# DECOLOURING BLOODMEAL: CONSUMPTION AND POTENTIAL RECYCLING OF PERACETIC ACID

**Talia Hicks[1], C.J.R. Verbeek[1], M. Lay[1] and M. Manley-Harris[2]**

[1]School of Engineering
Faculty of Science and Engineering
tmh20@waikato.ac.nz
[2]Department of Chemistry
University of Waikato

## ABSTRACT

A method of deodorizing and decolouring bloodmeal using an equilibrium mixture of peracetic acid, hydrogen peroxide, acetic acid and water has been developed to improve its marketability as a source of protein for bioplastics.

The objective of this study was to determine what quantity of peracetic acid is required to give reasonable bleaching of the bloodmeal and determine whether there is potential for the wastewater to be recycled. This was carried out by measuring the quantity of chemical species in the initial equilibrium mixture and the resulting wastewater upon bleaching using volumetric analysis. Bleaching efficacy was determined after exposing 100 g bloodmeal to 1.1, 2.5, 3.6, 4.5 and 5.6 wt% peracetic acid solutions as either 300 g total solution or a constant molar equivalent of 2.2 mmol peracetic acid/g bloodmeal and using a chromameter to measure colour change.

Addition of 300 g 5.6 wt% peracetic acid solution resulted in effective bleaching. This represented a ratio of 2.20 mmol peracetic acid/g bloodmeal of which 1.4 mmol peracetic acid/g bloodmeal was consumed (63%). If 300 g <2.5 wt% peracetic acid solution was used, there was insufficient bleaching. If >300 g of <2.5 wt% solution is added such that there is still 2.2 mmol peracetic acid/g bloodmeal, bleaching is still insufficient.

These results suggest that an excess of peracetic is required for bleaching to occur, and that its concentration is paramount to bleaching efficacy. Due to the excess of peracetic acid used in the bleaching process, there is potential for wastewater recycling to be carried out provided that the wastewater is not diluted.

## INTRODUCTION

Conventional petroleum derived plastics are versatile and durable materials with many applications. Worldwide, approximately 140 million tonnes of petroleum-based polymer materials are produced each year, which due to their resistance to chemical and microbial degradation, have begun accumulating in the environment (Barnes et al., 2009, José, 2002, Shah et al., 2008, Shimao, 2001).

Due to the increasing environmental impact of discarded plastics and the increasing cost of petroleum feedstocks, there is a large demand for plastic products which are both biodegradable and sustainable, such as those derived from proteins (Jerez et al., 2005).

Proteins that are currently used in the bioplastics industry are generally obtained from food crops (e.g. corn). Ethical concerns about using potential food resources for the production of bioplastic, together with competition from the biofuels industry has

shifted interest to the development of Second Generation Bioplastics which utilize non-food resources such as bloodmeal, as raw materials (Verbeek and van den Berg, 2011).

Bloodmeal is a dried protein powder produced from inedible blood collected in meat processing plants. It is a low value by-product and is currently used as fertiliser or in small fractions as an additive in animal feed. This sustainable non-food protein has been used to produce bioplastic resin at Waikato University (Pickering et al., 2010, Verbeek and van den Berg, 2011).

To improve the marketability of this bioplastic, it can be deodourised and decoloured via chemical oxidation using a commercial mixture of peracetic acid. Due to the oxidative power of peracetic acid (theoretical reduction potential 1.76 V *vs.* Ag/AgCl (Awad et al., 2004)) and its ability to form reactive oxygen species (Zhao et al., 2008a), the decolouring reaction occurs very rapidly.

The objective of this study was to determine what quantity of PAA is required to give reasonable decolouring of the bloodmeal (BM), the composition of the equilibrium mixture before and after decolouring and due to the expense of peracetic acid, to determine whether there is potential for the wastewater to be recycled.

## Literature Review

Peracetic acid is an equilibrium mixture, prepared by the reaction of hydrogen peroxide with acetic acid in water with a mineral acid catalyst, such as ~1 wt% sulphuric acid (Rangarajan et al., 1995). The mineral acid catalyst remains in the equilibrium mixture leading to a pH of ≤1 and chelating agents are added to minimise the transition metal ion catalysed decomposition of peracetic acid to acetic acid and oxygen (Dul'neva and Moskvin, 2005, Peragen Systems, 2004).

