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This is the submitted version of the following article:

Scholes, E., Verheyen, V., Brook-Carter, P. (2016) A review of practical tools for rapid monitoring of membrane bioreactors. *Water Research*, 102, 252-262.

Which has been published in final form at:

<http://doi.org/10.1016/j.watres.2016.06.031>

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A REVIEW OF PRACTICAL TOOLS FOR RAPID MONITORING OF MEMBRANE BIOREACTORS.

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ABSTRACT

The production of high quality effluent from membrane bioreactors (MBRs) arguably requires less supervision than conventional activated sludge (CAS) processes. Nevertheless, the use of membranes brings additional issues of activated sludge filterability, cake layer formation and membrane fouling. From a practical standpoint, process engineers and operators require simple tools which offer timely information about the biological health and filterability of the mixed liquor as well as risks of membrane fouling. To this end, a range of analytical tools and biological assays are critically reviewed from this perspective. This review recommends that Capillary Suction Time (CST) analysis along with Total Suspended and Volatile Solids (TSS/VSS) analysis is used daily. For broad characterisation, total carbon and nitrogen analysis offer significant advantages over the commonly used chemical and biological oxygen demand (COD/BOD) analyses. Of the technologies for determining the vitality of the microbial biomass the most robust and reproducible, are the second generation adenosine-5'-triphosphate (ATP) test kits. Extracellular polymer concentrations are best monitored by measurement of turbidity after centrifugation. Taken collectively these tools can be used routinely to ensure timely intervention and smoother operation of MBR systems

Key Words

MBR, Activated Sludge, process monitoring, CST, TOC/TNb, ATP,

Introduction

27 According to the Food and Agriculture Organisation of the United Nations (FAO) the volume of
28 wastewater produced globally is unknown, largely due to a lack of data in many countries
29 (FAO 2012). It is known however, that many hundreds of cubic kilometres of wastewater are
30 produced. This immense volume of wastewater represents both a potential hazard, and a
31 vital resource for humanity and the environment. In developed countries the bulk of this
32 wastewater is treated in centralised plants where pathogen and nutrient content are reduced
33 before it is either released into the environment or disinfected for reuse.

34 For over one hundred years the conventional activated sludge (CAS) process has been used
35 to treat both domestic and industrial wastewaters (Lofrano and Brown 2010, Tilley 2011).
36 This likely represents the largest use of industrial bioprocesses worldwide (Seviour and
37 Nielsen 2010). Many permeations of the CAS process have been implemented to suit varying
38 wastewater streams and local environmental conditions. CAS processes can suffer from
39 biological phenomena such as bulking and reduced settling in clarifiers which result in poor
40 quality effluent which contains suspended biomass (Jenkins, Richard *et al.* 2004).

41 The rise of membrane bioreactors (MBRs) over the last 15 years has been driven by the
42 desire for smaller plant footprints, higher quality effluent, the necessity of water reuse and
43 advances in membrane technology (Judd and Judd 2011). Although an aerobic MBR contains
44 an activated sludge process, effluent suspended solids issues are mitigated by membrane
45 separation. MBRs are an established technology now approaching maturity with 'fifth
46 generation' plants currently being built (Kraemer, Menniti *et al.* 2012). MBRs can however
47 fail to process the desired volume of wastewater if extreme caking, fouling or low biomass
48 filterability occurs (Judd and Judd 2011). Additionally due to the necessity of constant
49 membrane agitation, and chemical cleaning for removal of fouling, the capital and operational
50 costs of ownership of MBRs is higher than for CAS processes (Le-Clech 2010, Kraemer,
51 Menniti *et al.* 2012, Li, Yang *et al.* 2012).

52 Membrane fouling is typically categorised as either inorganic or organic, with the latter being
53 less well understood. A large proportion of the literature regarding MBRs concerns mixed
54 liquor properties and fouling propensity (Chang and Lee 1998, Rosenberger and Kraume

2002, Ng and Hermanowicz 2005, Pollice, Brookes *et al.* 2005, Rosenberger, Evenblij *et al.* 2005, Choi, Zhang *et al.* 2006, Le-Clech, Chen *et al.* 2006, Lebegue, Heran *et al.* 2008, Pan, Su *et al.* 2010, Tian, Chen *et al.* 2011, Ma, Wen *et al.* 2013). Research evidence strongly indicates that higher concentrations of extracellular polymers (ECPs) and / or soluble microbial polymers (SMP) are the keys to explaining low biomass filterability and a consequent high fouling tendency (Sheng, Yu *et al.* 2010). The literature is complex and marred by the variation in extraction methods and analytical techniques.

Given the uncertainties over operational issues affecting biomass filterability and the opex costs of MBR ownership, the question of how to best monitor the operation of an MBR and its biomass becomes more urgent. This review critically examines the range of analytical techniques (tools) now available for engineers and operators to monitor the biomass and treatment efficiency of an MBR. The techniques evaluated (Table 1 below) include both current analytical techniques as well as a range of newer techniques. In this review we have considered process chemistry methods for ion and sum parameter analysis and methods for the bulk assessment of solids. A particular focus is placed on the determination of extracellular polymers and viability and vitality by respiration indicators, dehydrogenase quantification and ATP measurement. Lastly, methods for microbial ecology are reviewed, with discussion limited to the number of techniques currently able to provide timely feedback.

Analysis	Main Application	Secondary Application	Notes
Ion Analysis			
Colorimetric	Compliance and process monitoring	n/a	Substantial historical use, well established parameters
Ion Chromatograph		Scale formation potential	Only viable for larger MBR plants
Sum Total Analysis			
COD	Compliance Monitoring Organics Reduction Evaluation & Mass Balance over processes	n/a	Substantial historical use, well established parameters
BOD		Influent toxicity monitoring	
TOC / TNb		Biomass C:N ratio monitoring	Emerging best available technology
Solids			
TSS/VSS	Inventory Management	Mass loading calculations	Substantial historical use, well established parameters
CST	MLSS Filterability & Dewaterability	MLSS Stress Response Detection	
Extra-Cellular Polymers			
Via Extraction	Fouling potential indicator	Tool for undersanding what operational conditions cause biological stress	Historical data is required to provide context for results
Via TOC			
Via Turbidity			
Biomass Viability and Vitality			
OUR	Biomass Health Assessment	Influent toxicity monitoring	Historical data is required to provide context for results
DHA - Tetrazolium		n/a	
DHA- Resazurin		n/a	
DHA - NADH		BNR DO control	
ATP (2nd Gen)		Biomass Stress Index (BSI) Active VSS ratio (AVSS)	BSI and AVSS comparable over different dates and treatment plants.
Ecology			
Microscopy	Filamentous bacteria monitoring	ECP monitoring	Historical data is required to provide context for results

74

75 Table 1: Evaluated analytical tools, their main and secondary applications in the operation of
76 an MBR.

77 The analytical tools identified in Table 1 have been evaluated from a practical standpoint, and
78 scored according to equipment and reagent cost, ability for point of testing use, ease of use
79 and repeatability and timeliness allowing for rapid (same day) management responses. See
80 Table 2 below for the scoring criteria and Table 3 for the results themselves. Although this
81 review is largely focused on MBR operation, many techniques are transferrable to the
82 operation of CAS plants.

