Real-time *in-situ* measurement of haemoglobin in wastewater
L. Sutherland-Stacey, R. Dexter, B. McWilliams and K. Watson

**ABSTRACT**

The meat processing industry generates large volumes of relatively high load wastewater. In New Zealand and Australia this wastewater is often pre-treated on site and then discharged to environmental waters or municipal sewers. Owing to the limited number of water quality parameters which can be measured in real-time it is often difficult for industry to optimise treatment processes or public bodies to monitor for water-quality compliance. Abattoir wastewater is often observed to be red in colour, owing to the presence of haemoglobin. Measurement of visible light absorption spectra of wastewater grab samples has for some time provided information about blood concentration. However such grab sampling techniques are piecemeal and cannot provide instantaneous time resolved signals which are required for process control or comprehensive monitoring. In this work an *in-situ* UV/VIS spectrometer is used to continuously determine the concentration of haemoglobin in wastewater arriving for treatment at two different Wastewater Treatment Plants (WWTPs). The data is of high temporal resolution—data recorded at the distant WWTPs allows for identification process events, such as the end of shift wash downs.

**Key words** | abattoir, haemoglobin, in-situ, UV/VIS, wastewater

**INTRODUCTION**

The meat processing industry generates large volumes of high strength wastewater. It has been estimated that about one cubic metre of water is required to process a cattle beast (Russel *et al.* 1991). In New Zealand slaughterhouse wastewater is often discharged into municipal sewers or directly to waterways, the ocean or applied to land.

When discharged to the environment the high load wastewaters from abattoirs can cause degradation of surface water quality (Cooke *et al.* 1980; Sangodoyin & Agbawhe 1992). Application of slaughterhouse wastewater to land allows valuable nutrients to be re-used by plants but excessive application can lead to groundwater contamination (Russel *et al.* 1993).

As a contributor to municipal sewer networks abattoir wastewater can at times constitute a significant fraction of the total volume of wastewater. This load can be detrimental to wastewater treatment systems as it increases nitrogen load and oxygen demand. It can be a significant challenge for Wastewater Treatment Plants (WWTPs) which receive slaughterhouse waste to deal with the short and long term fluctuations inherent to industrial processes.

Monitoring of water quality associated with slaughterhouse wastewater treatment processes and discharges is generally undertaken by grab or composite sampling, according to standard analysis techniques. This methodology does not capture the high temporal variability which inevitably exists in complex industrial discharges and hence makes it difficult to implement smart wastewater treatment strategies. Nor do standard tests allow for identification of times when discharges from industrial pollution are significant in waterways or municipal sewerage systems. It is therefore desirable to develop a real-time *in-situ* method.
for quantifying contributions from slaughterhouses against complex and time-varying background water matrices.

The UV/VIS spectra of various components of biological fluids are well defined in medical and analytical literature. Haemoglobin, or red blood, absorbs light strongly around 410 nm in the so-called Sorret Region and more weakly between 500 and 600 nm. The exact spectral shape varies between the common derivatives of haemoglobin such as deoxyhaemoglobin, oxyhaemoglobin, carboxyhaemoglobin and methaemoglobin (henceforth abbreviated Hb, O₂Hb, COHb and Hi respectively). Measurement of visible light absorption of wastewater grab samples at single wavelengths has for some time been an accepted methodology for determining haemoglobin concentration (Cooper 1976). With the advent of submersible field spectrometers it is natural to extend UV/VIS methods to automated monitoring.

Archive datasets collected with in-situ UV/VIS spectrometers were available from wastewater streams which were known to carry regular loadings from abattoirs. Preliminary examination of these spectra revealed strong absorption peaks at 280 and 410 nm and weaker peaks between 500 and 600 nm at times. Characteristic spectra from the datasets known to carry abattoir loads (Figure 1) compared qualitatively with those of haemoglobin from literature.

In this work a methodology for the de-convolution of the UV/VIS absorption spectra of haemoglobin from wastewater is developed. An empirical model of the UV/VIS spectra of wastewater accounting for the apparent absorption due to light scattering by particles and the absorption by haemoglobin is proposed. The difference between model coefficients and a series of observed spectra is then minimised to generate time series of haemoglobin concentration and exclude broad signals from the background wastewater matrix.

**METHODOLOGY AND METHODS**

**Equipment**

In this work UV/VIS measurement are made with a submersible field spectrometer (s::can Messentechnic GmbH, Austria), installed directly in the wastewater stream. The spectrometers used had a path length of 2 mm and recorded the absorption spectrum between 200 and 720 nm every 2 minutes. More information regarding the instrument can be found in the literature (e.g Langergraber et al. 2003).

