tank design highlighting pipe section to be replaced by the protein skimmer in this study.

4.1.2 Component Selection

A number of ideas based on the criteria, measurement limits specified and estimations for column dimensions were considered and are shown in Appendix A. The number of options was constrained by space limitations within the fish tank design as well as the limited components sizes available to order from the QVF catalogue (QVF-Process-Systems, 2005).

The few designs considered differed mainly according to the components used for the base of the skimmer which would replace the pipe section. The base also has an effect on positioning of the air inlet into the column whereby, air needs to be introduced into the column from the bottom so that the foam can rise to the top.

For the column base, an unequal tee shaped piece, a y-piece angled at a 45° and cross shaped piece shown in Figure 12 were considered as they fulfilled the length requirement for the design. The positioning of the air supply was evaluated for these three options. For the tee piece and the y- piece, the open end on the right side would be used as an air supply inlet and the bottom end of the crosspiece would be used if this option was chosen. However, due to the positioning of the airstone in the cross piece, foam would be forced back into the fish tank which is not a desirable effect. The y piece was not selected because the entire apparatus setup would have to be angled making this setup less stable.

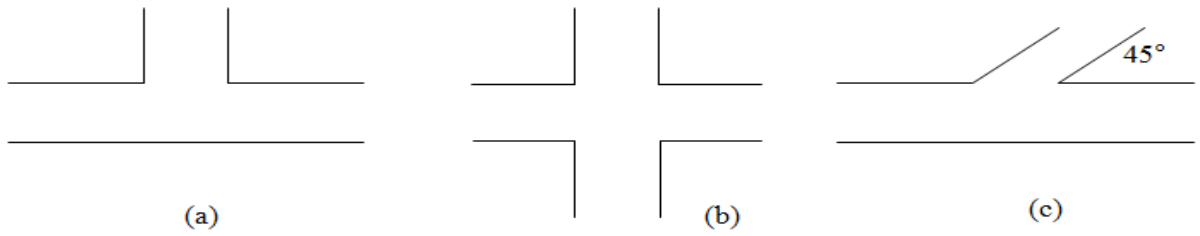


Figure 12: Protein skimmer base options

Therefore, the unequal tee piece was selected as the best option for the base. The tee piece option allows for the same air supply currently used to aerate the fish tank water to be used for the foaming process. Because the height of the tee piece was fairly small, a riser and an overflow component with reasonable heights were selected.

4.1.3 Final Design

The final design was chosen because of its simplicity and ease of assembly as compared to the other options. It consisted of an unequal tee piece; a pipe section and a U bend. The pipe section and the U-bend were chosen for the column to act as the riser and overflow pipe respectively. A curved pipe was chosen for the top to stop backflow of foam in the column when it reached the top especially at very low gas flow rates.
All three components were ordered from QVF Process Systems Ltd and were made of borosilicate glass. The final design is shown in Figure 13 and the dimensions of each component are shown in Appendix A. The total height of the final design was 315 mm, within the space limitations.

The fish tank under investigation was assembled by the University workshop. Therefore, assembly of the components was commissioned to the University workshop because of their experience and expertise in working with QVF glassware.

The setup in shown in Figure 13 had to be modified to become a closed unit so that experiments could be conducted. Two plates were attached to either side of the tee piece to seal it off. The plates were made of plastic with a 4mm thickness and 110 mm diameter. Two central holes were drilled 25mm apart on each plate so that the stainless steel hose barbs could be fitted. The two ports to the right were used as inlets for air and feed respectively and the top port on the left was used for overflow. The bottom left port was blocked so no liquid would flow out.

To ensure that the plates were tightly fitted, gaskets were placed between the plates and the glass. The gaskets had an outside diameter of 110 mm and an inside diameter of 70 mm. The inside diameter of the gasket was designed to be smaller than that the glass diameter to provide a better seal and avoid any leaks during experimental runs. A schematic of the protein skimmer configuration with the plate and gasket attachments are shown in Figure 14. The final design of the protein skimmer is shown in Figure 15.

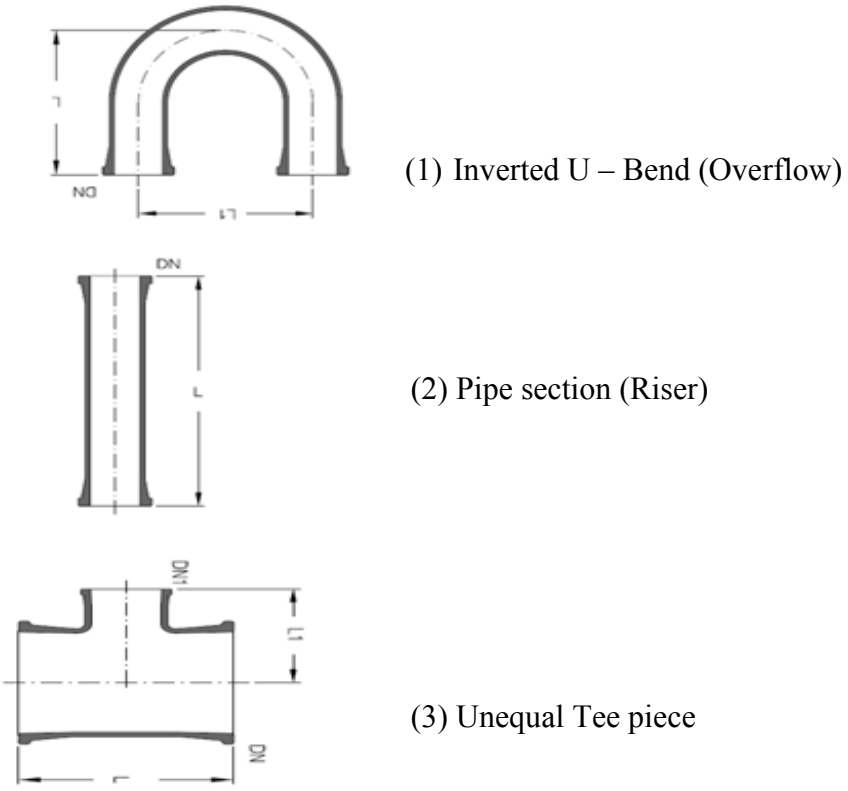


Figure 13: Column components

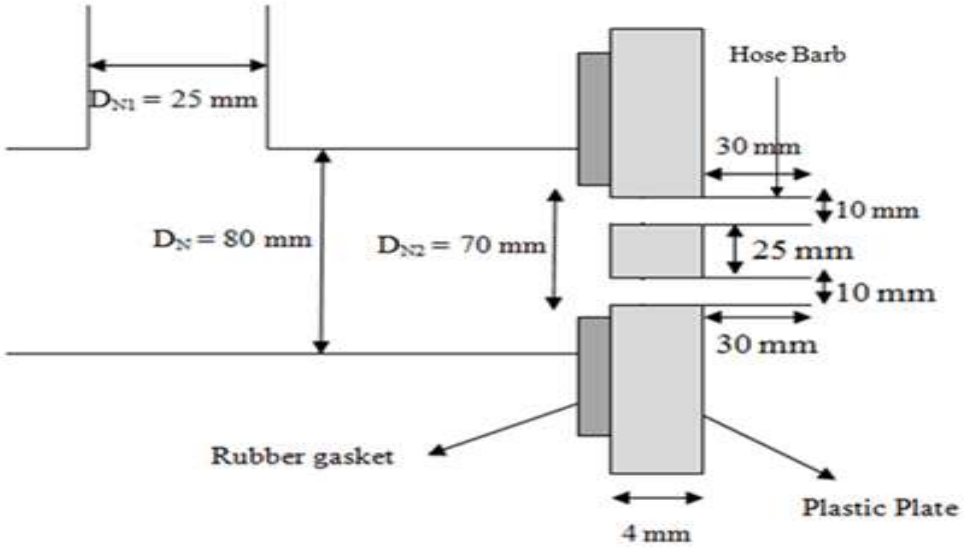


Figure 14: Schematic of cross section of gasket and plate arrangement on unequal t piece base. Where D_N and D_{N1} are the nominal diameters of the tee piece and D_{N2} is the gasket nominal diameter.

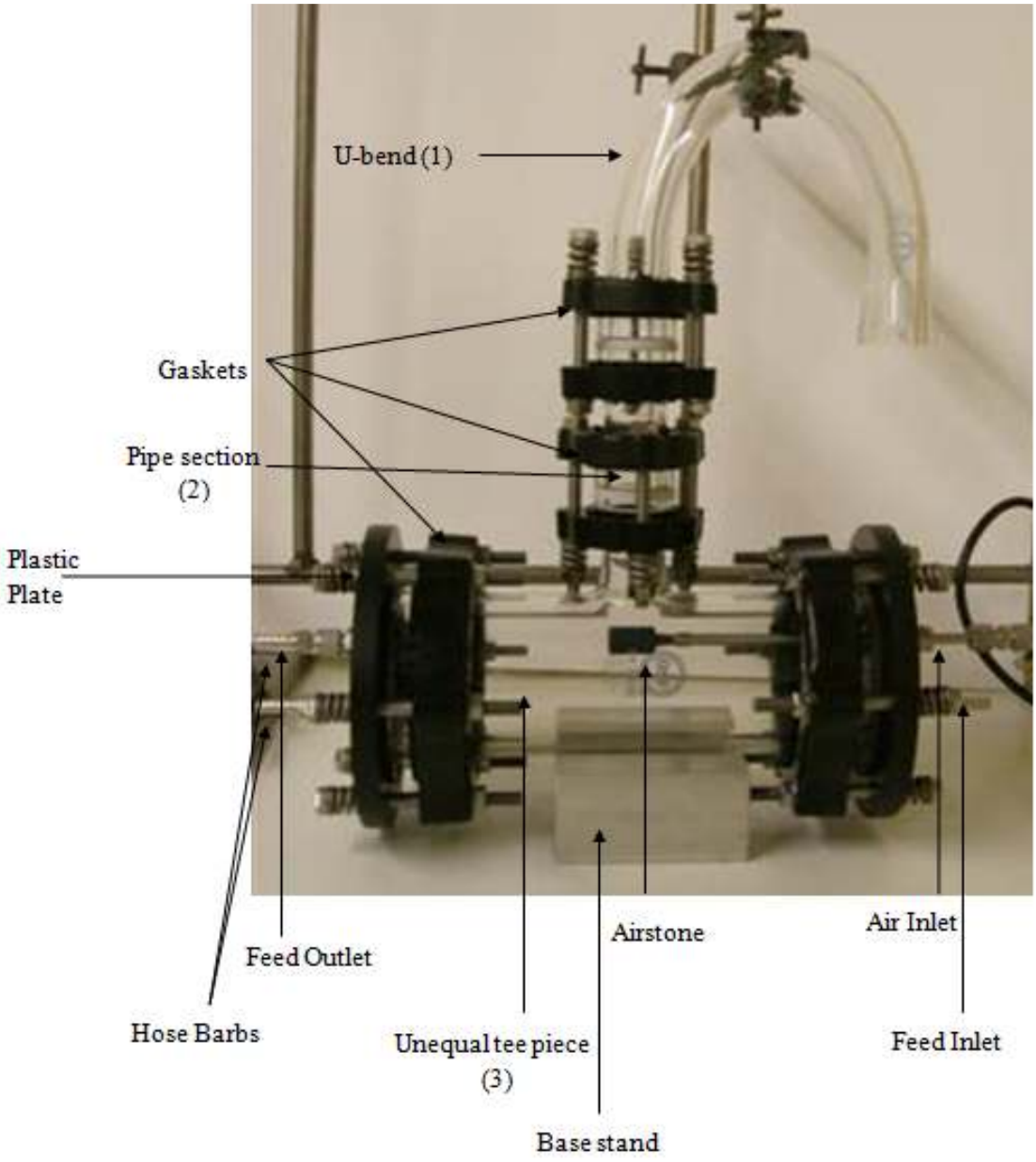


Figure 15: A photograph of the final Protein Skimmer design. (1), Represents the inverted U-bend, (2) Represents the Pipe section and (3) represents the inverted T-piece base.

