Activated sludge bioassays for rapid biochemical oxygen demand.

A dissertation submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy

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For Jimi and Charlie,
Shine on you crazy diamonds.
- C. Montgomery Burns
Acknowledgements

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Finally I would like to thank my family and my ex-missus Tanya. You were very patient, thank you.
Statement of Originality

The material in this thesis has not previously been submitted for a degree in any University, and to the best of my knowledge contains no material previously published or written by another person except where due acknowledgement is made in the thesis itself.

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Mark A. Jordan

January 2015
Publications

This thesis consists of 3 published manuscripts, as Chapters 3, 4 and 5, which are co-authored with fellow researchers. My contribution to each co-authored paper is outlined at the front of each relevant chapter.


**Chapter 5:** Jordan MA, Welsh DT, Teasdale PR, Ubiquity of activated sludge ferricyanide-mediated BOD methods: a comparison of sludge seeds across wastewater treatment plants, *Talanta* 2014, **125**, 293-300.
Abstract

A number of recent studies have described new rapid biochemical oxygen demand (BOD) methods. However, most have not maintained the features that make the 5-day standard BOD assay particularly relevant to wastewater management – a high level of substrate bio-oxidation and use of wastewater treatment plant (WWTP) sludge as the biocatalyst. In a critical breakthrough, return activated sludge (RAS) from Coombabah WWTP, southeast Queensland, was successfully incorporated as the biocatalyst in a ferricyanide mediated-BOD (FM-BOD) bioassay. The bioassay was initially optimized for the measurement of highly variable and complex wastewaters, particularly trade wastes, by maximizing the analytical working range (10 – 170 mg BOD\textsubscript{5} L\textsuperscript{-1}) and extent of substrate degradation (96 ± 23% of measured BOD\textsubscript{5} oxidation). A highly significant correlation (n = 35; slope = 1.07; R = 0.95; incubation time = 6 h) was found between this RAS FM-BOD and standard BOD\textsubscript{5} assays using a range of real trade waste samples.

The activated sludge FM-BOD bioassay was re-examined with the goal of measuring low–mid range wastewaters (i.e. treated effluents and WWTP influents) that comprise the bulk of all BOD samples analyzed worldwide. All experimental parameters were re-optimized, primarily to improve the detection limit of the FM-BOD assay to approximate that of the standard BOD\textsubscript{5} assay (i.e. ≈2 mg BOD\textsubscript{5} L\textsuperscript{-1}). Primary influent sludge (PIS) from Coombabah WWTP was the most favorable sludge trialed, with the new bioassay having an analytical range of 2 – 40 mg BOD\textsubscript{5} L\textsuperscript{-1}. A highly significant correlation (n = 33; slope = 0.94; R = 0.96; incubation time = 3 – 4 h) was observed between the PIS FM-BOD and standard BOD\textsubscript{5} assays using a range of treated effluent, influent and grey water samples.

The industry-wide applicability of the new FM-BOD assays was investigated using activated sludge seeds from 11 diverse WWTPs in southeast Queensland. FM-respiration was proportional to substrate concentration for BOD standards and in most cases linear, particularly for the PIS FM-BOD assay. This demonstrated that the new FM-BOD assays can be seeded with activated sludge sourced from any WWTP, with very little modification, as the standard BOD\textsubscript{5} method can be. FM-respiration of real samples was calibrated with standard solutions using several different FM-BOD and BOD\textsubscript{5} sludge seeds in parallel; mean FM-BOD and mean BOD\textsubscript{5} concentrations compared very well. These results have confirmed that both FM-BOD assays may be applied widely throughout the wastewater industry.
The use of activated sludge with the previously developed FM-Tox assay was also investigated. Activated sludge IC\textsubscript{50} values for all inorganic toxicants studied compared very well with values from the research literature. Difficulties arose however when evaluating organic toxicants. In their presence, an unknown non-respiratory mechanism also produced an analytical response, thus masking any toxic effect. However, the toxicity of the organic toxicants was confirmed for the same sludge community using standard oxygen based respiration bioassays. Glutathione was investigated as a non-respiratory redox compound that is known to reduce ferricyanide. This was confirmed experimentally, however appreciable concentrations of glutathione were not recorded in activated sludge. These results require further investigation.