The peracetic acid equilibrium mixture used for decolouring bloodmeal has a composition of 5-6 wt% peracetic acid (PAA), 21-23 wt% hydrogen peroxide (HP), 10-11 wt% acetic acid (AA) and 63-65 wt% water (FMC Corporation, 2006). The process of generating peracetic acid is described by the following scheme:

$$CH_3COOH + H_2O_2 \rightleftharpoons CH_3COOOH + H_2O$$

The concentration equilibrium constant (K) for this reversible reaction is reported to be between 0.7 and 5, depending on the temperature and the catalyst concentration (Dul'neva and Moskvin, 2005, Janković and Sinadinović-Fišer, 2005, Rangarajan et al., 1995, Zhao et al., 2008b).

Based on the assumption that the forward reaction is first-order in acetic acid and in hydrogen peroxide and that the reverse reaction is first-order in peracetic acid and water the reaction kinetics is described:

$$\frac{\partial[\mathrm{PAA}]}{\partial t} = k_1([HP]_0 - [PAA])([AA]_0 - [PAA]) - k_2[PAA]([H_2O]_0 + [PAA])$$

Where, [PAA] is the concentration of peracetic acid, $[HP]_0$, $[AA]_0$ and $[H_2O]_0$ are the initial concentrations of hydrogen peroxide, acetic acid and water, ($molL^{-1}$); and $k_1$ and $k_2$ are the rate constants of the forward and reverse (hydrolysis) reactions, respectively ($Lmol^{-1}s^{-1}$).

In an equilibrium mixture, peracetic acid is able to be consumed in three reactions (Kitis, 2004, Križman et al., 2005, Yuan et al., 1997b, Yuan et al., 1997a):

1. Hydrolysis:

$$CH_3COOOH + H_2O \longrightarrow CH_3COOH + H_2O_2$$

2. Spontaneous decomposition:

$$2CH_3COOOH \longrightarrow 2CH_3COOH + O_2$$

3. Transition metal catalysed decomposition

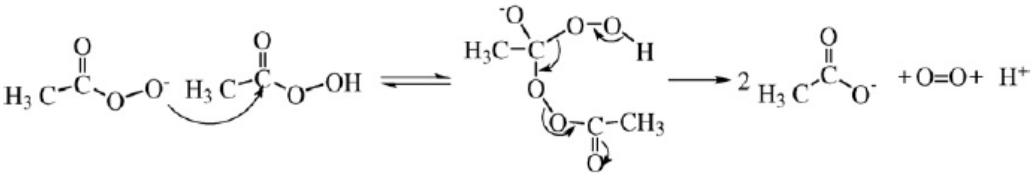
$$2CH_3COOOH \xrightarrow{M^{n+}} 2CH_3COOH + O_2 + \text{other products}$$

The equilibrium constant for generating peracetic acid at 20 $^oC$ results in an equilibrium mixture containing ~5 wt% PAA and has been calculated as approximately K= 2, indicating the formation of the products is favoured but the reaction is easily reversed (Dul'neva and Moskvin, 2005). The rates of the forward and reverse reaction have been calculated for a reaction carried out at 20, 40 and 60 $^oC$ (Table 1).

Tab.1: Summary of the composition of the reaction mixture, equilibrium state, equilibration time, equilibrium constant and forward and reverse reaction rates for the formation of peracetic acid, at 20, 40 and 60 $^oC$ at pH ~ 1.2 (Dul'neva and Moskvin, 2005).

| T ($^oC$) | Equilibration Time (h) | Initial concentration ($molL^{-1}$) | | Equilibrium concentration ($molL^{-1}$) | | | K | $k_1$ (x $10^{-5}$ $Lmol^{-1}s^{-1}$) | $k_2$ (x $10^{-5}$ $Lmol^{-1}s^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| | | HP | AA | PAA | HP | AA | | | |
| **20** | 36 | 9.88 | 2.47 | 0.840 | 9.04 | 1.63 | 2.10 | 6.81 | 3.25 |
| **40** | 28 | 9.88 | 2.47 | 0.678 | 9.20 | 1.80 | 1.46 | 8.55 | 5.85 |
| **60** | 22 | 9.88 | 2.47 | 0.544 | 9.33 | 1.93 | 1.07 | 13.82 | 12.92 |