4.2 Experiment Design

Materials

Preliminary experiments were conducted using a cationic surfactant, Cetylpyridinium chloride (CPC) of 96.0% purity purchased from Acros Organics. The preliminary experiments were conducted to determine optimum operating conditions.

A test protein was used to make the protein solutions instead of using fish culture water because the water present in the tank is already filtered. Bovine Serum Albium (BSA) protein was chosen for the study because it is relatively cheap, easily acquired, stable under foaming conditions and its concentration can easily be measured using a protein assay and a spectrophotometer at very low concentrations. BSA also has a low CMC of 25 mg/L at a pH of 5 because of its large structure with a molecular weight of 67000 g mol^{-1} (Hossain and Fenton, 1998). BSA in the form of lyophilized powder (≥ 98% electrophoresis) was purchased from Sigma-Aldrich Chemical Co.

The chemicals used to make the acidic buffer and adjust the pH of the solution were acetic acid glacial and sodium acetate both of an Analytical Reagent grade. The optimum pH is the isoelectric point as discussed in Section 2.2.3.3. The buffer was used to maintain the pH of the protein solutions at the isoelectric point which is pH 4.8 for BSA.
All the experiments were carried out using distilled water with a conductivity of 1μS. cm^{-1}as the diluent. Sample dilutions and solution preparation were all carried out using distilled water.

Operating conditions

The column design allowed for both batch and continuous experiments to be conducted. The aim was to mirror the conditions occurring in the fish tank hence the operating conditions were determined based on estimates of fish tank conditions within the fish tank in shown in Section 4.1.1. The effect of varying the different operating conditions is discussed in Section 2.2.3.3.
The fish tank set up in Figure 11 shows a continuous water recirculating system. The experiments were conducted with a continuous feed supply to the column at a flow rate 10.32 ml min^{-1}. This flow rate was chosen based on the fish tank recirculation rate.

The chosen feed rate increases the residence time of the bulk liquid in the column for protein adsorption and also minimised the liquid volume needed to conduct the experiments. The effect of varying the feed flow rate is discussed in Section 2.2.3.3.

The liquid height was maintained at a constant level of 8 cm above the airstone. This height was chosen based on the findings of Ahmad (1975) and Uraizee and Narsimhan (1996). Both studies found that using very low liquid pool heights increased the enrichment due to increased coalescence and drainage.
The results obtained by Ahmad (1975) showed that an enrichment of 1 was obtained at a liquid pool height of 10cm using BSA at 100ppm concentration, pH 4.9, air flowrate 333 ml min^{-1} and feed rate of 30 ml min^{-1}. Similar results can be expected with the chosen height for this study. It was preferable to have a higher foam height than feed height to minimise the amount of liquid hold up in the foam and the amount of water removed from the system. Higher foam height increases foam drainage time. Excessive removal of water from the fish tank is not desirable as a regular water changes would be required.

The operational pH of the protein solution was chosen at the isoelectric pH of 4.8 based on the discussions in Section 2.2.3.3.

The gas flow rate was estimated using the amount of protein that had to be removed from the fish tank and the bubble diameter as shown in Section 4.1.1.2. Although a gas flowrate of 133 ml min^{-1} was estimated, a lower gas flowrate of 130ml min^{-1} to ensure all protein is removed as lowering the gas flow rate increases protein removal.

The concentration range used for the experiments was determined using literature values for the typical fish culture concentration (Chen *et al*., 1993). The concentration limit for total dissolved solids (proteins) within the culture water is 400 mg/L (Timmons, 2002) as shown in Table 1. A study conducted using fish culture water showed that the CMC was 100 mg/L (Chen *et al*., 1993). The concentration investigated for this study was therefore varied between 100 mg/L and 500 mg/L to determine the effect of concentration on protein enrichment within the column. The maximum concentration investigated was 500 mg/L to observe the behaviour of the system above the limit.

4.2.1 Equipment Setup

The setup of the column as it was operated for the protein experiments is shown in Figure 16.

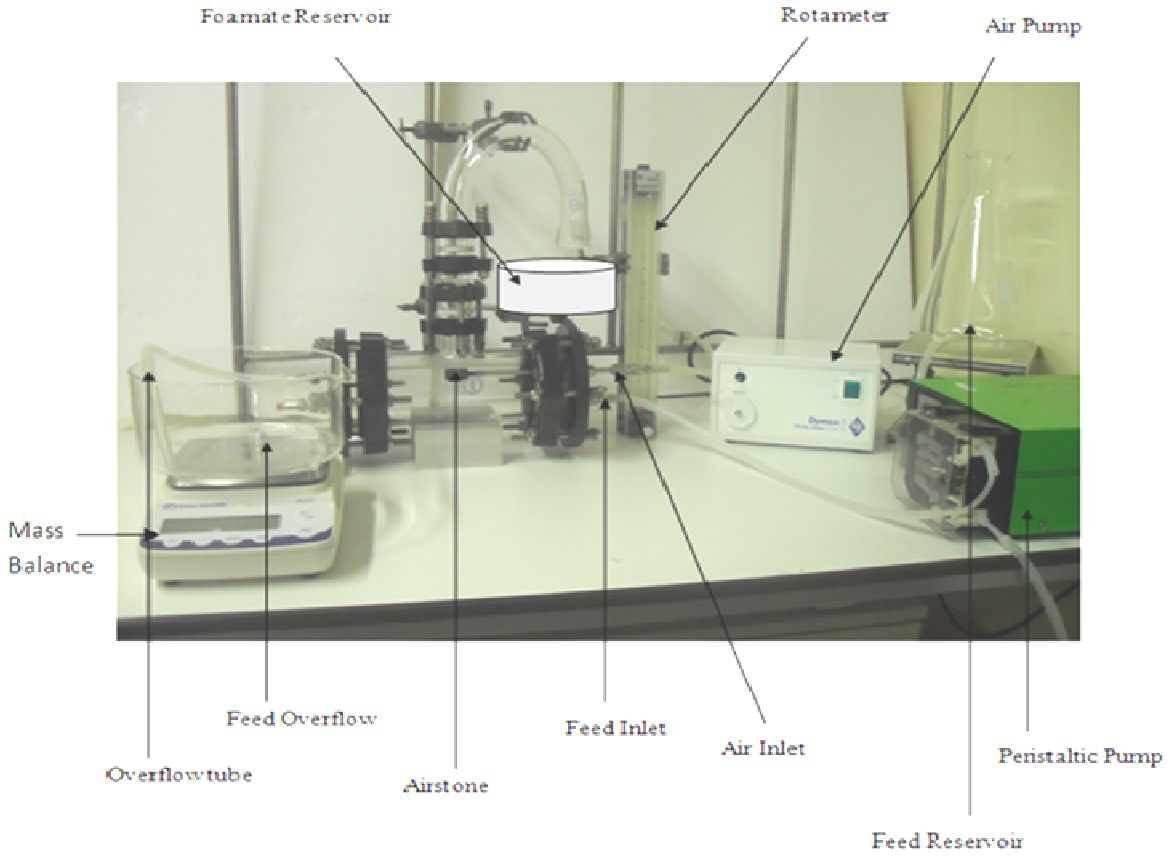


Figure 16: Apparatus for continuous foam fractionation set up.

4.2.2 Column Performance Criteria

The criteria used to assess the protein skimmer performance in this study are shown in Equations (21), (22) and (23. The criteria were chosen because they are commonly encountered in literature. All three factors were calculated for each time interval to determine steady state conditions.

The enrichment is a ratio of protein in the foam produced to the protein concentration in the bulk feed solution at any given time. This was the best measure of efficiency for the stripping process undertaken in this study.

$$Enrichment = \frac{C_f}{C_r} \quad \textbf{(21)}$$

The removal factor measures the efficiency of the column at removing surfactant from the liquid pool. High removal rates approaching unity are preferable for all experiments.

$$Removal\ factor = \frac{C_i - C_o}{C_i} \quad \textbf{(22)}$$

Finally the recovery was also calculated which is the ratio of the mass of surfactant in the foam to the mass of surfactant in the initial feed. High surfactant recovery is preferable as less surfactant is left in the bulk liquid pool.

$$Recovery\ factor = \frac{M_f}{M_i} \quad \textbf{(23)}$$

Where C represents protein concentration and M represents the mass of the protein. Subscripts f, r, i and o represent the foam, residual feed, initial feed and overflow respectively.

5.0 Methodology

5.1 Feed solution preparation

The mass of CPC needed for each concentration was calculated using the Equation (24) which relates the mass to the desired solution volume and mole concentration of the solution. For each experiment, a 1 litre volume of CPC solution was made up to a concentration of 1mM. The molecular weight of CPC is 358.01 g mol^{-1}.

$$\text{Mass} = \text{Volume} \times \text{Concentration} \times \text{Molecular Weight} \qquad (24)$$

The calculated mass of CPC powder was weighed using a mass balance with an accuracy of ± 0.001 g and dissolved in distilled water to the desired volume. A new solution was made up for each experiment conducted.