83 Having placed a focus on the use of analytical tools in everyday plant operation, the review
 84 does not include the time consuming methods typically employed by researchers. For a
 85 review of molecular techniques in use for wastewater treatment the reader is directed to
 86 (Sanz and Köchling 2007, Seviour and Nielsen 2010). For the use of flow cytometry (Díaz,
 87 Herrero *et al.* 2010, Davey 2011) and for advanced image analysis of biomass (Costa,
 88 Mesquita *et al.* 2013). Moreover, this review does not cover the use of conventional on-line
 89 membrane engineering parameters such as monitoring of flux, permeability and trans-
 90 membrane pressure. For information on these see either the MBR Book or WEF Manual of
 91 Practice No 36 (Judd and Judd 2011, Water Environment Federation 2012). This review also
 92 omits the basic measurements of pH, Conductivity and Temperature, as they are assumed to
 93 be ubiquitous in all water labs.

94 NOTES ON THE CRITERIA FOR EVALUATION

95 Tools are evaluated under six categories, detailed in Table 2. Each category is rated 1 to 5
 96 with 5 being the best.

Score Awarded	Reagents (\$AUD per test)	Equipment (\$AUD per test)	Location	Training	Confidence and Utility	Time
1	>101	>100,001	Large research facility (at a university or commercial provider of this service)	Requires full time personell dedicated to this equipment	Low repeatability (low confidence data) or results difficult to use without extensive research and prior results	>7 days
2	51-100	50,001-100,000	Large equipment, or requires a dedicated room (eg dark or clean rooms)	Requires scientifically trained personnel to oversee operation	Less repeatability (variation ~20%) or less usable results, where meaning of the test results is ambiguous	2-7 days
3	11-20	10,001-50,000	Well equipped on site laboratory (e.g. fume cupboard, incubators)	Requires extensive training. Best run by regular users	Moderate repeatability (variation ~15%), or where data is only suitable to guide operational decisions in context of past results	up to 1 day
4	6-10	5001-10,000	Larger portable equipment or basic on site laboratory	Can be performed by operators with moderate training	Reasonable repeatability (variation ~10%), gives clear process information	<1 hour
5	0-5	<5000	On site on portable or requires ubiquitous instrumentation	Can be performed by operators with little training	Highly repeatable (variation ~5%), suitable for immediate action/use	<10 mins

98 Table 2: Scoring matrix of key criteria detailed in the text

99 Cost of Reagents & Capital Cost of Equipment

The first two categories concern cost of the analysis. Firstly, the cost of consumable reagents and secondly the capital investment required. The lower these costs, the more likely the tests are to be incorporated into routine analysis. These costs may vary significantly depending on manufacturer, specification, volume purchased, purchasing power and location of purchase. The prices are in the experience of the reviewers, as very little in the way of price information is available publically. One exception is Hach Australia (<http://au.hach.com/>) where the recommended retail prices for most common wastewater equipment is available without signing in as an account holder. Please note, Hach do not sell all the analytical tools discussed in this review, nor are their products endorsed by the reviewers. Additionally, the retail prices given are likely to be higher than that actually paid by account holders. That being said, our ratings of cost have been given using these prices as a guide. Where cost of reagents vary significantly enough that they span several categories (for example colorimetric kit chemistry fall both below and above the \$5 mark) half points will be used.

Location

In terms of producing data which can be used on the day of sampling, technologies which can be used onsite are clearly the most beneficial. The location score rates the requirements of the technology in terms of the sophistication of the laboratory required.

Time

The time required to perform each analysis or prepare it for automated processing is another determinant of suitability for routine use.

Training

Analysis which requires significant scientific expertise to perform is less desirable than that which are straightforward to use. To this end, procedures which are simple and little training is required are more highly rated.

Confidence and Utility

This category is designed to distinguish between tests with low repeatability and little clear operational meaning, and those which can be used with confidence and have well defined operational responses.

THE EVALUATION RESULTS

Analysis	Reagent Cost	Equipment Cost	Location Score	Time Score	Training Score	Confidence and Utility	Total Score
Ion Analysis							
Colorimetric	4.5	3.5	5	4	4	4	25
Ion Chromatograph	5	2	4	4	3	5	23
Sum Total Analysis							
COD	5	4	5	3	4	4	25
BOD	5	3.5	3	2	3	2	18.5
TOC / TNb	5	2	4	5	3	5	25
Solids							
TSS (MLSS) /VSS	5	4	5	4	4	5	27
CST	5	5	5	5	5	4	29
Extra-Cellular Polymers							
Via Extraction	4	3.5	3	3	3	3	19.5
TOC based bulk Characterisation	5	2	4	5	3	3	22
Turbidity based bulk Characterisation	5	5	5	5	5	3	28
Biomass Viability and Vitality							
OUR	5	4	4	5	3	3	24
DHA - Tetrazolium	3	3	2	3	2	3	16
DHA- Resazurin	5	4	4	4	4	3	24
DHA - NADH	3	3	2	3	2	3	16
ATP (2 nd Gen)	3	5	5	5	4	5	27
Ecology							
Microscopy	5	4	4	5	3	3	24

Table 3: Results of the Evaluation of MBR monitoring techniques against key criteria

INFLUENT / FILTRATE

CHEMISTRY

An established set of analytical chemical tests are routinely used to monitor both plant influent and filtrate to ensure that the required chemical transformations and nutrient removals are taking place. Without examining the internal process, data obtained from this ‘black box’ approach to process chemistry is able to give an operator confidence that there

are no major process upsets and that the effluent is likely to meet regulatory requirements for discharge. Typically, the analysis of plant influent and effluent include determination of various ions (e.g. NH_4^+ , PO_4^{2-}) and sum total measurements (e.g. COD and TOC)

ION ANALYSES

Determination of nitrogen and phosphate ion concentrations is frequently carried out via colorimetric analysis using commercially available test kits. Whilst these kits are a quick and simple option, large or complex processes can require multiple parameters to be tested on many samples. In this case, the economic cost of test kit use can multiply rapidly given the cost and time involved in ordering, shipping, storing, actual use and finally disposal (Table 3). Different chemistries are available from a variety of suppliers at a range of costs typically between \$2-\$6AUD per test. The environmental cost of test kit use can also be quite high where vials are packaged in foam boxes or where the reagents themselves include hazardous chemicals.

A recommended alternative for high throughput, is the ion analysis using ion chromatography (IC). IC offers lower limits of detection (APHA, AWWA *et al.* 2005) and interference free analysis for highly coloured or sulfide containing waters, for which colorimetric determination is often unsuitable. IC can also quantify the full range of major cations (Li^+ , Na^+ , NH_4^+ , K^+ , Ca^{2+} and Mg^{2+}) and anions (F^- , Cl^- , Br^- , NO_2^- , NO_3^- , PO_4^{2-} , SO_4^{2-}) and can also be used to detect transition metals such as iron and manganese (Cardellicchio, Ragone *et al.* 1997). Although the capital expense of ion chromatography is significant, the advantages of having comprehensive on-site ion analysis are valuable for membrane processes which have a risk of (inorganic) scale formation. The ability to quickly check the Langelier Saturation Index (LSI) or Calcium Carbonate Precipitation Potential (CCPP), and adjust or initiate acid dosing could save many tens of thousands of dollars in cleaning chemicals, extend membrane life and reduce lost process time (Jefferies and Comstock 2001). Preparation of samples for IC is similar to that of test kit chemistry, with sample filtering and dilution of concentrated wastewaters. Through the use of an auto-sampler, IC determinations can proceed without the need for the continual attention of an analyst,

166 reducing staffing costs. The actual instrument time taken for the determination of each
167 sample depends on the length of the columns used, but typically this would be between 15
168 and 30 minutes. Although ion analysis is important, the need to characterise plant influents
169 and reductions across a processes, necessitates the use of 'sum total' analysis.