The RAS and PIS FM-BOD assays described above have demonstrated that in conjunction they may be used for simple, same-day BOD analysis of all typical wastewaters analyzed for BOD. At present the BOD\textsubscript{5} standard assay is the only recognized measure of BOD worldwide and as such the industry potential of the new FM-BOD assays is huge; this was demonstrated clearly by the successful use of sludge from 11 different WWTPs with both FM-BOD assays.
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ampere</td>
</tr>
<tr>
<td>ADS</td>
<td>Aerobic digester sludge</td>
</tr>
<tr>
<td>APHA</td>
<td>American Public Health Association</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BOD</td>
<td>Biochemical oxygen demand</td>
</tr>
<tr>
<td>BOD&lt;sub&gt;5&lt;/sub&gt;</td>
<td>5-day Biochemical oxygen demand assay</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>3,5-DCP</td>
<td>3,5-Dichlorophenol</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>E</td>
<td>Potential</td>
</tr>
<tr>
<td>E&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Standard reduction potential</td>
</tr>
<tr>
<td>E&lt;sub&gt;app&lt;/sub&gt;</td>
<td>Applied potential</td>
</tr>
<tr>
<td>ETS</td>
<td>Electron transport system</td>
</tr>
<tr>
<td>FADH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Flavine adenine dinucleotide</td>
</tr>
<tr>
<td>F:M</td>
<td>Food-microorganism-ratio</td>
</tr>
<tr>
<td>FM</td>
<td>Ferricyanide mediated</td>
</tr>
<tr>
<td>FM-BOD</td>
<td>Ferricyanide mediated biochemical oxygen demand</td>
</tr>
<tr>
<td>FM-Tox</td>
<td>Ferricyanide mediated toxicity</td>
</tr>
<tr>
<td>GGA</td>
<td>Glucose/glutamic acid</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>GSSG</td>
<td>Glutathione disulfide</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HACCP</td>
<td>Hazard analysis and critical control points</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>i</td>
<td>Current</td>
</tr>
<tr>
<td>i&lt;sub&gt;lim&lt;/sub&gt;</td>
<td>Diffusion limiting current</td>
</tr>
<tr>
<td>IC&lt;sub&gt;20&lt;/sub&gt;</td>
<td>Concentration causing 20% inhibition of the test population</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration causing 50% inhibition of the test population</td>
</tr>
<tr>
<td>IC&lt;sub&gt;80&lt;/sub&gt;</td>
<td>Concentration causing 80% inhibition of the test population</td>
</tr>
</tbody>
</table>
LC₅₀  Concentration causing 50% mortality of the test population
LOD   Limit of detection
LOEC  Lowest observable effect concentration
m     Milli (prefix)
M     Molar concentration
ML    Mixed liquor
MLSS  Mixed liquor suspended solids
MLVSS Mixed liquor volatile suspended solids
NADH  Nicotinamide adenine dinucleotide
n     Nano (prefix)
NOEC  No observable effect concentration
OD    Optical density
OECD  Organization for Economic Cooperation and Development
PB    Phosphate buffer
PIS   Primary influent sludge
R     Correlation coefficient
RAS   Return activated sludge
rcf   Relative centrifugal force
S     Standard deviation
SDS   Sodium dodecyl sulfate
SSVI  Stirred settled volume index
SVI   Sludge volume index
TCA   Tricarboxylic acid
TOC   Total organic carbon
UEWD  Upset early warning device
µ     Micro (prefix)
V     Volt(s)
WWTP  Wastewater treatment plant
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