The protein solutions were prepared using the same procedure stated for CPC but the protein powder was dissolved in a buffer with a concentration of 1 mM solution instead of distilled water. The molecular weight of BSA is reported in literature as 67000 g mol^{-1} (Hossain and Fenton, 1998). However, because a mass concentration was used, the mass of BSA was simply the product of the mass concentration and the volume.

Acetic acid and sodium acetate buffer was chosen as it had a working pH range of 3 – 6. It was used to maintain the protein solution pH at 4.8 by minimising any changes in pH due in the presence or any acid of alkali.

5.2 Column Operation

Preliminary experiments were carried out using a cationic surfactant, CPC, to validate foam fractionation as a method of surfactant enrichment. The preliminary experiments were repeated at least twice to ensure reproducibility of results.

Two different modes of operation were investigated. The first set of experiments was conducted in batch mode followed by a set of experiments in batch mode with a reflux stream. The reflux experiments were operated under total reflux conditions with a stream flowing from the foamate reservoir back into the top of the inverted J shaped column as shown in Figure 17.

The operating conditions used for both modes of operations are shown in Table 6 unless otherwise stated. The power output for the foam breaker varied slightly across the experiments. The voltage was set at 12V initially but it tended to fluctuate during the experiment which is why the voltage, current and power outputs are given as a range.

Table 6: Experimental Operating Conditions of CPC Batch Experiments

Parameters	Range	
Mode of Operation	Batch – No Reflux	Batch - Reflux
Feed concentration (mM)	1.00	1.00
Gas Flowrate (L min^{-1})	1	1
Power Output to Foam Breaker (W)	0.624	0.567 – 0.866
Initial Liquid Pool Volume (L)	0.9417	0.800
Initial Top dish Volume (L)	0	0.150
Peristaltic pump set point for foamate (g)	N/A	120 – 140

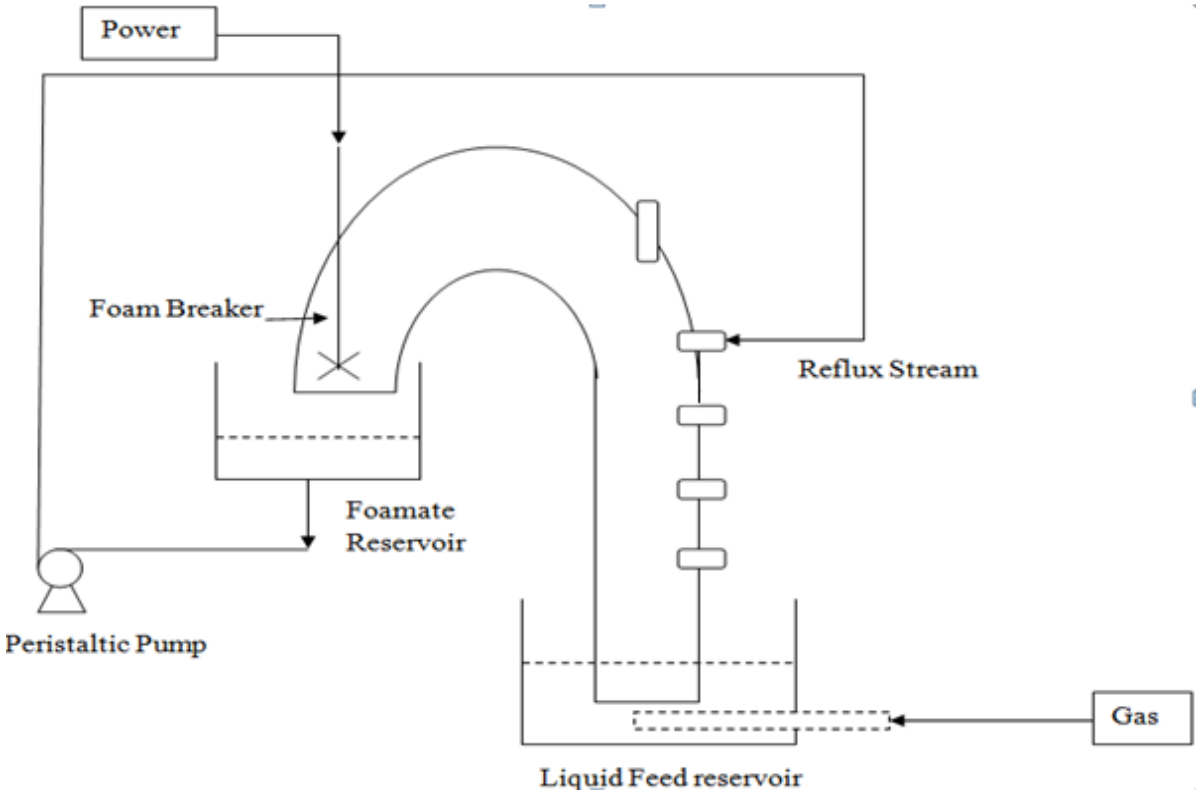


Figure 17: CPC experiment Setup

Protein experiments

The protein experiments were carried out in simple continuous mode and the protein concentrations in the feed were varied in the range of 0.1to 0.5 mg/ml in each experiment. The feed was continuously supplied using a peristaltic pump (Watson-Marlow 503U) at a flow rate of 10.32 ml min^{-1}.

The liquid level was maintained at 8 cm above the sparger by having an overflow stream. The overflow was controlled by positioning the overflow at the same height as the liquid level in the protein skimmer. Excess liquid above the selected liquid height would flow into the overflow reservoir. The flow rate of the overflow was estimated from the change in mass of the reservoir with time.

Compressed air at 0.8 bar pressure was supplied into the column by an air pump (Charles-Austen) and regulated using a valve. The air flow rate measured with a rotameter (GEC Elliot Press instruments Ltd) was kept constant at 130 ml min^{-1} for all experiments. An airstone (123-Aquatics) was attached to the air outlet to aid the formation of small uniform bubbles. A bubble diameter of 1mm was estimated was using the protein skimmer column diameter of 25 mm in Equation (9) (Winterson, 1994).

Sampling

Sampling was carried out at 15 minute intervals. Foam samples were collected in a sampling dish for 1 minute at each interval and left to collapse before the concentration and volume of the samples was measured. The empty mass of the foam sampling dish was known so the mass of the foam could be deduced. Samples were also taken from the foamate reservoir using a pipette.

The samples of the feed solution in the column were collected directly from the overflow tube whereby a small amount of liquid was collected in sampling dish.

The mass of all samples taken from the system was measured and recorded for the mass balance calculations.

5.3 Concentration Measurement

The concentration of both CPC and BSA protein was determined using a calibration plot of concentration against absorbance.

Absorbance measurements were carried out using a UVmini-1240 Spectrophotometer (Shimadzu) with a wavelength range of 190.0-1100.0nm. The spectrophotometer uses light of a given wavelength to measure the absorbance of the samples using single beam measurement. Absorbance was measured by placing the sample in a cuvette and into the spectrophotometer. The absorbance was measured at a wavelength accuracy of ± 1.0nm and the reading was given to a photometric accuracy of ± 0.003 ABS.

Only absorbencies within the linear range were used to calculate the concentration. Solutions with absorbencies outside the linear region were diluted with distilled water to get an absorbance within the linear range and the concentration was then calculated by multiplying the concentration calculated from the diluted sample by the dilution factor.

CPC Calibration

The peak absorbance of CPC lies at a wavelength of 259 nm so all measurements were carried out at this wavelength. A 1 litre volume of 1mM CPC solution was made up; samples were taken and diluted appropriately using distilled water to obtain nine samples with concentrations in the range of 0.1 – 0.9 mM. Distilled water with no analyte was used as the blank of the calibration curve to remove the error in measurements due to impurities in the distilled water. The absorbance of the distilled water was measured then zeroed with the sample still in the spectrophotometer before all other measurements were carried out. Three dilutions were carried out from each sample at each concentration and the average was used to plot the calibration curve.

Protein Calibration

BSA solution of 2 mg ml^{-1} was diluted according to the manufacturer's instructions with distilled water to obtain a range of concentrations between 0.025 mg ml^{-1} to 2 mg ml^{-1}. For each sample, 30 µL of the protein solution of a given concentration was added to a cuvette then 1.5 ml of Coomassie Bradford Protein Assay (Pierce-Biotechnology) was added. The assay was used at room temperature and the measurements were carried 10 minutes after the assay was added to obtain more consistent results for all protein samples.

The absorbance of the samples was measured at 595nm because of maximum sensitivity of the assay at this wavelength (Pierce-Biotechnology).

A blank was used to eliminate the absorbance contribution of the Bradford assay. This ensures that only the protein absorbance is accounted for. The blank solution for the protein calibration curve contained distilled water and Bradford reagent only but no protein.
Two measurements were made from each sample at a given concentration and the average was used to plot the calibration curve.

5.4 Mass balance

Mass balances were all performed using Equation (24) and Equation (25). The change in surfactant mass in the liquid feed should be equal to the surfactant mass change in the foamate.

$$\Delta \text{Mass}_{\text{surfactant,Foamate}} = \Delta \text{Mass}_{\text{Surfactant,Liquid Pool}} \qquad (25)$$

The density of both the CPC solution and the protein solution were approximated to 1g/ mL. Electronic scales connected to a computer programme (Scout Pro) were used to measure and monitor mass change of the feed, foamate and control reflux during the CPC experiments. Scout Pro control software uses derivative control to obtain total reflux. A mass set point for the foamate was entered into the programme which was kept constant by varying the pump speed. The pump speed increased when foamate mass was above the set point and decreases when foamate mass was below the set point.
Electronic mass balances were used to measure change in mass of the foamate and overflow as well as the samples taken during the protein experiments. All mass measurements were made to an accuracy of ± 0.001g.