170 ***SUM TOTAL ANALYSES***

171 Sum total analyses are tests which seek to give an overall assessment of a sample. These
172 typically include BOD₅, COD and TOC. They are often used for estimating the organic load or
173 removal efficiency of a process (Frimmel and Abbt-Braun 2011). BOD₅ limits frequently
174 feature in regulatory requirements (Higgins, Warnken *et al.* 2004). Conversion factors to
175 determine one parameter from another (for example BOD from a COD measurement) should
176 be used with extreme care and are not valid across different wastewater types (Aziz and
177 Tebbutt 1980).

178 BOD₅

179 Biological oxygen demand (BOD₅) is a test of how much oxygen is consumed in a five-day
180 period by microbes inoculated into a sample. It is thus a relatively crude indicator of the
181 degradable material in a sample and is dependent on the viability of the microbial population
182 seeded. BOD₅ has a substantial historical use (Jouanneau, Recoules *et al.* 2014), and is
183 valuable for quantifying the potential biodegradability of a sample. For daily plant operation
184 BOD₅ is impractical due to the five day incubation (and thus scores 2 for time) (Henze and
185 Comeau 2008). Interferences to BOD can include ferrous iron, sulfides or reduced nitrogen
186 compounds (Frimmel and Abbt-Braun 2011). In the latter case, the 'carbonaceous BOD' can
187 be established by suppression of the nitrogenous bacteria with nitrification inhibitors.
188 Unsurprisingly for a microbially mediated analysis, the variation in BOD₅ values for cross
189 laboratory studies is up to 20% (Jouanneau, Recoules *et al.* 2014) and therefore scores 2 for
190 confidence. For BOD₅ analysis of wastewaters with unusually complex or toxic properties, the
191 use of standard microbial inoculum is likely to lead to under reporting due to the absence of
192 metabolic capabilities required to degrade that sample (Jordan, Welsh *et al.* 2014). Despite
193 the drawbacks of BOD it is useful to compare the COD:BOD₅ ratios of plant influent over time.

194 This can indicate a change in the treatability, or toxicity of the influent. Reagent costs are
195 minimal (score 5), equipment requirements include incubators and dissolved oxygen or
196 pressure measurement devices (score 3.5 for cost and 3 for location). The total score
197 accorded to conventional BOD is 18.5. The search for a replacement technology which
198 directly measures BOD₅ in a short time-frame or which can be used as an on-line sensor
199 continues. Numerous faster methods, most popularly microbial fuel cells (MFC) (Abrevaya,
200 Sacco *et al.* 2015) have been developed, and some commercialised (Namour and Jaffrezic-
201 Renault 2010). As yet none of these has seen widespread uptake by industry possibly due to
202 insufficiently rugged designs or unrealistic maintenance requirements.

203 COD

204 Chemical Oxygen Demand (COD) is a measurement of the concentration of organic
205 compounds able to be oxidised using heat, dichromate and sulfuric acid. This test gives
206 results in under three hours (therefore a time score of 3). Reagents are generally cheap
207 amounting to \$1-\$2AUD a test (Reagent cost rate of 5) but disposal costs may also be in the
208 same order of magnitude.

209 COD as a measurement of organic load can be artificially high in the presence of reactive
210 inorganic species such as Fe²⁺ (Frimmel and Abbt-Braun 2011) or high levels of halides.
211 Despite this, COD is the most commonly used method of assessment of oxidation demand (da
212 Silva, da Silva *et al.* 2011). Whilst interference due to oxidation of inorganic compounds may
213 not be a significant issue for domestic wastewaters, industrial wastewater may contain high
214 concentrations of inorganic compounds in a reduced state (e.g. H₂S). In an aerobic MBR
215 process H₂S will be oxidised to form SO₄²⁻ ions. Therefore, an organic mass balance based on
216 COD will require influent and effluent measurement of the various sulfur species. For these
217 reasons confidence and utility are rated a 4. Despite these shortcomings, COD is the most
218 commonly used method of assessment of oxidation demand (da Silva, da Silva *et al.* 2011).
219 The equipment required is a heating block and a spectrophotometer (score of 4) and is able
220 to be done in even basic laboratory spaces (location score 5). The training required relatively

221 little however OHS&E aspects have to be stressed (training score 4). In our evaluation
222 summary (Table 3) COD scores 25/30.

223 TOC

224 Given the shortcomings of BOD₅ and COD measurement there is growing movement to
225 directly analyse Total Organic Carbon (TOC) compounds in wastewater (Aziz and Tebbutt
226 1980, Thomas, El Khorassani *et al.* 1999, Bisutti, Hilke *et al.* 2004, Gray 2010). The method
227 utilised for the widest range of TOC analysis is High Temperature / Infrared (SM 5310 B),
228 however for clean waters (ie MBR filtrate) the UV/persulfate method (SM 5310 C) has lower
229 limits of detection (APHA, AWWA *et al.* 2005). TOC analysers which include simultaneous
230 measurement of total 'bound' nitrogen (TNb) are available, making this a very attractive
231 primary instrument for larger treatment plant labs. It should be noted that not all TOC
232 analysers are sufficiently robust to handle samples with particulate material (Vanrolleghem
233 and Lee 2003, Visco, Campanella *et al.* 2005), therefore samples run on these instruments
234 require 0.45µm filtration prior to TOC analysis. The results would therefore more accurately
235 be called *total dissolved organic carbon* (dTOC) rather than *total organic carbon* (TOC). More
236 robust instruments with the ability to perform both TOC and dTOC will produce (via
237 difference) the particulate TOC (pTOC), and therefore a much more complete picture of
238 carbon movement and degradation through a treatment system. Additionally, the ability of a
239 TOC analyser to handle particulate material means that it can be used to quantify the TOC
240 (and TN if fitted) of both process waters and the biomass solids itself. This latter ability is
241 useful to identify variations in normal carbon to nutrient ratios which may result in greater
242 microbial polymer production and membrane fouling rates (Wang, Han *et al.* 2013). Whilst
243 the confidence and utility are high (score 5), the capital cost of TOC analysis is the most
244 significant detractor (score 2) however the cost of consumables is a minimal amount of high
245 purity oxygen (score 5). Laboratory requirements are a benchtop space, access to bottle
246 oxygen and reasonable grade pure water (Location Score 4). The training required is rated a
247 3 but is dependent on the product and its software. Sample preparation is quick (filtration for
248 dissolved samples, dilution for sludges) and the use of an autosampler means many analyses

249 can be performed over a day without need for operator intervention (time 5). TOC is
250 awarded a 24 /30.