**Field sites**

Historic datasets of time resolved UV/VIS spectra were available from a large number (c2) of WWTPs in New Zealand, Australia and Europe. Field work had been undertaken at these WWTPs to characterise the wastewater Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and nitrate (NO₃) concentration according to established methodologies (e.g Rieger et al. 2004). The datasets were not collected with the intent of measuring haemoglobin. A subset of these sites received non trivial industrial contributions which made instrument calibration for COD measurement difficult. Two WWTPs from this smaller dataset were associated with abattoirs and are considered in this work.

Site A was the inlet structure of a WWTP which received the wastewater of domestic and industrial origin from the city of Timaru, New Zealand. Indicative ranges for commonly quoted wastewater parameters were 300–1,500 mg/l TSS and 1,000–7,000 mg/l COD. Large volumes of animal blood enters the sewer from an upstream slaughterhouse. The plant has since been replaced by a
Site B is the inlet structure at the Clive East wastewater treatment plant in Hastings. At the outset of monitoring in 2005 the site received wastewater from a combined industrial and domestic sewer. Influent characterisation at that time returned values of 100–700 mg/l TSS, 0–3 mg/l NO3, 200–2,000 mg/l COD, with significant variability attributed to variable industrial contributions. Civil works were undertaken between 2006 and 2007 to separate the industrial and domestic streams.

**Instrument calibration**

Owing to the retrospective nature of the analysis, a calibration procedure was required to estimate the relative haemoglobin concentration without resorting to standard Partial Least Squares (PLS) calibration techniques, which require corresponding analytical tests.

Human haemoglobin derivative spectra spanning the measurement range of the spectrometer are more readily available from medical sources than animal haemoglobin spectra and hence are used in this study. For this work it is assumed that the haemoglobin absorption spectra relevant to the meat industry does not vary significantly from that reported for humans or from animal to animal. This assumption is consistent with the results of Zijlstra & Buursma (1987, 1997) and Zijlstra et al. (1991, 1994), who observed only slight variations. Also the change in spectra with pH are neglected as they have been observed to be small (Wimberley et al. 1988; Fogh-Andersen et al. 1990).

Some success has already been reported in the use of curve fitting techniques to extract information from wastewater UV/VIS spectra when the target species are well understood (Sutherland-Stacey et al. 2008). A curve fitting model was developed to fit the expected apparent absorption due to scattering from suspended material (after Huber & Forst 1998 and Langergraber et al. 2003) and the absorption of various haemoglobin derivatives. Absorption of visible light by other dissolved components that are present in wastewater is not included in the model under the assumption that their contribution is relatively small in the visible region and has a similar shape to the light scattering curve. Analysis of the larger ($c^2$) dataset showed that the majority of variability in the visible region has a shape consistent with apparent absorption due to scattering.

It was found that only one haemoglobin derivative spectra was chosen by the model for either site. It is expected that particular characteristics of the wastewater network tend to stabilise haemoglobin into one or other of its derivative states. This observation significantly reduced the complexity of the minimisation, which took the form of Equation (1).

$$\text{Abs}(\lambda) = A\lambda^{-D} + C\epsilon_{\text{Hb}}(\lambda)$$

Equation (1). Model of the haemoglobin and wastewater spectrum between 400 and 700 nm used in this paper. The first term accounts for light scattering and the second for the absorption due to haemoglobin where $\epsilon_{\text{Hb}}(\lambda)$ is the extinction coefficient for the particular haemoglobin derivative present which varies as a function of wavelength $\lambda$.

Initial estimates of the coefficients were obtained to reduce the number of iterations required to obtain a good fit between the observations and the model. Guesses of $A$ and $D$ were obtained by a least-squares regression over the region of the visible spectrum only marginally affected by haemoglobin absorption. To accelerate the fitting procedure initial guesses of $C$ were obtained from the previous $(t − 1)$ fit, observing that new spectra tended to be correlated with the previous measurement.

The MATLAB® (The MathsWorks Inc, Nitack Massachusetts, United States of America) function fminsearch.m which, uses the simplex search method of Lagarias et al. (1998), was then employed to minimise the problem with $C$ constrained to be positive. We found that the residuals were more uniform across the wavelength dimension if the minimisation was also allowed to search for a fixed offset of up to 2 nm in the reference haemoglobin spectra. A systematic bias of this magnitude in observations of the location of peak absorption is consistent with the manufacturing precision of the instrument. A graphical example of the information obtained from the minimisation process is presented in Figure 2.

An estimate of the goodness of fit was obtained by considering the residual variation not explained by the model. In most cases this was below 2% and almost never
observed to be large enough to indicate significant disagreement between the modelled and observed spectra.