6.0 Results and Discussion

6.1 CPC Experiments

CPC Calibration Results

Figure 18 shows the CPC concentration calibration, with the linear region indicated by the black line. The equation of the line which lies below an absorbance of 2 is also shown. The error measurements were calculated from three repeats shown on the graph. However, because the error is less than 1%, the error bars are not fully visible.

$$Concentration = \frac{Absorbance}{3.9647} \qquad (26)$$

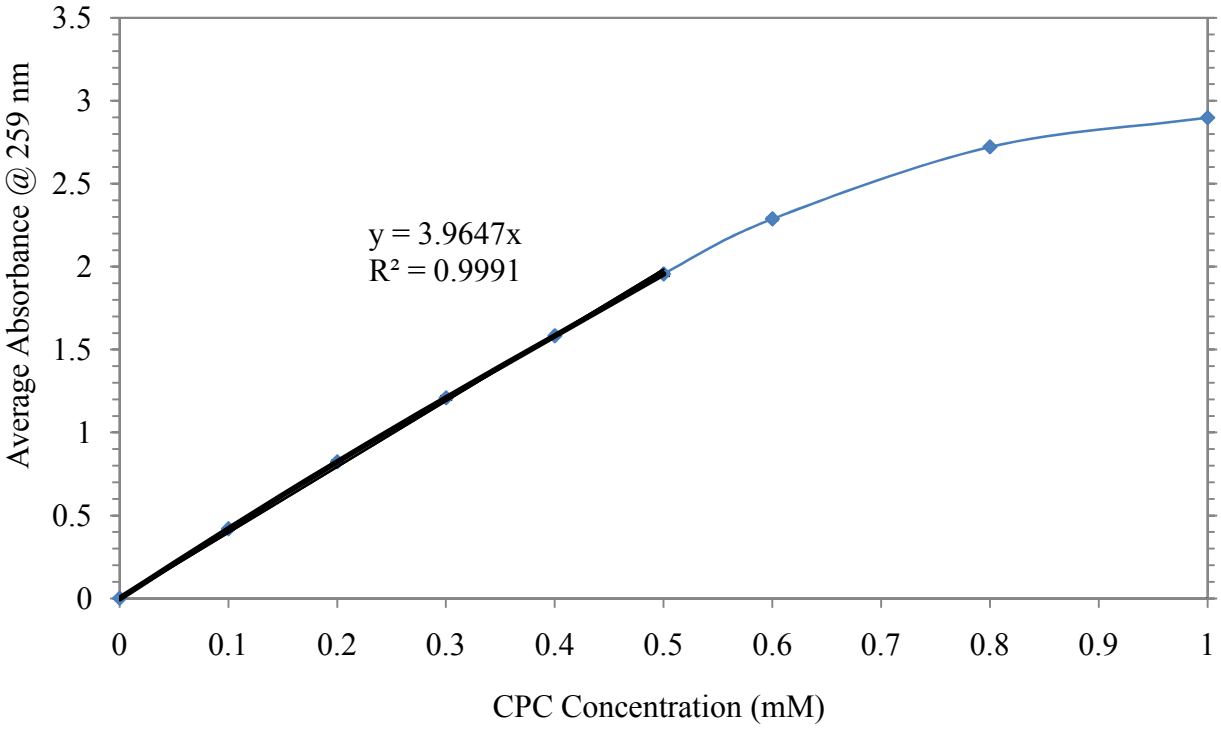


Figure 18: Calibration Curve showing average absorbance of CPC over a range of concentrations

6.1.1 CPC Foam Fractionation Results

All experiments were conducted using 1mmM CPC feed solutions and an air flow rate of 1 L min^{-1}. Five experiments were conducted; one at a no reflux (R = 0) with an initial volume of 900 mL, three repeats at total reflux (R=1) with an initial volume of 800 mL and one at total reflux with a lower initial volume of 530 mL.

The concentration of the foamate increased with respect to time and the concentration of the bulk liquid pool decreased with respect to time for both the reflux and non reflux experiments.

Figure 19 shows the enrichment ratio achieved by varying the reflux ratio and the initial feed volume. Higher enrichment was achieved with the no reflux experiment which had a higher initial volume than at total reflux were lower initial feed volumes were used. The initial feed volume used is indicated in brackets in the figure legend.

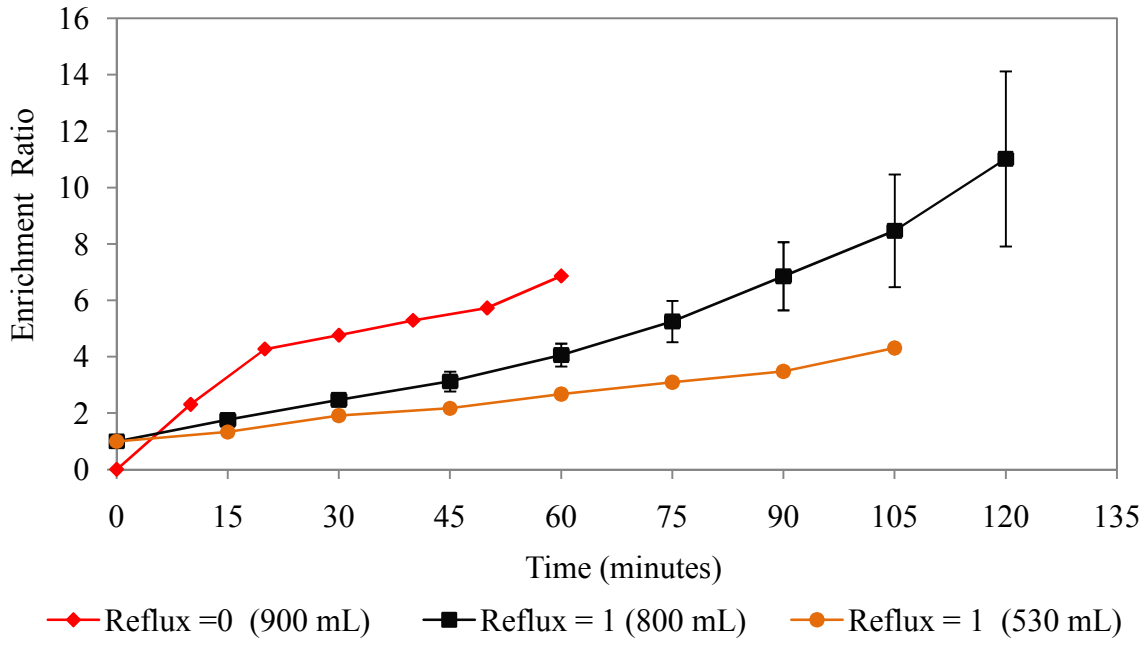


Figure 19: Enrichment ratio for CPC batch experiments with varying initial feed volumes and reflux ratios.

The experiment with no reflux was conducted for an hour to determine the concentration profile. The total reflux experiment with an initial feed volume of 530 mL was stopped after 90 minutes of operation because the feed height above the sparger dropped significantly. The foam formed was unstable with coalescing air bubbles and collapsed before reaching the top of the column.

The results from the total reflux experiment with a feed volume of 530 mL were used to set the minimum allowable volume in the liquid pool for stable foam formation at a 1mM concentration. The initial volume was increased from 530 mL to 800 mL for all subsequent experiments in an attempt to foamability and foam stability.

Figure 20 shows that recovery increases with respect to time. This increase is expected as more CPC is removed by the foam so the mass of CPC in the feed decreases with time. Recovery is higher with no reflux than with total reflux which was not expected as it doesn't match the trends found in literature.

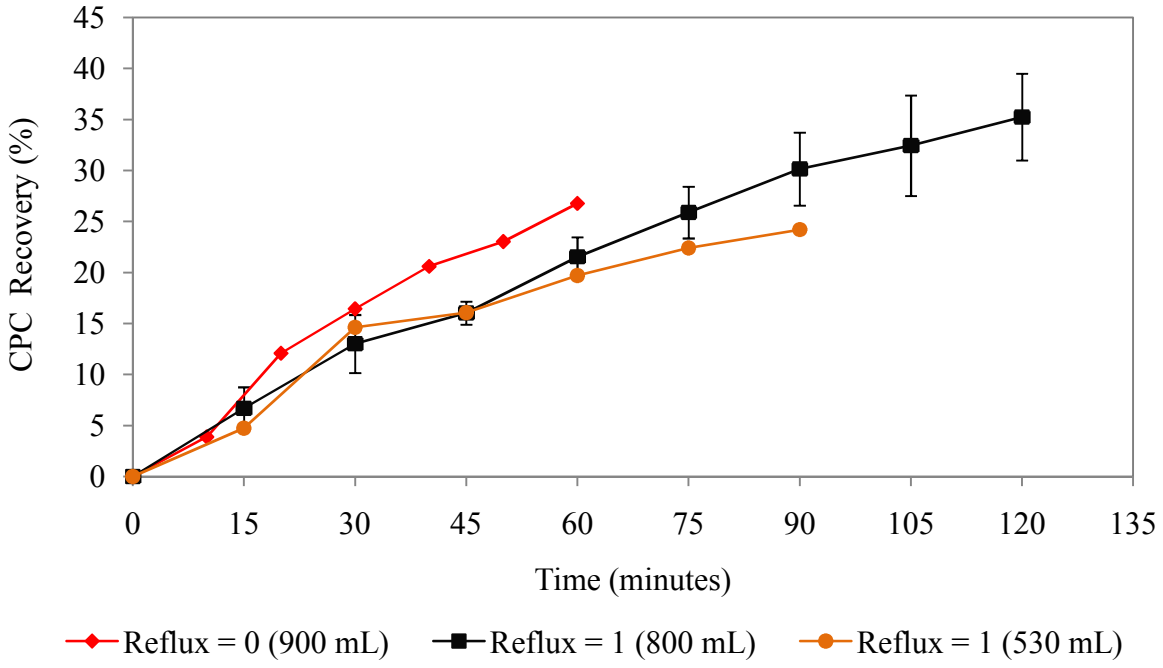


Figure 20: Recovery of CPC with varying reflux ratio and initial feed volume.

The maximum standard error for enrichment was calculated as ± 3 at 120 minute and 3.5 in recovery at 90 minutes for total reflux (800mL). These values and the size of the error bars in Figure 19 and Figure 20 suggest that there is some variation in the results obtained. The modest precision achieved from the results collected may be due to the dynamic nature of the system and experimental error. Sources of error in the experiment were interruptions due to foam breaker malfunctioning and minor errors in measurement of initial feed concentrations.

6.1.2 Discussion

A comparison of the enrichment ratios and recovery were carried out for all experiments at 60 minutes as the non reflux experiment was only conducted for an hour.

A higher enrichment ratio of 6.9 was observed for no reflux compared to 4.06 and 2.67 at total reflux for 800 mL and 530 mL initial volumes respectively. The results don't follow the expected trend.

Although very few studies have conducted on foam fractionation with external reflux, the results obtained from the few have shown external reflux increases the enrichment of foam (Lemlich, 1961), (Lucena *et al*., 1996). Higher foam enrichment is expected because the surfactant concentration in the reflux is higher than the residual concentration in the feed. The reflux creates a counter current effect with the rising liquid entrained by the rising foam allowing the surfactant in the reflux to adsorb to the rising foam. This mechanism should generate significantly enriched foam for reflux experiments than in those without reflux.