251

252 **ANALYSIS OF MIXED LIQUOR SUSPENDED SOLIDS (MLSS) SUPERNATANT**

253 In an MBR system analysis of the supernatant (in which solids are suspended) can provide
254 important information either about nutrient removal or fouling propensity. For example, the
255 ammonia, nitrate and orthophosphate levels in the supernatant taken from various zones can
256 help diagnose the cause of nitrification / denitrification or phosphate removal issues. Typically
257 the supernatant is separated from the biomass via centrifugation and then filtered (0.45um)
258 prior to analysis (Rosenberger, Evenblij *et al.* 2005).

259 The supernatant may be significantly different to the filtrate of the MBR process in terms of
260 organics and colloidal materials. This is due to the retention of all materials which are
261 insufficiently soluble to pass through the MBR membrane. Depending on the pore size of the
262 MBR membrane and the filter used to prepare the supernatant, a range of soluble and
263 colloidal polymeric substances could be present.

264 The sampling and analysis of activated sludge supernatant requires more care than simply
265 measuring the filtrate. Foam present on the top of MBR reactors can contaminate samples
266 and care needs to be taken not to include foam in the MLSS sample. Secondly, centrifugation
267 and filtering should occur as quickly as possible after sampling (Jenkins, Richard *et al.* 2004),
268 or the results will be affected by continuing microbial removal of substrates. Phosphate
269 accumulating organisms (PAO's) for example can release phosphate under low dissolved
270 oxygen conditions. If supernatant analysis is performed regularly an operational "normal"
271 profile can be established. Analysis showing deviations from this norm can assist in locating
272 problems (such as loss of effective aeration or mixing).

273

274 ***ANALYSIS OF EXTRACELLULAR & SOLUBLE MICROBIAL POLYMERS***

275 The analysis of MLSS can also involve determination/characterisation of extra-cellular
276 polymers (ECPs), a generic term for bio-polymeric substances. Soluble microbial products
277 (SMPs) are the soluble fraction of ECPs. The measurement of ECPs/SMPs is discussed
278 frequently in MBR literature particularly when the mechanisms of membrane fouling are
279 under investigation. Many authors link ECPs/SMPs to low filterability conditions in MBRs
280 (Drews, Vocks *et al.* 2008, Lyko, Wintgens *et al.* 2008, Pan, Su *et al.* 2010). The terms ECP
281 and SMP apply to a wide range of molecules such as polysaccharides, proteins, nucleic acids,
282 humic acids, lipids and other compounds which have been found at or outside the cell surface
283 and in the intercellular space of microbial aggregates. This inclusive categorisation applies
284 regardless of where these compounds originated. Disagreement exists over whether SMPs or
285 ECPs play the more important role in filterability decrease and bio-fouling. Additionally, there
286 are various opinions on the relative importance of protein or carbohydrate fractions. The
287 often contrary nature of research findings is noted by other reviewers (Rosenberger, Evenblij
288 *et al.* 2005, Drews 2010, Wang, Mei *et al.* 2013). It is also likely that some of the contrasting
289 research findings are due to the variety in MBR designs, scale, design of experiments,
290 membrane types and manufacturers, wastewater characteristics and the microbial
291 populations present and active.

292 The exact definition and hence properties of ECPs and SMPs is heavily dependent on the
293 methods used to obtain and characterize these biopolymers (Domínguez, Rodríguez *et al.*
294 2010a, Domínguez, Rodríguez *et al.* 2010b). ECP study is widespread however there are no
295 standard methods for extraction making cross-comparison of study results difficult
296 (Rosenberger, Evenblij *et al.* 2005). A comprehensive list of the various extraction methods
297 can be found in (Sheng, Yu *et al.* 2010). Factors that negatively impact ECP studies arise
298 initially in the extraction methods in which there is nearly always some degree of cell lysis
299 and hence uncertainty over the actual amount of genuine ECPs in the original mixed liquor.

300 The origin and purpose of ECPs has also been the subject of much speculation. Firstly, ECPs
301 can act as adhesives, assisting in floc and biofilm formation. In conventional activated sludge
302 systems ECPs are considered vital for flocculation and their absence correlates with poor

303 settleability (Bala Subramanian, Yan *et al.* 2010). ECPs also serve as a protective barrier;
304 providing resistance to toxins, temperature shocks and osmotic pressure changes and prevent
305 desiccation. Thirdly ECPs assist in nutrient acquisition by sorption of organic compounds and
306 metal ions, as well as assisting to retain enzymes involved in the digestion of exogenous
307 macromolecules or those used for quorum communications (Raszka, Chorvatova *et al.* 2006).
308 Lastly it has been noted by numerous authors that the production of ECPs increases when
309 nutrient limitations occur, with most finding that the carbohydrate portion is particularly
310 elevated (Janga, Ren *et al.* 2007, Sheng, Yu *et al.* 2010). There is also a consensus that lower
311 temperatures (particularly shocks) tend to result in less biological degradation and a higher
312 potential for colloidal material to accumulate and affect MBR filterability (van den Brink,
313 Satpradit *et al.* 2011, Ma, Wen *et al.* 2013).

314 Once the extraction of ECPs or SMPs has been completed, it is typical for the protein and humic
315 substances present to be determined according to the method described by Frølund *et al.*
316 (Frølund, Griebe *et al.* 1995), itself a modification of the classic protein only method of Lowry
317 (Lowry, Rosebrough *et al.* 1951). The carbohydrate content of ECPs is often measured using
318 another older traditional colorimetric method (Dubois, Gilles *et al.* 1956). In 2012 Silva and
319 colleagues used sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and
320 Matrix Assisted Laser desorption/ionization time-of-flight mass spectroscopy (MALDI -ToF/MS)
321 to identify proteins isolated from bulk MLSS ECPs (Silva, Carvalho *et al.* 2012). The results of
322 this study suggested that very few of the ECP proteins (~1% of soluble and none of the bound)
323 were secreted extracellularly, and the vast majority were cellular break-down products. The
324 conclusion to be drawn from this is that ECP formation occurs largely as a result of the death
325 of microbial biomass. The concept that microbial cell death is an important consideration for
326 the filterability of MLSS is supported by the work of several other authors (Hwang, Lee *et al.*
327 2008, Azami, Sarrafzadeh *et al.* 2011). Another study using MALDI- ToF/MS investigated the
328 proteins found in the cake layer of MBR membranes identifying many as 'sticky' outer
329 membrane proteins (OMP) of *Gamma* and *Betaproteobacteria*. (Zhou, Meng *et al.* 2015). At
330 this stage the results for proteome studies of environmental samples are limited by the

331 databases of sequenced organisms, which are heavily dominated by the proteins of human
332 related bacteria (Miyoshi, Aizawa *et al.* 2012). Attempts to use MALDI -ToF/MS to study
333 polysaccharides in MBR fouling has so far been of limited success due to matrix effects (Kimura,
334 Tanaka *et al.* 2012).