Peracetic acid is also a weak acid, with a pKa of 8.2 and is able to partially dissociate at higher pH. This enables the peracetic acid molecule to undergo spontaneous decomposition via the nucleophilic attack of the peracetate anion upon another peracetic acid molecule (or hydrogen peroxide), to form two acetate anions and oxygen (Zhao et al., 2008a):



From this scheme the rate of PAA degradation owing to spontaneous decomposition is:

$$-\frac{\partial[\mathrm{PAA}]}{\partial t} = k_3[PAA]^2$$

Due to the high pKa of peracetic acid (little dissociation at low pH), the rate of spontaneous decomposition of peracetic acid at low pH such as that of commercial PAA, <1, should be small. It was found that the dependence of kinetics of decomposition on total peracetic acid concentration was second-order, and that at 40 $^oC$ for pH 5.5 $k_3$ is 7.1 x $10^{-5}$ $Lmol^{-1}s^{-1}$, comparable with the rate of hydrolysis at the same temperature (Tab. 1), and increases to its maximum observed rate, 7.4 x $10^{-3}$ $Lmol^{-1}s^{-1}$ at pH 8.2 the pKa of peracetic acid (Yuan et al., 1997a). This indicates that at low pH, for an equilibrium mixture spontaneous decomposition of PAA is unlikely to cause

significant changes in the concentration of PAA compared with the rate of formation and hydrolysis, thus giving a stable quantity of PAA over time.

During the decolouring reaction PAA is consumed by the protein and due to the presence of the sulphuric acid catalyst in the commercial PAA used, the depletion during the reaction may lead to the renewal of PAA to begin to re-establish the equilibrium concentration.

## Experimental

### Reagents

*Decolouring:* Bloodmeal (Agricultural Grade, Wallace Corporation Ltd), Peracetic acid (Proxitane Sanitiser 5%, Solvay Interox Pty Ltd).
*Volumetric analysis:* Analytic grade reagents ~ 0.1molL$^{-1}$ ammonium iron (II) sulphate hexahydrate, (Merck), ~0.2 molL$^{-1}$ ceric sulphate prepared in 0.05 molL-1 sulphuric acid, Ferroin indicator, Starch Lintner's (BDH Chemicals Ltd), ~0.05 molL$^{-1}$ potassium dichromate, hydrochloric acid, sulphuric acid, 10 wt% potassium iodide, iodate free, sodium hydroxide, ~0.01-0.02 molL$^{-1}$ sodium thiosulphate (Ajax Finechem Pty Ltd), potassium iodate (May & Baker Ltd), sodium carbonate decahydrate (Riedel-de Haen), phenolphthalein (Labchem Pty Ltd).

### Procedure

*Decolouring:* The effect of PAA concentration was investigated by using a constant ratio of PAA solution to bloodmeal (300 g PAA: 100 g BM) at prepared at ~1, 2, 3, 4 and 5 wt% PAA with distilled water. Under these conditions BM was treated using a variable molar ratio of PAA to BM as indicated in Table 2.

To further investigate the effect of concentration, BM was also treated at a constant ratio of 2.2 mmol PAA per gram of BM, at ~1 to 5 wt% PAA solution (Table 2). All samples were mixed for 10 minutes, bought to pH 7 by sodium hydroxide and oven dried overnight at 75 $^{o}$C to determine colour change by use of a chromameter.

Tab.2: Preparation of decoloured bloodmeal, molar quantity and concentration are given from volumetric assay. The pH of the PAA solutions is 1-2.8.