The foamate reservoir contained a CPC solution of 1mM initially. This was done to minimise time wasted, waiting for the foamate to build up for reflux. However, the foamate produced was diluted in the foamate reservoir which limited the increase in foamate concentration for the first hour. This affected the enrichment ratio and limited the effect of the reflux stream on foam enrichment within the column.
The experiments with no reflux were performed with an empty foamate reservoir so the concentration measurements were representative of the concentration of the foamate produced with time which resulted in higher enrichment values.

After the first hour, a significant increase in the enrichment ratio was observed for the total reflux experiment (800 mL) reaching a maximum of 11 after 120 minutes of operation which is a threefold increase in an hour. However, because the batch only experiment was only conducted for 1 hour, a more accurate comparison cannot be carried out. The enrichment ratio would however be expected to reach a maximum that is lower than that of the reflux experiments.

The effect of using a smaller feed volume was also investigated and the results are shown in Figure 19. Liquid volume has an effect on both the enrichment and the recovery of CPC where both factors increased with increasing feed volume. Figure 20 shows that a higher recovery was obtained with increasing feed volume. At a low feed volume, the bubbles were formed at the liquid surface which significantly reduced the residence time of bubbles in the liquid pool. This reduces both the time for adsorption and the total bubble surface area available for the CPC to adsorb to. Less CPC is removed from the liquid pool therefore the recovery is low.

Figure 20 shows that there was very little variation in surfactant recovery between the non reflux and reflux experiments. A comparison of the experiments at 60 minutes showed that the recovery for no reflux was 27% and was slightly higher that attained at total reflux which were 22% and 20% for feed volumes 800 mL and 530 mL respectively. This may be because the high concentration reflux was drained back into the liquid pool returning the surfactant that had been removed which caused a lower recovery. The maximum recovery achieved within two hours was 35% for total reflux at 800mL initial feed volume.

Surfactant recovery increased with increasing feed volume which is supported by the findings of Uraizee and Narsimhan (1996). This effect is due to the reasons given for the surfactant enrichment increase with a larger feed volume.

The overall results obtained from these experiments showed that foam fractionation could be successfully used as a separation technique. A maximum recovery of 35% and an enrichment ratio of 11 were obtained at total reflux. These results showed that foam fractionation has potential and could be applied to the problem addressed in the current study which was the removal of protein from fish culture water. The results obtained for these experiments were also used in the selection of the operating conditions for the protein experiments. Using a lower feed concentration and gas flow rate would increase the maximum enrichment and recovery.

6.2 Protein Experiments

Protein Calibration Results

The calibration curve for BSA protein is shown in Figure 21. The linear region and equation of the line are shown more clearly in Figure 22. The unknown concentration of samples can be determined by rearranging the equation shown by Equation (27).

$$Concentration = \frac{Absorbance}{1.091} \qquad (27)$$

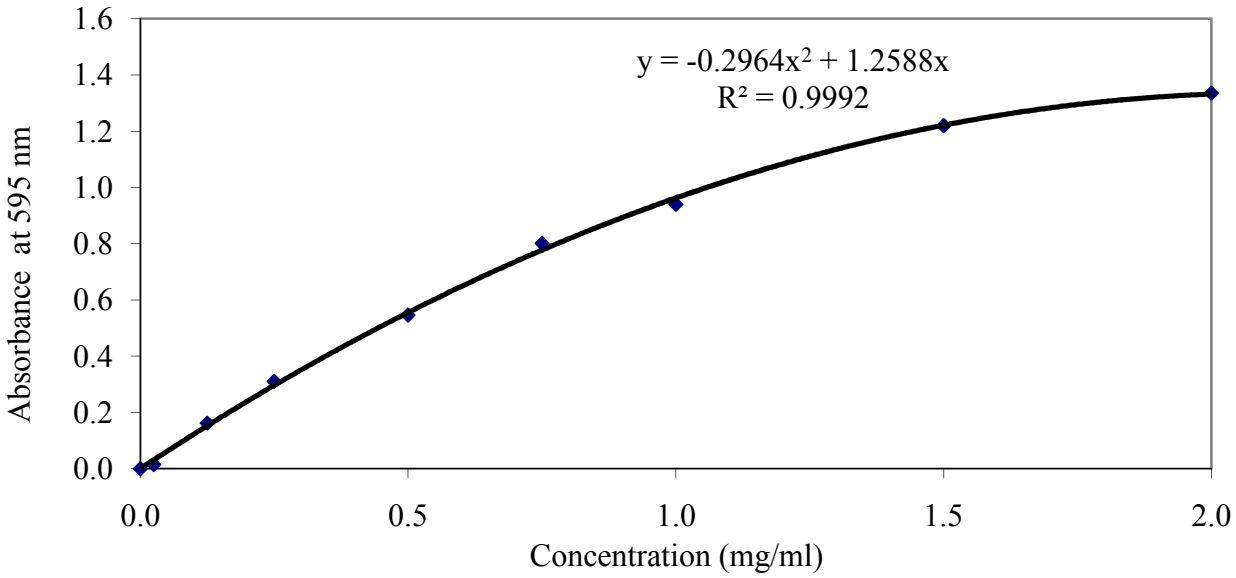


Figure 21: BSA Calibration Curve

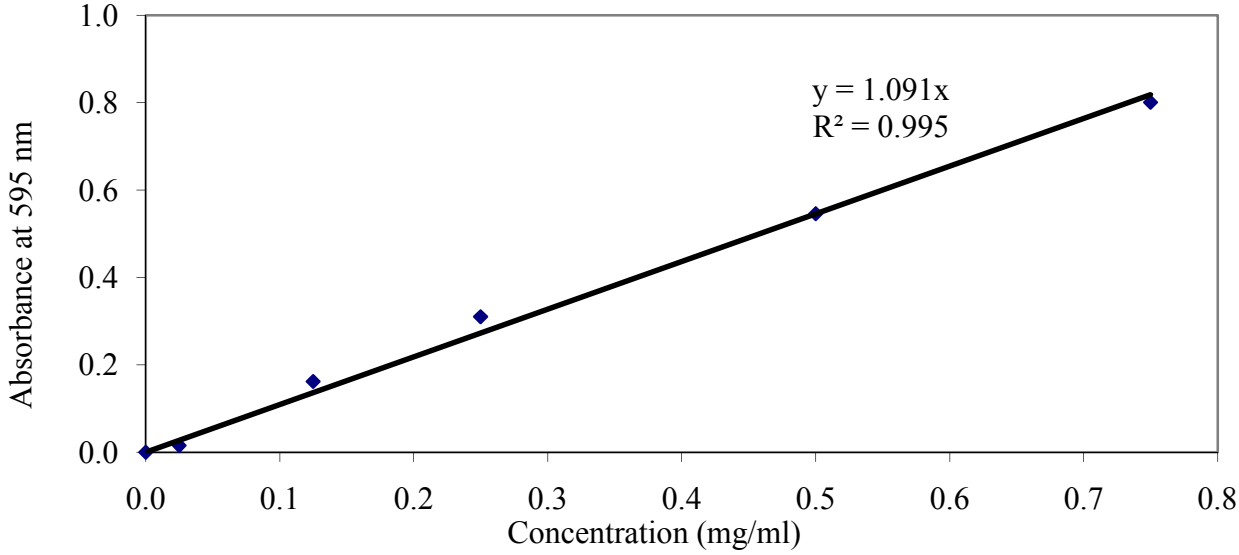


Figure 22: Linear region of BSA Calibration Curve

6.2.1 Protein Foam Fractionation Results

The effect of varying the initial feed concentration was investigated using BSA. A total of six experiments were conducted. The first was a batch experiment at 0.5 mg/mL feed concentration, then four continuous experiments at 0.1, 0.2, 0.4, 0.5 mg/mL and finally; a continuous recirculation experiment at 0.1 mg/mL. All experiments were conducted with an air flow rate of 130 mL min^{-1} and a sodium acetate-acetic acid buffer at pH 4.8. A feed flow rate of 10.32 mL min^{-1} was used for the continuous and recirculation experiments.
All experiments were conducted for a minimum of 2 hours using 3 litres of feed with one exception at 0.5 mg/mL feed concentration. The performance indicators in Figure 23 were also plotted with respect to time and are shown in Appendix D. Steady state was reached within 45 minutes for all experiments. A mass balance carried on all experiments was closed within 90%.

Figure 23 shows the effect of varying concentration on protein enrichment ratio, recovery and removal fraction.

The removal fraction decreased very slightly with increasing feed concentration. At 0.4 and 0.5 mg/mL feed concentrations, more foam was produced therefore more protein was removed from the continuous feed stream than at the lower concentrations. Results are shown in Appendix D.

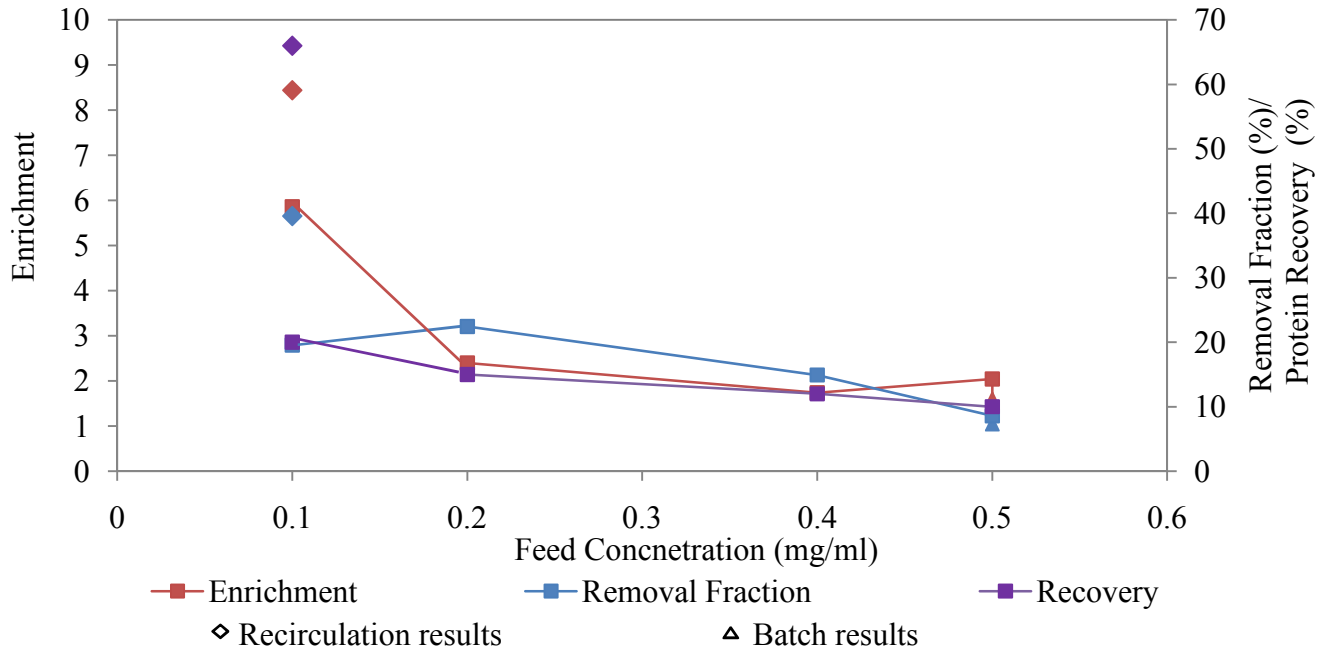


Figure 23: Shows the effect of initial feed concentration on the three performance criteria.