As the minimisation scheme is numerically demanding a simpler PLS calibration has been developed employing the curve fitting results as training data (after methods described in Sutherland-Stacey et al. 2008). The PLS can be run on the small field control computer and provide a real-time output to plant operators or end users.

![Figure 2](https://iwaponline.com/wst/article-pdf/60/7/1683/448612/1683.pdf)

Figure 2 | Top left: time resolved UV/VIS spectrum of a wastewater stream with O2Hb contribution. The spectra has been post-processed to remove the first term in Equation (1) to aid viewing and four example measurements are highlighted (and labelled 1–4). Bottom: The example measurements (solid blue) are plotted with the model (dotted black and red for the first and second terms in Equation (1) respectively). Top Right: Time series of coefficients $C$, $A$ and $D$, (top to bottom), from Equation (1), $C$ is related to the concentration of O2Hb.
RESULTS AND DISCUSSION

Data from each site was treated according to Equation (1) to generate time series of haemoglobin concentration. In both the data sets the minimisation system converged readily and there appeared to be no interfering signals from other wastewater components.

WWTP inlet time series

Haemoglobin concentration ranged from 0 to over 100 mg/l at both WWTP sites. The minimisation scheme only required one haemoglobin derivative for each site, probably indicating that in-sewer processes or pre-treatment preferentially converts haemoglobin to a particular state. Characteristic time series from the WWTP sites can be found in Figure 3 and are discussed below.

Site A

At the WWTP inlet only O₂Hb was detected. Haemoglobin concentration is generally low at night but high during the day. At around 6 pm on weekdays concentration increases suddenly then drops away to zero. On Sundays haemoglobin concentration is usually lower. This pattern is not followed on Easter weekend (in 2005, the year this data was collected, Easter weekend fell on 27th March–1st April). Instead, when public holidays occurred, haemoglobin concentration was much lower and on the Easter Sunday haemoglobin concentration was unusually high. All these features are consistent with a daytime shift production with most work carried out on weekdays and followed by a daily final wash down. The plant operates at reduced capacity over public holidays accounting for the observed reductions in haemoglobin load.

The slaughterhouse upstream of Site A operates seasonally. In mid 2005 the plant shut for seasonal maintenance. Corresponding to the shut there is an absence of haemoglobin signal in the wastewater influent for this time period.

Site B

Haemoglobin discharge into the sewer appears to be similarly very low over the weekends and planned shut-downs due to public holidays (for example the Queens Birthday, which in 2005 was on Monday 6th June). At this site the minimisation scheme found a fit using only HHb. Monitoring at this site began in 2005 and is still under way at the time of publication. During 2006 and 2007 works were undertaken to separate the industrial and domestic streams. After separation was concluded there was a significant reduction in haemoglobin concentration,
although some is occasionally detected, suggesting there are still linkages between the two systems.

The variability in haemoglobin concentration observed at the inlets is noteworthy. Most widely used wastewater quality sampling methodologies involve grab sampling followed by laboratory based analysis. Spot sampling, no matter how thorough, cannot be expected to produce data at the temporal scales which were captured with this methodology.

**Other remarks**

The filtered COD (CODf) or filtered Biological Oxygen Demand (BODf) concentration associated with haemoglobin can be readily calculated from first principles as 0.16 \( \text{O}_2 : 1 \text{Hb} \). In the case of the WWTPs represented here, haemoglobin accounts for up to 20 mg/l additional CODf or BODf load. However it is almost certain that additional invisible load is associated with slaughterhouse effluent in the form of proteins which do not absorb in regions of the spectrum used in this study (Stoscheck 1990).

Existing factory CODf calibrations on the spectrometer used in this study do not account for absorption in the region of the UV/VIS spectrum where haemoglobin absorbs or correctly treat absorption from proteins. Therefore error of several 10s of mg/l is introduced into existing UV/VIS CODf calibrations in the presence of haemoglobin and proteins. Linear regression against the factory calibration for CODf at Site B (not surprisingly) returned statistics indicating no correlation even when the slaughterhouse was closed. It is suspected that a fish processing plant also contributed significant protein load during the plant shut, a contribution is observed in the UV region and has further disrupted the CODf calibration.

**CONCLUSION**

A new technique for real-time quantification of haemoglobin in wastewater has been demonstrated. The methodology has been tested over a data set spanning some two years of measurements and two different locations. Because this technique exploits a field tested commercially available probe it is immediately suitable for a variety of end users such municipal bodies and abattoirs.

Monitoring of discharges from the meat processing industry has perhaps been neglected for lack of a simple sensor system which is sensitive to slaughterhouse waste products. The authors hope the advent of such a system can be employed by technical users to affect changes to waste discharge practice and minimise losses.

**REFERENCES**