| Experiment # | Actual PAA Concentration (wt%) | Quantity PAA (mmol) | Quantity HP (mmol) | Quantity AA (mmol) | Mass PAA solution (g) | Ratio of PAA solution: BM (g/g) |
|---|---|---|---|---|---|---|
| 1 | 1.1 | 45.85 | 455.10 | 107.51 | 300 | 3 |
| 2 | 2.5 | 97.70 | 923.93 | 215.60 | 300 | 3 |
| 3 | 3.6 | 142.87 | 1587.91 | 323.28 | 300 | 3 |
| 4 | 4.5 | 177.15 | 1885.50 | 425.17 | 300 | 3 |
| 5 | 5.6 | 220.89 | 2292.06 | 539.16 | 300 | 3 |
| 6 | 1.1 | 22.09 | 229.21 | 53.92 | 150 | 15 |
| 7 | 2.5 | 22.09 | 229.21 | 53.92 | 75 | 7.5 |
| 8 | 3.6 | 22.09 | 229.21 | 53.92 | 50 | 5 |
| 9 | 4.5 | 22.09 | 229.21 | 53.92 | 37.5 | 3.75 |
| 10 | 5.6 | 22.09 | 229.21 | 53.92 | 30 | 3 |

Filtered wastewater from the experiments conducted was subjected to volumetric analysis to determine HP, PAA and AA content.

*Recycling:* Wastewater from bloodmeal bleached with ~4 and 5 wt% PAA was diluted with water in a ratio of 3:2. Dilution was required for treatments using 4 and 5 wt% due to swelling of the bloodmeal making filtration difficult. The PAA wastewater was used to decolour bloodmeal in the same ratio as the initial bleaching step. The bloodmeal decoloured using recycled wastewater was then analysed for colour change, as above.

*Volumetric analysis:* All regents were prepared in water unless otherwise stated. A method of analysis using ceric sulphate and sodium thiosulphate was established based on a previously reported method (Greenspan and MacKellar, 1948). A sample of PAA solution was accurately weighed to 5 d.p. (0.1-2.0 g) and placed in a 500 mL Erlenmeyer flask containing 5 wt% sulphuric acid (150 mL) and sufficient cracked ice to maintain a temperature of 0 to 10 $^{o}$C. Three drops of ferroin indicator were added and the flask contents were titrated with a standardised ceric sulphate solution (~0.1-0.2 molL$^{-1}$) containing 0.05 molL$^{-1}$ sulphuric acid, to the disappearance of the salmon colour of the indicator. Upon completion, 10 wt% potassium iodide solution (~10 mL) was added and the liberated iodine titrated with standardised sodium thiosulphate (~0.01-0.02 molL$^{-1}$). Starch indicator was added near the end point for the thiosulphate titration. Error limits have been based upon the highest experimental error calculated for the series of titrations

*Colour change:* The quality of decolouring was determined by monitoring the relative colour change using the Chromameter CR-410 (ThermoFisher Scientific) for bloodmeal exposed to solutions of PAA in the concentration range of ~1 to 5 wt%. The dried samples were ground and sieved to 1 mm, pressed flat, and analysed with the chromameter. Results have been presented as percentage whiteness, (the sum of the R G B values as a percentage of total white, 765).

## RESULTS AND DISCUSSION

### Decolouring

Decolouring was first investigated using a constant ratio of PAA solution to BM (3:1) at increasing PAA concentration (Figure 1).

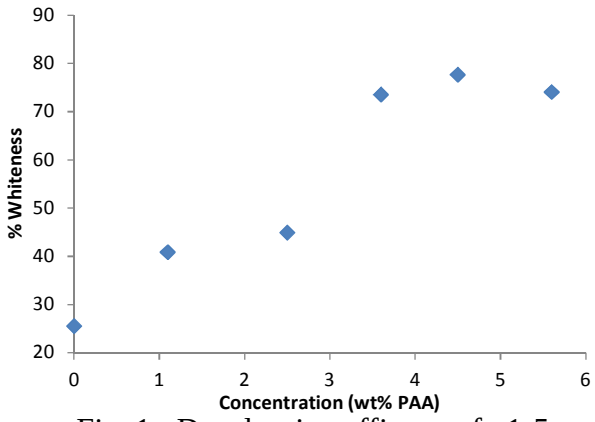



Fig. 1: Decolouring efficacy of ~1-5 wt% PAA, in a ratio of 3:1 PAA solution: BM.