Increasing the initial feed concentration lowered the enrichment ratio. The enrichment dropped significantly by 4 between 0.1 mg/mL and 0.2 mg/mL and remained constant for higher feed concentrations. This may be due to production of a large number of small bubbles at high concentrations with greater liquid hold up. The production rate increases 2 fold between 0.1 and 0.2 mg/mL and increases 3 fold between 0.1 and 0.5 mg/mL which would support this theory.

The results obtained from continuous mode with recirculation were all significantly higher than the results obtained from the continuous experiment at the same concentration with respect to the enrichment ratio, removal fraction and protein recovery. This trend was as expected and will be discussed later. Normal operating procedure in section 5.2 was followed initially to confirm steady state. Recirculation was started after three hours whereby the recirculation stream returned the overflow to the column as feed. The experiment lasted six hours before it stopped foaming. The maximum removal rate was reached after two hours and remained fairly constant. These results are shown in Appendix D.

Figure 23 also shows that protein recovery increased with increasing feed concentration. Additional protein was present in the initial feed at higher concentrations so more protein was likely to be recovered in the foam. The results obtained for the batch experiment were similar to those obtained at the same concentration in continuous mode for both the enrichment and removal fraction within the first two hours. The batch results are slightly lower which shows that the mode of operation had little effect in the first 2 hours of the experiment.

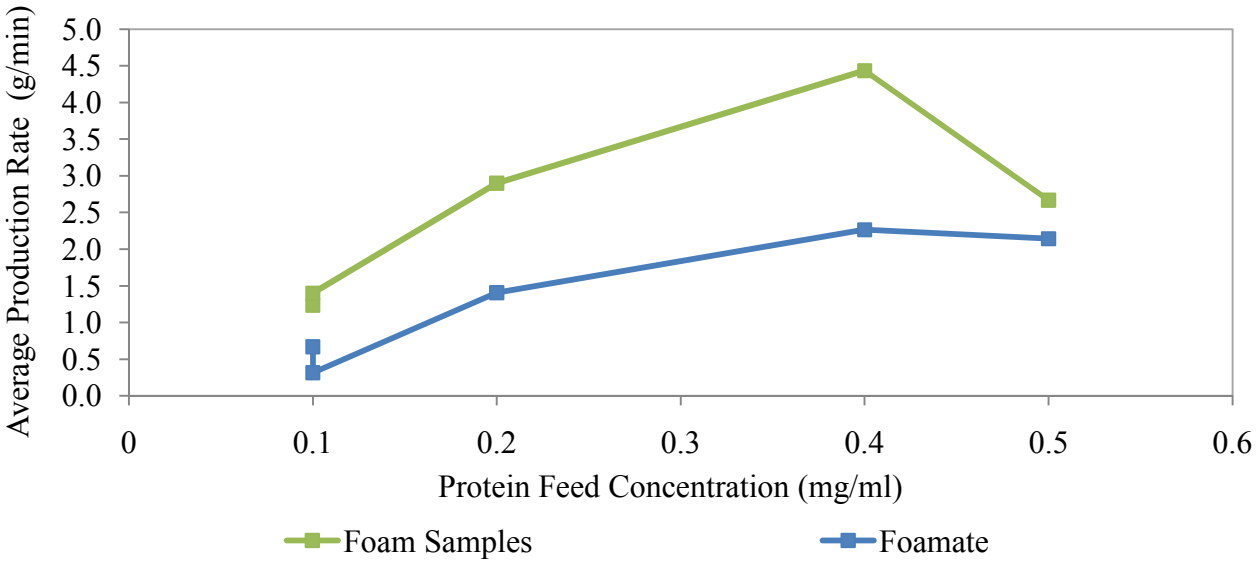


Figure 24: The effect of initial feed concentration on foam production rates.

Figure 24 shows the average foam production rate. Foam production was estimated from the foamate accumulation rate and from the foam samples collected for 1 minute at each sampling interval. The average for each experiment was based on its duration.

The foam production rate increased with concentration which is expected as the liquid hold up in foam is greater at high concentrations. However, a significant decrease at 0.5 mg/mL is an anomalous result caused by a significant drop of feed height above the sparger. The foam produced was not very stable so foam accumulation was affected. The experiment was only run for 90 minutes using an initial feed volume of 2 litres.
This experiment was used to set the minimum allowable operation volume to 3L instead of 2L for all other experiments. All feed solution foamed easily and produced foam at the rate shown in Figure 24.

6.2.2 Discussion

The proteins experiments were only carried out once at the same operating conditions. No repeats at the same operating conditions were carried out because it was more important to test performance of equipment for foam fractionation under different operating conditions. This was done due to time constraints and limited experimental resources. The assay needed to determine the concentration of the protein was limited so only a fraction of the planned experiments were conducted. The concentration values given were obtained by taking two measurements from the same sample and the average was plotted.

Enrichment decreased with increasing feed concentration due to production of small bubbles at higher concentrations. Although small bubbles increase the total surface area available for protein adsorption at high concentrations, this is counteracted by increased foam production rates and stability. There is limited drainage due to the stabilising nature of proteins hence a larger liquid hold up is present in the foam. The liquid entrained was at the concentration of the residual solution so foam enrichment was also restricted. Similar trends in literature were observed for investigations conducted using BSA (Uraizee and Narsimhan, 1996) and (Hossain and Fenton, 1998).

Decreasing enrichment could also be explained by the linear Langmuir isotherm in Equation (3). Enrichment is the ratio of surface concentration to bulk feed concentration. Therefore, high bulk feed concentrations correspond to low separation and vice versa (Lemlich, 1972). At high bulk concentrations in equilibrium, adsorption at the gas-liquid interface is higher due to lower surface tension. This is why equilibrium is reached quicker with concentrated solutions than with dilute solutions as shown in Appendix D.

The enrichment ratios obtained for feed concentrations 0.5mg/ml and 0.4 mg/ml were very similar. At high concentration above the CMC, proteins form micelles. The micelles may interfere with the foaming process and could significantly affect protein enrichment. This could explain the small difference between the two higher concentrations relative to that between the two lowest concentrations within the range studied.
The recovery at the different initial feed concentrations was compared at 90 minutes and showed very small differences between 10% and 18% for the four different feed concentrations. A bigger difference is observed at 120 minutes however, because the experiment conducted at 0.5mg/ml was only conducted for 90 minutes, a complete comparison of all experiments cannot be carried out.

Samples of the effluent were taken from the side of the inverted T-shaped column base. However, because the foaming occurred at the centre of the column, the concentration of the bulk liquid in the centre was likely to be lower than that at the side so the measurement were not representative of the actual concentration in the entire column. This is due to the non uniform mixing within the column especially after a short period of time. This affected the removal fraction calculated and resulted in negative values for the removal fraction. This trend was observed for 0.1mg/ml, 0.4.mg/ml and 0.5mg/ml shown in Appendix D. However, due to the protein skimmer design, this was the only method of measuring the concentration of the liquid in the column. Therefore error incurred should be accounted for in the results.

A maximum recovery of 65% was achieved within 6 hours of column operation using a recirculation stream. Enrichment ratio and the recovery increased significantly by 1.5 and 46 % respectively relative the results obtained at 0.1 mg/mL without recirculation. The higher enrichment was most likely due to the continuously decreasing concentration of the feed from the recirculation stream. Enrichment is higher with lower concentrations as previously explained.

Enrichment increased significantly in the last hour of operation because of the extremely dry foam production with a very small liquid hold up which increased foam concentration.

A high recovery is important for this study as the aim is to remove as much waste protein from the fish tank system as possible. The results obtained from this study showed that the protein skimmer could successfully be used to separate protein from dilute solutions. A maximum enrichment ratio of 8.6 and a maximum recovery of 65% were achieved at the lowest concentration of 100 mg/L when the recirculating system in the fish tank was mimicked using the protein skimmer.

Table 7: Predicted concentrations in fish tank using a recovery rate of 65% determined from the experiments using the protein skimmer.

Protein feed concentration (mg/mL)	Foam Concentration (mg/mL)	Residual feed concentration (mg/mL)
0.1	0.07	0.035
0.2	0.13	0.070
0.3	0.20	0.105
0.4	0.26	0.140
0.5	0.33	0.175

The suitability of the protein skimmer at removing organic material from the tank was determined using the recovery rate obtained from the recirculation experiment. The upper limit of Total Dissolved Solids (proteins) within fish culture water is 0.4 mg mL^{-1} (Timmons, 2002). The experimentally determined recovery was used to predict the residual protein concentration that would be present in the fish tank at different initial feed concentrations. The residual concentrations were all within the limits as shown in Table 7. Even an initial starting concentration above the upper TDS limit (0.5 mg mL-1) would be reduced significantly to a safe residual concentration of 0.175 mg mL^{-1} in the tank. Typical safe levels of TDS in the tank range between 0.1 – 0.2 mg/mL.

These results satisfy the objective of the study which was to determine the efficiency of the protein skimmer and its ability to improve water quality. Further studies would have to be conducted using fish culture water to confirm the trends observed in this study.

7.0 Conclusion

The purpose of this study was to design and characterise a protein skimmer to improve the quality of water in the CEAS fish tank. The protein skimmer designed successfully removed a significant amount of from the protein solutions used for the study. The design of the protein skimmer met the criteria specified in the design section and can easily be integrated into the current fish tank installation.