335 The lack of consensus on the causes of ECP production in the scientific literature is unsurprising
336 given the variation in wastewater influent and microbial populations. It is likely that each MBR
337 will have its own triggers for ECP production and heightened membrane fouling. Operational
338 staff should monitor their MBR to elucidate which conditions correlate with ECP production.
339 Whilst the characterisation of ECPs may be highly relevant to applied MBR research, regular
340 operational determination of protein or carbohydrate portions is unlikely to be viable due to the
341 time required for extraction and analytical characterisation (time score 3, training score 3,
342 laboratory requirements location score 3, equipment cost 3.5), for a total of 19.5. We assigned
343 all ECP / SMP methods a 3 for confidence and utility as results need to be interpreted in light
344 of past results. Extraction free methods are likely to provide more timely data and so some
345 authors have used measurements of the TOC or turbidity after centrifugation as a crude
346 indicator of ECP/SMP content (Lyko, Wintgens *et al.* 2008). Bulk determination via TOC
347 (reagent cost 5, equipment cost 2, location 4, time 5, training 3) for a total score of 22.
348 ECP/SMP estimation via a turbidity assay is even more highly rated (reagent cost 5, equipment
349 cost 5, location 5, time 5, training 5) for a total score of 28). ECPs in MLSS may be quantified
350 by the use of direct light microscopy following reverse staining with India Ink (Jenkins, Richard
351 *et al.* 2004). The subjective nature of this analysis without prolific replication is a significant
352 drawback.

353

354 BIOSOLIDS ANALYSIS

355 ***TOTAL SUSPENDED SOLIDS (TSS) & VOLATILE SUSPENDED SOLIDS (VSS)***

356 In MBR engineering and operational terms, the total microbial 'inventory' is usually taken as
357 being the mass of the volatile suspended solids (VSS). The VSS is a subset of total suspended
358 solids (TSS also commonly called mixed liquor suspended solids (MLSS)), and is effectively the
359 organic compounds which volatilise at 550°C (APHA, AWWA *et al.* 2005). VSS is commonly
360 used when assessing the food to microorganism ratio and managing solids inventory (Metcalf
361 and Eddy 2003). The cost of each test is small (score 5) with GFC filter papers being only a
362 few dollars (recommend 90mm diameter for MBR TSS). Laboratory requirements are a drying
363 oven (105°C), a furnace (550°C) and a desiccator (location score 4). Although relatively
364 simple this test requires careful laboratory technique, (the filter papers must be moisture free,
365 and samples need to be weighed in a timely manner, particularly for low solids samples (training
366 4).

367 Despite the limitations of TSS and VSS measurements (discussed below), these measurements
368 are the most valuable practical daily data for MBR operation and control (total score 27/30 in
369 Table 3). In an MBR no measurable solids will exit with the effluent therefore regulation of the
370 TSS/VSS depends entirely on the 'wasting rate' of excess sludge. High TSS/VSS loading (12-
371 15000mg/l) is a recognised cause of significant cake layer formation (Le-Clech, Chen *et al.*
372 2006) and a dominant influence in the filterability of the MLSS.

373 The assumption implicit in the use of VSS as a measure of 'organisms' is that all the volatile
374 material in the MLSS is microbial biomass. This is a problematic assumption on a number of
375 levels. On a purely chemical level, the VSS removed at between 105°C and 550°C also includes
376 chemically bound water, and the weight of the non-volatiles are impacted by the oxidation of
377 inorganics. Additionally, phosphorous remains in the ash (as a 'non-volatile') despite
378 originating inside cellular material. Another complication can be the accumulation of (non-
379 microbial) organic compounds that are resistant to breakdown. ECPs are also produced and
380 secreted into the MLSS by the microbial populations but could not be said to be living biology.

381 It is plain that TSS/VSS measurement are also insufficient to allow much understanding of the
382 biological status of microbes in the mixed liquor (Andreottola, Foladori *et al.* 2002). In terms

of the actual microbial biomass in the reactor, a significant portion is not categorically 'alive' in that it not metabolically active (effectively dormant, or dead but intact), or alternatively alive but metabolising at an undetectable level. One study that biomass volume accounted for only 10-15% of the mixed liquor VSS (Frølund, Palmgren *et al.* 1996). Similarly a later (2010) study found that approximately 11.1% (on a COD basis) of the activated sludge was living biomass with only 45% of this being metabolically active (Foladori, Bruni *et al.* 2010). Whilst conventional solids testing is the backbone of MBR operation, it offers very little information of the actual viability, vitality and composition of the microbial population.

CAPILLARY SUCTION TIME (CST)

Capillary Suction Time (CST) is a simple chromatography based method used to measure how quickly the MLSS supernatant takes to travel between two points through a filter paper by capillary action. CST was initially developed as a tool to predict the potential to de-water sludge (dewaterability) (Gale and Baskerville 1967), it has since been found to be informative with regard to filterability of mixed liquor in an MBR and scores highest (29/30) in our evaluation summary (Table 3).

CST is better suited to evaluation of thickened sludge and MBR biomass than CAS biomass due to the higher MLSS concentrations (lower solids concentrations produce readings of less than 10 seconds which lack resolution). In MBR MLSS, the CST has a good demonstrated correlation with Specific Resistance to Filtration (SRF) and is a quicker test to perform (Chen, Lin *et al.* 1996, Higgins and Novak 1997, Scholz 2005).

The CST is influenced by the colloidal loading of the supernatant. As the sludge contacts directly with the filter paper, a 'cake layer' forms and acts as a barrier for further capillary action along the paper. The presence of macromolecules and fine particles which can increase the cake layer formed at the interface and hence increase the CST (Sawalha 2010). The concentration of suspended solids has a significant influence on the CST, therefore Standard Methods (2710 G) recommends normalising the result against TSS. This produces a CST in seconds per gram of TSS.

Although cheap (score 5 for reagent and capital costs) and quick (time score 5), CST can suffer from repeatability issues often due to leakage of the MLSS between the funnel and the paper (score 4 confidence and utility). A 2010 doctoral thesis investigated causes of CST variability and found that the type and pore size of filter-papers used, the temperature, and shape and size of the funnel all contributed to variation in the measured values (Sawalha 2010). Swalha recommended the use of a sealant between the paper and funnel to reduce the variability. An alternative solution is to run samples in triplicate and exclude outlying results.

MICROBIAL VIABILITY AND ACTIVITY TESTS

In order to move beyond the use of VSS as a measure of 'organisms' additional testing focusing on the viability and activity of microbes is required. These analyses can be based on respiration, cell membrane integrity, enzyme levels or cellular energy storage detection.

RESPIRATION BASED TESTS FOR MICROBIAL ACTIVITY

The oxygen uptake rate (OUR) also known as the oxygen consumption or respiration rate, is a simple measurement of the oxygen consumed by the total biomass. OUR as a monitoring tool, scores well in our evaluation largely due to low reagent and equipment costs (score 5 and 4 respectively) but does require careful implementation as outlined below. The specific oxygen uptake rate (SOUR) is the OUR normalised against VSS concentration. Additionally the portion of oxygen consumed by nitrification can be established by performing the test with and without a nitrification inhibitor (Strotmann, Keinath *et al.* 1995).