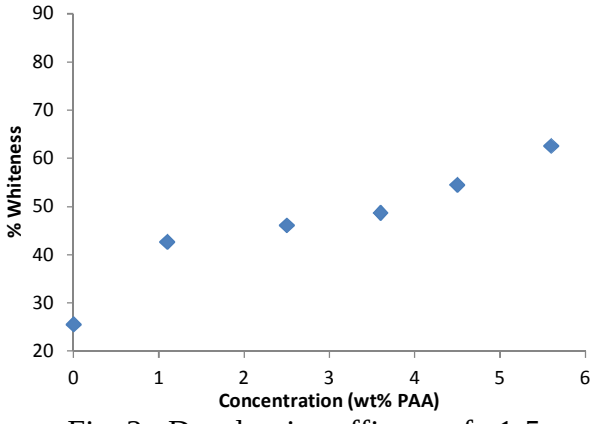



Fig. 2: Decolouring efficacy of ~1-5 wt% PAA, using ~2.2 mmol PAA per gram of BM.

It was found that the percentage whiteness improved with increasing PAA, but plateaued after about 3 wt%. There is a large shift in the obtained percentage whiteness occurring between 2.5 and 3.6 wt% PAA, potentially indicating a critical point and perhaps a change in kinetics; this is discussed further below. The plateau beyond this

point would suggest that above 3 wt% any further excess of PAA is redundant. In this experiment the ratio of PAA to BM was varied, and to assess the effect of this ratio, a constant molar ratio was also considered at different dilution ratios (Figure 2). At a constant PAA: BM ratio, an almost linear increase in whiteness was observed with increasing concentration.

Peracetic acid can be consumed in four different classes of reactions:

1. Decomposition reactions (degradation/hydrolysis)
2. Protein oxidation (cross-linking, fragmentation, side-chain modifications)
3. Haem degradation
4. Deodourising

The major products of these reactions include modified proteins, acetic acid, carbon dioxide and oxygen. It was observed during the decolouring reaction that the mixture foams, indicative of evolved gases.

Protein oxidation is thought to be caused by the formation of active oxygen species (AOS) from PAA and HP. These highly reactive AOS react almost instantaneously at the site of formation and lead to non-specific changes in the protein substrate (Kitis, 2004, Liberti et al., 2000, Sanderson, 1995). If the AOS access the haem moiety, they induce decolouring by randomly attacking the carbon methene bridges in the haem tetrapyrrole producing various pyrrole products and releasing inorganic iron (Nagababu and Rifkind, 2004).

During this time, bloodmeal is also deodorized. This is thought to occur by partial dissolution of the odorous compounds into the reaction mixture followed by their oxidation by PAA and HP to highly soluble compounds (Dell'Erbaa et al., 2007, Hei et al., 2001), and these together with the liberated inorganic iron, are removed upon decanting.

From the combined results (Fig.1 and Fig.2) it can be concluded that the ratio of PAA to BM is important and sufficient excess was required to overcome competing reactions that involve PAA and achieve decolouring. This excess quantity of PAA appears sufficient for decolouring with ~3 wt% PAA.

## Peroxide Consumption

The consumption of the reagents when using ~1 to 5 wt% PAA was determined volumetrically and the results are presented in Figure 3. Both HP and PAA are consumed in greater quantities as the initial concentration increases. More HP was consumed than PAA although it has been shown that without PAA, HP is not capable of producing a well decoloured, bright BM product with or without the addition of glacial acetic acid. Therefore it is thought that HP is mainly consumed by competing reactions such as those involving protein oxidation and the removal of volatile organic compounds. Although the number of moles of both PAA and HP consumed increased, the percentage consumption decreased for both.

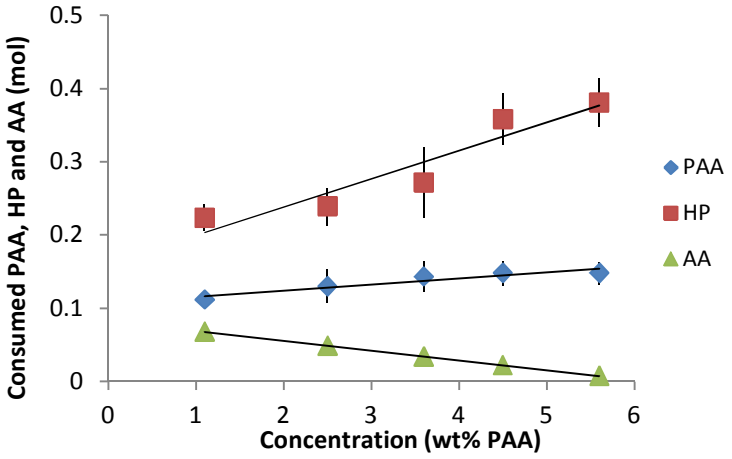



Fig. 3: Consumption of PAA, HP and AA per 100 g bloodmeal during decolouring.