The trends observed when the initial feed concentration was varied were; enrichment decreased with increasing feed concentration while the recovery and protein removal from the liquid pool increased. Maximum values of 8.9 and 65% and 35% were observed for the enrichment, recovery and removal fraction respectively at a feed concentration of 0.1 mg mL^{-1}. These results were due to increased residence time of the feed in the column when a recirculation stream was incorporated into the continuous set up. Estimations calculated using this experimentally determine recovery showed that the protein skimmer had the potential to significantly reduce protein concentration with the fish tank. A 65% recovery factor can effectively improve water quality to maintain safe levels of protein in the fish culture water. Starting concentrations as high as 0.4 mg/mL, the upper concentration limit for organics in the tank could be reduced to 0.14 mg/mL in the residual tank water when the experimentally determined recovery factor was applied. The results also showed that better results were obtained when recirculation of feed was applied in the experiments.
The protein skimmer designed therefore effectively met the main objective as effective separation is evident from the results and experimental findings were supported by literature.

Further work

Only one variable was investigated in this study. The results obtained could be improved by investigating other variables that have an effect on skimmer performance. These include the feed flow rate, gas flow rate, pH and feed height above the sparger. The results obtained would be used to deduce the optimum conditions for the fish tank

The experiments at the operating conditions could also be investigated to validate the reproducibility of the results obtained.

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9.0 APPENDICES

9.1 Appendix A

Table A1: Table showing the components and dimensions of protein skimmer

QVF glass U-bend		Length /Nominal diameter (mm)	
	Nominal diameter – D_N (mm)	25	
	Length - L (mm)	140	
	Length - L1 (mm)	140	
QVF glass Pipe section			
	Number of items	1	
	Nominal Diameter – D_N (mm)	25	
	Length - L (mm)	75	
QVF glass Unequal tee piece			
	Nominal diameter – D_N (mm)	80	
	Nominal diameter – D_{N1} (mm)	25	
	Length – L (mm)	250	
	Length – L1 (mm)	100	
Couplings with Stainless steel flanges			
	Flange Nominal Diameter – D_N (mm)	25	80
	Coupling sets	2	2
	Screw Nominal Diameter – D_N (mm)	8	8
	Outside diameter – D_o (mm)	85	155
	Distance between opposite screws (mm)	70	133
	Number of screws	3	6
	Distance between flanges (mm)	59	83
	Free length of spring (mm)	20	20
	Compressed spring length (mm)	14	14
PTFE ‘O’ Ring Gaskets			
	Nominal diameter (mm)	25	80
	Number of Gaskets	2	2
	Gasket outer diameter (mm)	34	88
Plastic plates	Number of plates	2 plates	
	Plate Thickness (mm)	4	
	Plate diameter (mm)	110	
	Number of centre holes	2	
	Distance between centre holes (mm)	25	
Centre hole diameter (mm)	8		
Number of outer holes for couplings	6		
Outer hole diameter (mm)	10		
Rubber Gaskets x2			
	Gasket outer diameter – d_o (mm)	110	
	Gasket inner diameter – d_i (mm)	70	
	Component 6	Stainless steel Hose barbs	
	Number of items	4	
	Diameter (mm)	8	

9.2 Appendix B

Fish Tank Waste Estimations

Estimating Liquid waste produced by the different types of fish in the tank. The estimation was carried out using the average weight of the different types of fish and also with the individual weights of the fish. The results show that there is very little difference in the estimate for urine production.

Table B1: Fish tank liquid waste estimations

Urine (mm^3/kg of fish. day)	3
Urine (mm^3/g of fish. day)	0.003
Average weight of all fish (g)	0.26
Number of fish	30
Total urine produced in tank (mm^3/.day)	0.0234795
Total urine produced in tank (mL/day)	0.0000235

Table B2: Fish waste estimations according to individual weight of fish

Fish type	Tiger barbs	Neon tetras
Number of Fish	17	13
Mean Weight (g)	0.33	0.1705
Urine per fish (mm^3/g of fish. day)	0.01683	0.0066495
Total urine produced in tank (mm^3/.day)	0.0234795	
Total urine produced in tank (mL/day)	0.0000235	

It was estimated that it would take 7 days of feeding (0.2273g feed of 48% protein) with no waste removal for the system to reach the concentration limits proposed by Timmons (2002).

Table B3: Change in concentration over a number of days with no waste removal.

Concentration	1 days	2 days	7 days	Concentration Limits (mg/L)
TVS or TDS (mg/L)	54.552	109.104	381.864	400
Protein (mg/L)	2.18208	4.36416	15.27456	
TSS (mg/L)	1.1365	2.273	7.9555	80
Fine solids generated daily (mg/L)	0.034095	0.06819	0.238665	2.4

9.3 Appendix C

Buffer Solution Calculations

The Henderson-Hasselbalch, Equation (28), was used to determine the amount of acid and salt needed to make a buffer with a given pH. The solutions were made with the amounts specified in Table C1.

$$pH = pK_a + log_{10}\left(\frac{[salt]}{[acid]}\right) \quad (28)$$

$$pH = -log_{10}[H^+] \quad (29)$$

$$pK_a = -log_{10}\,K_a \quad (30)$$

Where the pH can be determined from hydrogen ion concentration [H^+] in the solution, and pKa can be determined from Ka which is the dissociation constant. This constant is specific to the acid used in the buffer; for acetic acid, the Ka is 1.7×10^{-5}.

Table C1: Buffer calculation results

Buffer pH	4.8	Density of acid (g/ml)		1.05
Buffer Ka	1.70E-05	Volume of acid to be used (ml)		26.659
pKa	4.769551079			
Desired Molarity (M)	1			
[Salt]/[Acid]	1.072627486			
Component	Concentration	Buffer Volume	Mw	Mass
	(M)	(L)	(g/mol)	(g)
[Acid]	0.4661	1	60.05	27.992
[Salt]	0.5000	1	136.08	68.040

9.4 Appendix D

Stepwise Experimental Procedure

1) Set up apparatus as shown in Figure 16.
2) Make up protein solution to desired concentration, volume and pH.
3) Switch air pump and peristaltic pump and adjust them to the appropriate flow rates before liquid is pumped into column and then switch them off.
4) Pump liquid feed into column using the peristaltic pump and stop when desired feed height is reached.
5) The experiment can be started by switching on the air and feed pumps. Respective flow rates can be adjusted using the control valves.
6) Foam produced moves up the column and is collected in the foam reservoir at the top of the column and overflow is collected in the overflow reservoir.
7) Samples were taken at 15 minute intervals to determine steady state. Foamate samples were taken using a pipette from three different sample points at each sampling interval. Samples of the liquid feed within the column were taken directly from the overflow tube. Foam samples were collected for a minute directly from the column.
8) Foam, column feed and foamate samples were left to collapse completely before the absorbance was measured.
9) Mass of total foamate and total overflow, foam and overflow samples were all measured and recorded at the sampling intervals.

Experiment Steady state Results from protein experiments

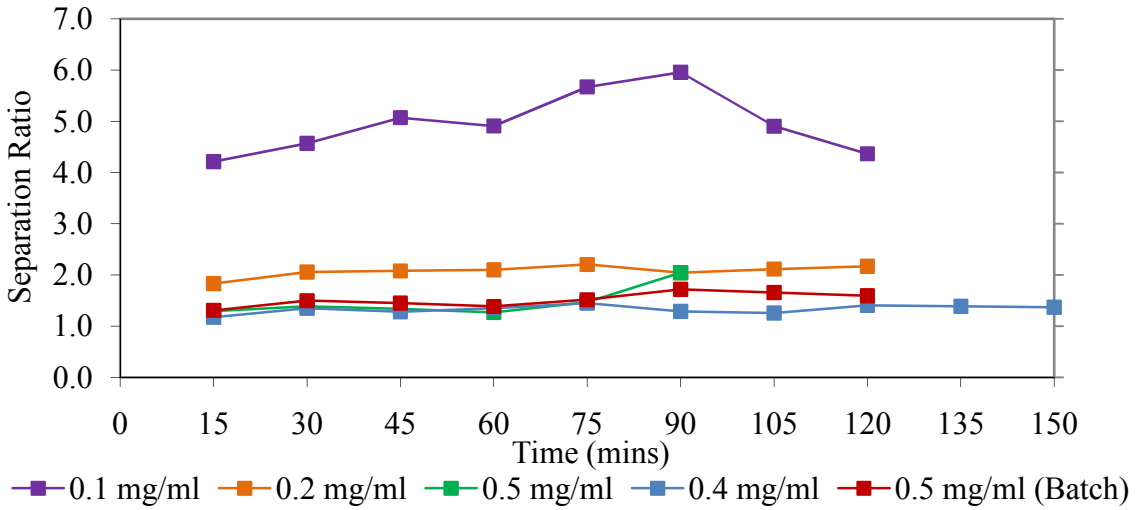

Figure D1: Enrichment Ratio at different feed concentrations.

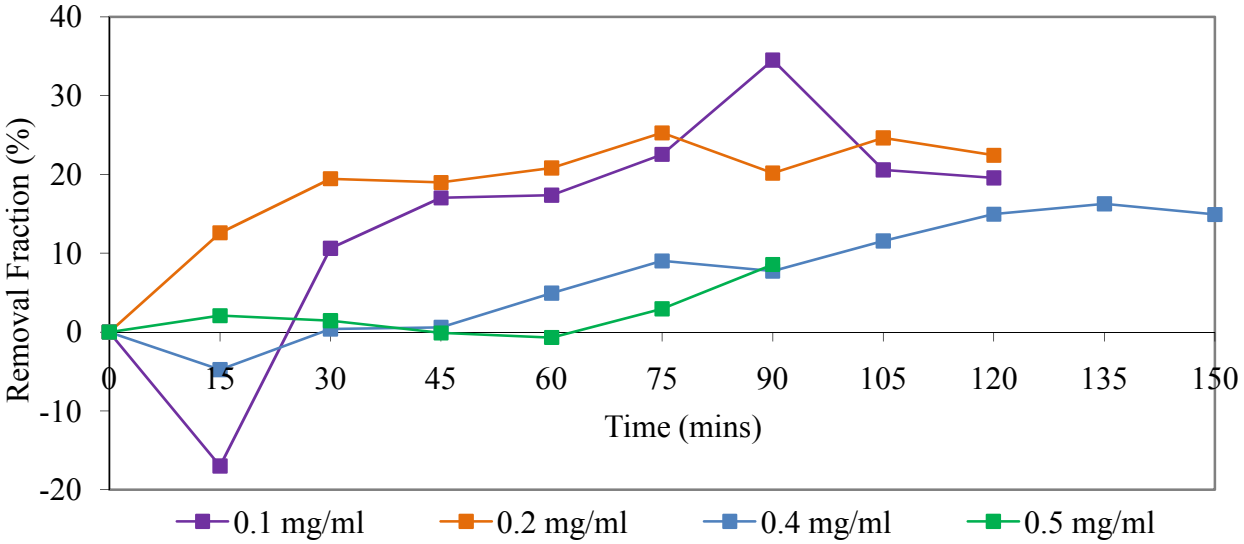

Figure D2: Removal Fraction of BSA protein from bulk liquid feed at different initial feed concentration.