In the OUR test a fresh MLSS sample is infused with air until the DO reaches 4 mg/l. Once the DO drops to 3.0 mg/l a timed period starts and the oxygen depletion is monitored until the DO drops below 1mg/l. The slope of the decline, in conjunction with the VSS previously measured is then used to calculate the specific OUR or SOUR. Whilst this appears to be a relatively simple

test to perform, it has numerous opportunities for the production of unreliable data (training score 3), especially if a comparable result from multiple occasions is desired (a confidence and utility score of 3). Standard Methods 2710 B notes that the result is 'quite sensitive' to temperature, therefore replicate determinations need to be made at $\pm 0.5^{\circ}\text{C}$, (necessitating a water bath). The test is normally performed at a temperature of 20°C , where this is not possible a mathematical compensation is able to be used. This compensation is only valid between 10 and 30°C . Additionally, the concentration of the sludge should be adjusted so that the test takes between 5-10 minutes. If the biomass is concentrated, insufficient data points will be obtained before the oxygen is depleted. In order to dilute the MLSS a (pre-warmed) buffer solution isotonic to the process supernatant should be used. Dilution may then result in settling out of the MLSS during the testing which can give uneven oxygen depletion. SOUR measurement will reflect the amount (or lack) of food in the wastewater supernatant, therefore some testing regimes will involve the addition of plant influent or acetate (Vollertsen, Jahn *et al.* 2001). The result is SOUR_{MAX} which is often a more helpful measurement than SOUR (Archibald and Young 2004).

Even with these method adjustments, other authors have noted that that the reliability of SOUR data depends on quality and maintenance of the dissolved oxygen electrodes used (Gernaey, Petersen *et al.* 2001). This assertion is supported by the experience of the reviewers. So whilst the study of biomass oxygen consumption can be a cost effective method for monitoring biomass activity, the test needs to be performed with a great deal of care. The total score for is 24/30.

Alternative respiration measurements (not based on oxygen consumption) can focus on the evolution of, carbon dioxide or nitrous oxide (Xiao, Araujo *et al.* 2015). Measurement of these species is more difficult and expensive than oxygen consumption but may be more appropriate for MLSS treating specific wastewaters. For anaerobic MBRs methane production is a good indicator of microbial activity.

DYE BASED METHODS FOR VIABILITY AND VITALITY

463 There are a wide range of assays for the testing of cellular metabolism using dyes which react
464 with metabolic products. There is also a range of dyes which can be used to determine
465 membrane integrity (and thus distinguish living and dead cells). Largely these dyes are
466 detectable by excitation at a specific wavelength and detection of the emission at another.
467 Whilst many research publications have used these techniques to good effect (Ziglio,
468 Andreottola *et al.* 2002, Berney, Vital *et al.* 2008, Foladori, Bruni *et al.* 2010), they are generally
469 not able to be performed on-site. Typically, they require expensive laboratory equipment such
470 as a fluorescence / laser microscope or a flow cytometer and staff with extensive experience in
471 these methodologies. These techniques are powerful however because individual cells are
472 visualised and counted automatically giving statistically significant data. These high tech
473 methods are very sensitive and therefore analysis of 'non-ideal' sample types such as MLSS
474 can be challenging. Typical hindrances are the tendency of biomass to clump together, the
475 presence of unknown materials, uneven staining of microbial populations and significant
476 background signals.

477 Some work has been done using dye based assay (LIVE/DEAD® BacLight™ Life Technologies)
478 for microbial viability and activity on less complex platforms. A plate reader based assay to
479 quantify the portion of living bacteria in probiotic solutions was used by Alakomi *et al.* in which
480 individual cells are not visualised or counted but red and green fluorescent signals are obtained
481 for the total solution (Alakomi, Mättö *et al.* 2005). They found that each of the different
482 bacterial strains required separate calibration curves. The inference is that LIVE/DEAD®
483 BacLight™ Bacterial Viability Kit may not be suitable for mixed populations of unknown species.
484 This view is supported by a study which investigated the proportions of live and dead bacteria
485 in soil samples which proved unsuccessful despite complex mathematical compensations and
486 careful dilution procedures (Pascaud, Amellal *et al.* 2009).

487 Dehydrogenase Activity (DHA)

488 A dehydrogenase is an oxidoreductase enzyme that oxidizes a substrate by a reduction reaction
489 that transfers one or more hydrides (H^-) to an electron acceptor. Nicotinamide Adenine
490 Dinucleotide (NAD^+) is reduced to $NADH_2$ (referred to as $NADH$) in one step of the electron

491 transport chain which generates ATP in bacteria. Measuring DHA is a direct measurement of
492 this rate of conversion.

493 has been found to correlate positively with OUR and substrate removal in activated sludge
494 (Awong, Bitton *et al.* 1985). DHA activity can be measured via a colorimetric or
495 spectrophotometric analysis of the change of colour of tetrazolium salts or resazurin, as well
496 as by direct measurement of NAD⁺/NADH.

497 *Tetrazolium salts*

498 Tetrazolium salts (MTT (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide),
499 INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride and XTT (2,3-bis-
500 (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) are a group of
501 compounds commonly used to measure dehydrogenase activity. Each of the tetrazolium salts
502 can be reduced by dehydrogenase to a highly coloured formazin compound which can be
503 measured using a colorimeter (Life Technologies 2010). The use of MTT and INT is limited
504 by the insolubility in water of the reduced product, therefore XTT is a better option for a
505 quick assay. Nevertheless the literature cites the use of INT for the determination of
506 dehydrogenase activity in domestic biomass using a solvent extraction of the product
507 compound (Liwarska-Bizukojc and Ledakowicz 2003). It was found that standard deviations
508 of this method were quite high (3-15%) (resulting in a confidence and utility score of 3).
509 Using XTT as an indicator of dehydrogenase activity Bensaid and Thierie found a good
510 correlation with OUR ($r^2 = 0.977$) (Bensaid, Thierie *et al.* 2000). The drawbacks of the XTT
511 assay include the requirement for facilities for solvent extraction and sterile preparation of an
512 electron decoupling reagent (location score 2, training 2), significant reagent preparation time
513 and rapid expiry of these reagents (time 3) and therefore the high cost of each set of these
514 reagents (reagent cost score 3). Combined, these factors result in a low evaluation score
515 (16/30) in Table 3 for biomass monitoring via Tetrazolium salts.

516 *Resazurin Reduction test*

517 Resazurin dye (7-hydroxy-3H-phenoxazin-3-one-10-oxide) can also be used as an indicator of
518 cellular dehydrogenase enzyme activity. The resazurin assay is moderately simple (time score
519 of 4), cheap (reagent cost of 5) and can be performed using the visible spectral range of a
520 spectrophotometer (equipment cost 4). Resazurin is blue in its oxidised state, when reduced
521 to resofurin it becomes pink. The method first used in the 1950's for the determination of
522 sperm health, was adapted as an assay to assess toxicity of chemicals by Liu (Liu 1986). This
523 method involves a solvent extraction step, making it a slow and insufficiently rugged for on-
524 site use. In 2007, McNicholl and co-workers modified this method to eliminate the solvent
525 extraction step and simplify it. They found that it was then ideal for regular use on site at
526 wastewater treatment plants (location score 4) (McNicholl, McGrath *et al.* 2007). Most recently
527 the resazurin assay has been used to evaluate the potential for toxicity to sewage treatment
528 plants from preservatives found in personal care products (Carbajo, Perdigón-Melón *et al.*
529 2015). Resazurin is the basis for the CellTiter-Blue® Cell Viability test kit (Promega) and also
530 for the ToxTrak™ Reagent Set (Hach). The use of resazurin as a basis for toxicity detection in
531 activated sludge is also detailed in the OECD Method 209 (OECD 2010). The numerous
532 commercial uses of resazurin assays would seem to support its use as a viability and toxicity
533 indicator, however Strotmann and co-workers found that "it did not always positively correlate
534 with the simultaneously estimated respiration activity" (Strotmann, Butz *et al.* 1993). This is
535 also the experience of the review authors, therefore a confidence and utility score of 3 and a
536 total of 24/30.