Upon completion of the reaction of treatments using higher PAA concentrations there was still significant quantities of peracetic acid remaining. Although it would appear there is still peracetic acid remaining after treatment with 1.1 wt% PAA, the uncertainty is proportionally greater due to interference of the free inorganic iron with the volumetric analysis employed. At higher concentrations, this percentage uncertainty is much lower and it is shown that for decolouring to occur there must be sufficient excess of peracetic acid. Under the conditions employed more than 3 wt% PAA was required, which meant significant quantities of PAA were left unreacted (Table 3).

At 2.5 wt% PAA there is a sufficient number of moles present to enable reasonable decolouring, however it was found that leaving the reaction to continue overnight did not lead to a material with any better degree of decolouring. This suggested that at low concentrations of PAA and HP, peractetic acid is involved in competing reactions, such as protein oxidation and possibly decomposition and hydrolysis, as well as haem degradation.

Tab.3: Consumption of peracetic acid and hydrogen peroxide (mol) for 300 g PAA solution per 100 g bloodmeal during decolouring across 1 to 5 wt% peracetic acid.

| Wt% PAA | Peracetic Acid Consumed (%) | Hydrogen Peroxide Consumed (%) | Acetic acid Consumed (%) |
|---|---|---|---|
| **1.1** | 95 | 49 | 37 |
| **2.5** | 81 | 26 | 23 |
| **3.6** | 77 | 17 | 11 |
| **4.5** | 71 | 19 | 5 |
| **5.6** | 62 | 16 | 1 |

An interesting observation is the apparent decrease in AA consumption with increasing PAA concentration. Acetic acid is consumed by adsorption of the acetic acid onto, and possibly into the bloodmeal particle but this is partially offset by increased production during decomposition of PAA which itself increases at higher concentrations. Acetic acid is known to cause swelling of proteins by disrupting hydrogen bonding interactions within the protein, and forming new hydrogen bonds between the acetic acid molecule and the amine groups on the protein backbone as well as with the amino acid side chains (Puchtler et al., 1968, Yadav and Tyagi, 2006).

Assuming the end products of oxidation with PAA, including its hydrolysis and decomposition lead to the formation of acetic acid, then the acetic acid that has been sorbed by bloodmeal could be calculated as the total of the consumed PAA plus consumed acetic acid. This sorption curve is given as Figure 4.

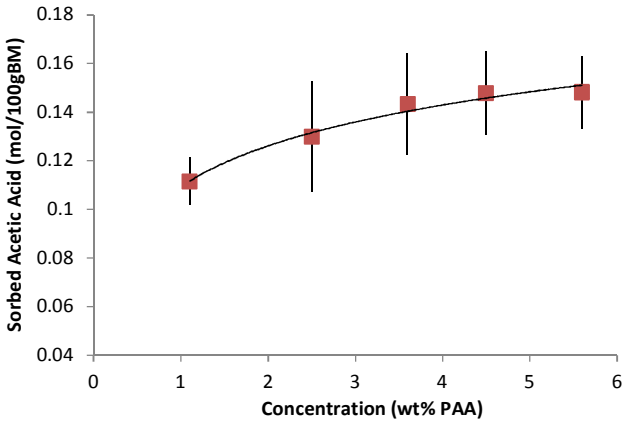



Fig. 4: Sorbed AA during decolouring with ~1 to 5 wt% PAA.

When comparing Figure 4 with that of the decolouring efficacy results from Figure 1, the plateau observed for percentage whiteness obtained occurring at ~3 wt% could be due to having reached maximum adsorption of acetic acid onto the bloodmeal (~1.4 mmol per gram of bloodmeal). This adsorption which has caused swelling of the protein chains gives peracetic acid greater access to the haem group, the kinetics of which will be determined by acetic acid concentration. At greater than ~3 wt% PAA, the concentration of acetic acid present is sufficiently high leading to rapid sorption and as mentioned earlier, this may explain the large shift in decolouring efficacy, and possible change in kinetics observed between 2.5 and 3.6 wt% PAA (Fig. 1).