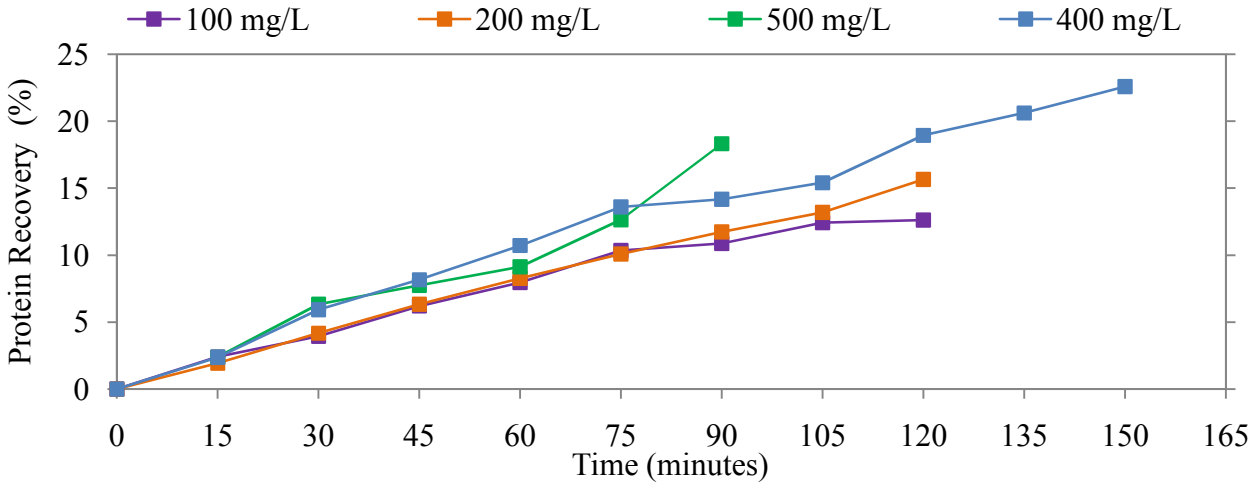

Figure D3: Protein Recovery in foamate collected.

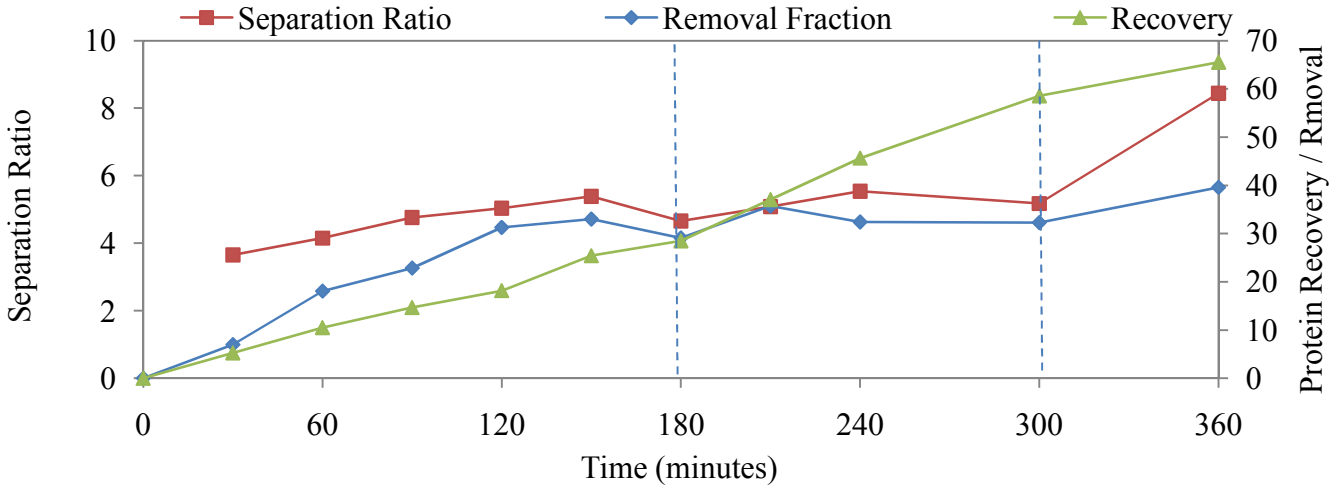

Figure D4: Continuous operation with feed recirculation at an initial concentration of 0.1 mg/ml.

9.5 Appendix E

Mass Balance results for CPC experiments and BSA experiments

Table E1: Mass balance on BSA for the different feed concentrations investigated.

Feed Concentration (mg/mL)	0.1	0.2	0.3	0.4
Feed Volume (L)	3	3	3	2
pH (range 4.8-5.0)	4.85	4.9	4.95	4.85
BSA in Samples (g)	0.0164	0.0232	0.0572	0.0282
BSA in Foamate (g)	0.0511	0.1041	0.3446	0.1377
BSA in Overflow (g)	0.0712	0.1449	0.2516	0.4760
BSA left in Column solution (g)	0.1117	0.2683	0.4956	0.3197
Total mass of BSA (g)	0.2503	0.5405	1.1490	0.9616
Initial mass of BSA	0.3017	0.6008	1.2004	1.005
Missing BSA (g)	0.0514	0.0603	0.0514	0.0434
Missing BSA (%)	5%	6%	5%	4%

Missing BSA is mostly likely due to experimental error as amount missing is consistent in all experiments. It may have been lost when the solutions was made up.

Table E2: CPC mass balance results

	Expt. 1	Expt. 2	Expt. 3	Expt.4	Expt. 5
Mode of Operation	No Reflux	Reflux	Reflux	Reflux	Reflux
Time taken for reflux to reach column (minutes)	0	12	10	13	36
Initial CPC in Bottom (g)	0.3313	0.1875	0.2909	0.3364	0.2829
Initial CPC in Top (g)	0.0000	0.0579	0.0488	0.0599	0.0510
Total Initial CPC (g)	0.3313	0.2453	0.3396	0.3963	0.3340
Final CPC in Bottom (g)	0.1736	0.0858	0.0648	0.0934	0.1527
Final CPC in Top (g)	0.0980	0.1251	0.1959	0.1937	0.1475
Total CPC in samples (g)	0.0147	0.0195	0.0287	0.0285	0.0273
Total Final CPC (g)	0.2862	0.2304	0.2893	0.3157	0.3275
Missing CPC (g)	0.0451	0.0149	0.0503	0.0806	0.0065
Missing CPC (%)	13.61	6.08	14.81	20.34	1.95

The mass balance for CPC was closed with a maximum of 20% missing CPC. The amount missing varied between experiments. This can be explained by CPC left in fractionating column when experiment is stopped, CPC lost during the process of making the CPC solution, some left on the apparatus used. The CPC discrepancy may also be due to inaccuracies in the measurement of the absorbance whereby a dilution error has to be accounted for.

9.6 Appendix F

Peristaltic Pump calibration data

Pump Setting	Pump output	Pump output
Arbitrary units	Flowrate of water (ml/min)	Flowrate of water (ml/min)
0	0	0
5	7.34	7.32
10	14.54	14.52
15	22.46	22.16
20	29.88	29.78
25	39.34	39.22
30	46.16	46.14
35	54.42	54.46
40	69.99	69.66
45	76.14	76.08
50	79.38	79.3

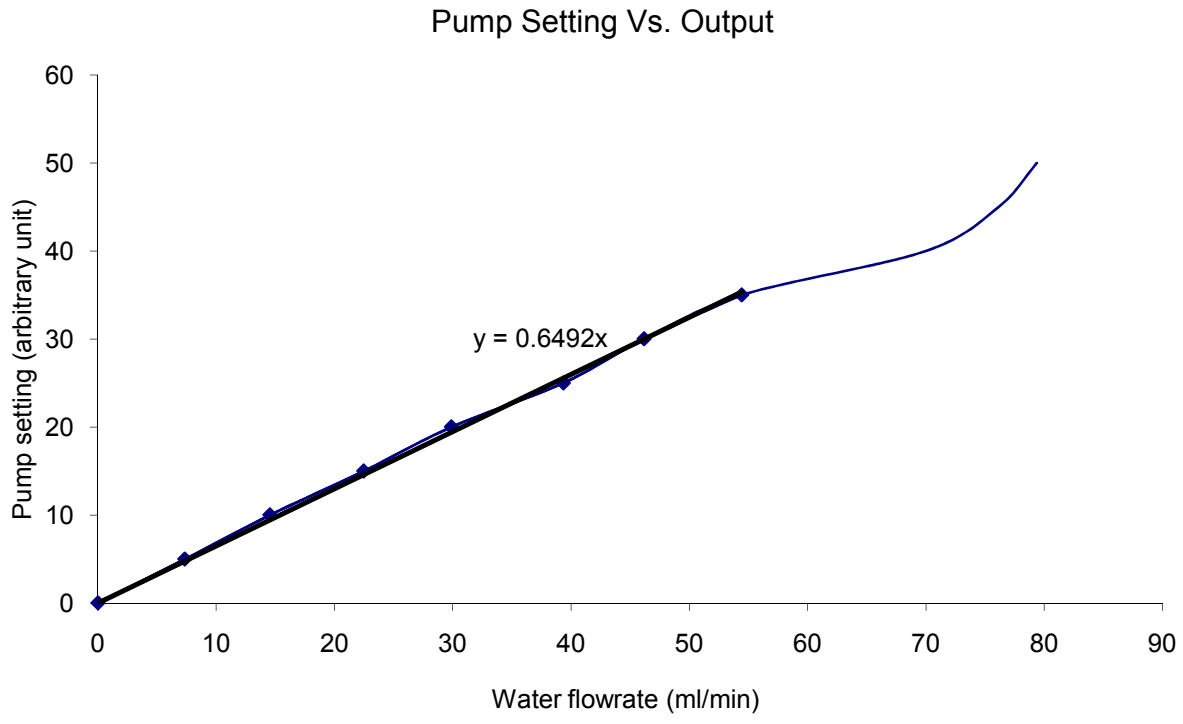

Figure F1: calibration curve used to determine pump setting corresponding to 10.31 ml min^{-1}