537 *Direct Measurement of NAD/H*

538 Nicotinamide Adenine Dinucleotide (NAD) is involved in many biological oxidation/reduction
539 reactions. It is alternately oxidised (NAD⁺) or reduced to NADH₂ (NADH) by the loss or gain
540 of two electrons respectively. The latter state can be detected when exposed to light at 340nm,
541 and the fluorescence measured at 460nm. In a laboratory study the fluorescence detected was
542 found to be proportional to the NADH concentration (König, Berns *et al.* 1997). Studies using
543 fluorescence probes outside the lab claim to provide a direct measurement of cellular metabolic
544 activity (Armiger, Lee *et al.* 1994, Farabegoli, Hellinga *et al.* 2003). Several studies have

outlined NAD/Hs on-line use potential for controlling dissolved oxygen in biological nutrient removal (BNR) where alternating aerobic and anoxic zones are required (Armiger, Lee *et al.* 1994, Parikh, Trivedi *et al.* 2011). An additional study found that the probe was a rapid indicator of fermentation imbalance with (anaerobic) reactors subjected to five different perturbations (Peck and Chynoweth 1992). Others however were slightly more reserved about the potential noting that NADH monitoring 'proved not to be as straightforward as suggested in the literature'(Farabegoli, Hellinga *et al.* 2003). This view is supported by Wos and Pollard who commented that both the studies supporting NADH probe use failed to separate extracellular from intracellular NADH (Wos and Pollard 2006). Furthermore that Armiger and Lee *et al.* did not address matrix issues such as turbidity which can cause light scattering thus having a negative impact on excitation of the NADH and detection of the emitted fluorescence (, fluorescence quenching) resulting in a significant under reporting of NADH (Wos and Pollard 2006). These concerns would be amplified when analysing MBR biomass due to the higher MLSS concentrations commonly used. Finally, there are currently no vendors of the on-line NADH process control equipment mentioned in the literature; Biobalance (Denmark), Fluoromeasure (BioChem) and Fluorosensor (Ingold)). There are a number of *ex-situ* assay measurements available, none of which are designed for wastewater matrices. Alternatively, a procedure developed for wastewater biomass by Wos and Pollard can be performed however this requires care in the making of reagents and standards (training score 2, time score 3, reagent cost 3) as well as a fluorimeter (equipment cost 3, location 2) (Wos and Pollard 2006). These factors explain the low score for NADH assays of 16/30.

ADENOSINE-5'-TRIPHOSPHATE (ATP)

Adenosine-5'-triphosphate (ATP) is the main energy molecule of living organisms (excluding viruses) and therefore the detection of ATP is indicative of cellular life. In the food industry ATP measurements have been widely used to evaluate the success of sterilisation techniques (Powitz 2007).

572 Numerous test kits requiring a luminometer are available commercially for biotechnology
573 laboratory use; BacTiter-Glo™ (Promega), EnzyLight™ (BioAssay Systems) and the
574 Calbiochem® ATP Assay Kit (Merck Millipore). These are all bioluminescent ATP Assay kits used
575 to determine the amount of ATP in a sample and thus the vitality of the microbial life present.
576 There are a number reports in the literature of the use of ATP tests for analysis of biological
577 activity in activated sludge, however most are dated pre 2000. Early work indicated that ATP
578 was a sensitive measurement of biomass viability (Patterson, Brezonik *et al.* 1970, Weddle and
579 Jenkins 1971, Jørgensen, Eriksen *et al.* 1992). Later work demonstrated that ATP analysis was
580 an instructive parameter for warning of toxic influents, undesirable process changes (Arretxe,
581 Heap *et al.* 1997, Dalzell and Christofi 2002) or determining the impact of pharmaceuticals on
582 MBR biomass health (Maeng, Choi *et al.* 2013). Despite these successes the use of ATP tests
583 in the daily operation of wastewater treatment has not been widespread due to the complexity
584 of wastewater matrices and the high level of scientific training required.

585 The analysis of biomass health can be made by determining the ATP content per gram of VSS.
586 Although some variations in the ATP content per cell have been reported (due to differences
587 in growth phase, cell size and microbial species), it is recognised as being more consistent for
588 cellular enumeration than other measurements such as protein, DNA, particulate nitrogen or
589 the fluorescent stains DiBAC4(3) and carboxyfluorescein (Weddle and Jenkins 1971, Berney,
590 Vital *et al.* 2008). Studies using flow cytometry have found an average ATP-per-cell value of
591 1.75×10^{-10} nmol/cell (Hammes, Goldschmidt *et al.* 2010).

592 Recently a range of test kits have been developed, including one specifically designed for
593 activated sludge health assessment (LuminUltra QG21W). Unlike first generation technologies
594 (mentioned above), this 2nd generation test measures both total and cellular ATP, so the
595 extracellular ATP content can be calculated. The proportion of ATP inside cells compared with
596 outside the cells (in the supernatant) can be used to measure the biomass stress index
597 (BSI). This dual determination addresses a major limitation of most ATP test kits, as noted by
598 Hammes *et al.* (Hammes, Goldschmidt *et al.* 2010). Although not a cheap option (with a reagent
599 score of 3), the advantages of this test kit are; a large sample size can be taken, the equipment

is portable and easy to use and the procedure is quick (<5 minutes), giving scores for location of 5, equipment cost of 5, and a training score of 4 and time score of 5. Standards included with the kit enable quantification and comparison of results over time (confidence=5) giving a final score of 27/30 in Table 3. Keasler *et al.* reported the use of a 2nd generation ATP test kit to assess the microbial content of oil-field systems (Keasler, Bennett *et al.* 2012). In their study ATP based determinations (used on the basis of 1 femtogram of ATP/cell) and quantitative PCR (see below) were in good agreement, whilst conventional serial dilution greatly underestimated the populations. A related test kit has also successfully been used for the early warning of activated sludge bulking/foaming (Brault, Whalen *et al.* 2011).

MICROBIAL ECOLOGY

MBR processes can be affected by the amount of biomass, the activity of the biomass and the microbial composition of the biomass. In an effort to understand the latter, a range of molecular microbial ecology methods have been developed. The most promising of these is Next Generation Sequencing (NGS), which is capable of providing insights into the composition of microbial communities and in some cases the metabolic consequences of this composition (Albertsen, Hansen *et al.* 2012, Sekiguchi, Ohashi *et al.* 2015, Beale, Karpe *et al.* 2016).

Whilst the cost of NGS has vastly reduced, and bioinformatics tools are becoming more user friendly, currently NGS cannot offer timely results for operators. However, given the rapid development of this field, in particular in 16s based molecular technology, there is hope that in the future this technology may provide water engineering relevant tools. Meanwhile basic microscopy is the best tool available.