Despite having recoverable quantities of PAA remaining in the reaction mixture upon completion of decolouring, protein swelling caused by acetic acid made its recovery by filtration or centrifugation difficult. For recycling to occur, the wastewater must be diluted by a minimum of 3:2, or dewatered using a press.

## Recycling and Economics

Decolouring has been quantified using percentage whiteness as well as using the RGB values (Table 4).

Tab.4: Quality of decolouring for 1-5 wt% PAA treatment of bloodmeal, the percentage of original wastewater recovered and the quality of decolouring obtained from recycling the recovered wastewater.

| | First pass | | | | Second Pass | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Wt% PAA | R | G | B | % Whiteness | % waste-water collected | R | G | B | % Whiteness |
| **0** | 79 | 52 | 64 | 26 | | | | | |
| **1.1** | 140 | 104 | 68 | 41 | | | | | |
| **2.5** | 153 | 113 | 77 | 45 | | | | | |
| **3.6** | 226 | 201 | 135 | 73 | | | | | |
| **4.5** | 233 | 214 | 146 | 78 | 23 | 135 | 99 | 67 | 39 |
| **5.6** | 228 | 205 | 133 | 74 | 22 | 129 | 102 | 73 | 40 |

It was found that the quality of decolouring was low when using recycled wastewater, with decolouring comparable to a 1.1 wt% PAA treatment. This was due to dilution effects as concentration determines decolouring efficacy. However, without dilution, it would be possible to recover wastewater with a maximum of 1-2 wt % PAA from treatments using 4 and 5 wt% PAA.

Decolouring bloodmeal with commercial ~4 or 5 wt% PAA costs $20.19 and $25.24/kg bloodmeal, respectively. This cost can be reduced to approximately $3.11/kg bloodmeal by the on-site production of PAA from HP and AA (Wadi et al., 2010). With dilution and filtration to recover the PAA for recycling, between 6 and 8% of the cost of commercial PAA can be recovered. Using a dewatering press rather than filtration or centrifugation, recovery could be improved and on a larger scale could be used for further decolouring steps by the addition of fresh PAA solution.

## CONCLUSION

Peracetic acid is able to be consumed in four classes of reactions; decomposition, protein oxidation, haem degradation and deodourising. These reactions lead to the formation of modified proteins, acetic acid, carbon dioxide and oxygen. Decolouring efficacy was strongly influenced by the concentration of the PAA solution used, and a large excess of PAA was required for decolouring to occur. This is because PAA must be present in sufficient excess to overcome competing reactions.

For decolouring reactions using ~1 to 5 wt% PAA, the number of moles of both PAA and HP consumed increased with increasing initial concentration of PAA, however the percentage consumption decreased for both. At low concentration, it appeared that competing reactions were more favourable leading to minimal decolouring.

The apparent consumption of acetic acid throughout the decolouring reaction may be caused by sorption of the acetic acid by bloodmeal. The saturation of bloodmeal with acetic acid occurred at the same concentration as the plateau observed for decolouring efficacy (~3 wt%). This would suggest that decolouring occurs most effectively for

bloodmeal having reached maximum sorption of acetic acid (~1.4 mmol per gram of bloodmeal), and as sorption is more efficient at high concentration, leads to rapid swelling of the protein chain. However, as all of the equilibrium components were varied in the decolouring efficacy experiment, further investigation is required for the role of acetic acid to be ascertained.

For the decolouring reactions involving ~4 and 5 wt% PAA, the resulting swelling of the bloodmeal proteins lead to difficulties recovering the PAA wastewater for recycling. A dewatering press could be used to avoid dilution, giving wastewater with a maximum of 2 wt% PAA. It was found that using the diluted wastewater caused some decolouring of bloodmeal, and based on the filtration method, between 6 and 8% of the cost of PAA was recovered. The addition of fresh PAA to the collected wastewater will be necessary for future decolouring steps to give reasonable decolouring.

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