MICROSCOPY BASED ECOLOGY

Direct examination of microbial population using a light microscope coupled with specific stains/dyes has long been a mainstay of wastewater treatment plant microbiology (Eikelboom 1975). An experienced operator can identify morphotypes (bacteria with the same appearance) regularly present and conversely any changes to the normal micro flora of the

627 MLSS. Given the relatively distinct morphotypes broadly associated with bulking and foaming
628 (Wanner and Grau 1989, Jenkins, Richard *et al.* 2004) the use of microscopy is understandably
629 widespread (Seviour and Nielsen 2010).

630 Light microscopy does represent a quick (time score of 5) and cost effective means of
631 monitoring biomass (reagent cost of 5), especially with the price of electronics and software
632 reducing rapidly (equipment cost 4). The microscope should include phase contrast and a 100x
633 oil immersion objective. To make the best of this technique the microscopist requires
634 experience and familiarity with the specific WWTP biomass (training score of 3). The use of
635 stains to assist in identification may not always be helpful. For example the presence of sulfide
636 can result in Gram variable and Neisser variable results for some morphotypes such as *Thiothrix*
637 and *Nostocoida* species (Jenkins, Richard *et al.* 2004).

638 With the advent of molecular methods for identification of microbes, the inexact nature of
639 morphological typing became has become evident (Müller, Schade *et al.* 2007). For example
640 some filamentous organisms are able to revert to a unicellular form at some stages of their
641 lifecycle (Ramothokang, Naidoo *et al.* 2006). Other morphological groups such as 'Eikelboom
642 Type 1863' have been shown to comprise of several unrelated taxa (Seviour, Blackall *et al.*
643 1997) (therefore a confidence and utility score 3). Although clearly basic light microscopy has
644 its limitations, it is currently the only practical microbial ecology tool available (overall score
645 24/30)

646 CONCLUSIONS

647 Timely and dependable analyses are vital for smooth and cost efficient operation of an MBR,
648 we have reviewed and evaluated many different technologies with a view to establishing a
649 standard set of timely and reliable analytical tools for monitoring and regulating MBRs. The
650 choice of monitoring technologies to employ for an MBR and its biomass must be considered
651 carefully and Table 3 summarises the relative advantages and disadvantages of the different
652 technologies assessed against factors such as cost, time, suitability for on-site location,
653 required training and the usefulness of the result.

654 In summary, TSS/VSS measurements will always remain important parameters, but for MBR
655 operation CST is also very valuable. For less equipped plants colorimetric analysis (including
656 COD) is likely to remain a mainstay of laboratory operations. It is recommended that larger
657 plants invest in on-site IC systems which measure nutrient and also assist in monitoring scaling
658 potential. Also for these sites a combination TOC/TNb systems offers results which are less
659 ambiguous and faster than BOD, and have less environmental impact than COD.

660 Of the methods for monitoring microbial viability and vitality, the standout analytical tool in
661 this field is ATP monitoring using 2nd Generation technology, which is now robust enough to
662 supply quality data, quickly without scientific training. Oxygen uptake rates can also be useful
663 however high quality well maintained DO probes and standardisation of temperature are key.
664 Extracellular polymer concentrations are best monitored by measurement of turbidity after
665 centrifugation.

666 From a practical standpoint monitoring microbial ecology of an MBR (via molecular technologies
667 being used currently) is not likely to reveal much about the health of the process within a
668 relevant timeframe. Neither is it a cost effective process control tool. Until these technologies
669 progress and our knowledge of microbial ecology is more complete, simple light microscope
670 observation will remain the best option.

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ACKNOWLEDGEMENTS

The authors would like to thank Gippsland Water for their financial support of this research. Additionally, to acknowledge LuminUltra Technologies who supplied reagents free of charge for evaluation. Thanks also to Clifford Jones for his proof reading.

REFERENCES

Abrevaya, X. C., *et. al.* (2015). "Analytical applications of microbial fuel cells. Part I: Biochemical oxygen demand." Biosensors and Bioelectronics **63**(0): 580-590.

Alakomi, H. L., *et. al.* (2005). "Application of a microplate scale fluorochrome staining assay for the assessment of viability of probiotic preparations." Journal of Microbiological Methods **62**(1): 25-35.

Albertsen, M., *et. al.* (2012). "A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal." ISME Journal **6**(6): 1094-1106.

Andreottola, G., *et. al.* (2002). "Biomass active fraction evaluated by a direct method and respirometric techniques." Water Science and Technology **Vol 46** (1-2): pp 371–379.

APHA, *et. al.* (2005). Standard Methods for the examination of water and wastewater. Washington, DC, USA.

Archibald, F. and F. Young (2004). "Common stresses affecting activated sludge health and performance – what the four-assay set can tell us." Water Science and Technology **50**(3): 49-55.

Armiger, W. B., *et. al.* (1994). "Control of BNR processes by regulation of the VFA:M ratio " Water Science & Technology **28**(11-12): 303-314.

Arretxe, M., *et. al.* (1997). "The effect of toxic discharges on ATP content in activated sludge." Environmental Toxicology and Water Quality **12**(1): 23-29.

Awong, J., *et. al.* (1985). "ATP, oxygen uptake rate and INT-dehydrogenase activity of actinomycete foams." Water Research **19**(7): 917-921.

Azami, H., *et. al.* (2011). "Fouling in membrane bioreactors with various concentrations of dead cells." Desalination **278**(1–3): 373-380.

Aziz, J. A. and T. H. Y. Tebbutt (1980). "Significance of COD, BOD and TOC correlations in kinetic models of biological oxidation." Water Research **14**(4): 319-324.

Bala Subramanian, S., *et. al.* (2010). "Extracellular polymeric substances (EPS) producing bacterial strains of municipal wastewater sludge: Isolation, molecular identification, EPS characterization and performance for sludge settling and dewatering." Water Research **44**(7): 2253-2266.

Beale, D. J., *et. al.* (2016). "An ‘omics’ approach towards the characterisation of laboratory scale anaerobic digesters treating municipal sewage sludge." Water Research **88**: 346-357.

Bensaid, A., *et. al.* (2000). "The use of the tetrazolium salt XTT for the estimation of biological activity of activated sludge cultivated under steady-state and transient regimes." Journal of Microbiological Methods **40**(3): 255-263.

Berney, M., *et. al.* (2008). "Rapid, cultivation-independent assessment of microbial viability in drinking water." Water Research **42**(14): 4010-4018.

Bisutti, I., *et. al.* (2004). "Determination of total organic carbon – an overview of current methods." TrAC Trends in Analytical Chemistry **23**(10–11): 716-726.

Brault, J.-M., *et. al.* (2011). "Early warning signs of bulking in an activated sludge system through interpretation of ATP data in a systems analysis context." Environmental Technology (Cranfield, UK) **32**(14): 1649-1660.

Carbajo, J. B., *et al.* (2015). "Personal care product preservatives: Risk assessment and mixture toxicities with an industrial wastewater." Water Research **72**(0): 174-185.

Cardellicchio, N., *et al.* (1997). "Use of ion chromatography for the determination of transition metals in the control of sewage-treatment-plant and related waters." Journal of Chromatography A **770**(1-2): 185-193.

Chang, I.-S. and C.-H. Lee (1998). "Membrane filtration characteristics in membrane-coupled activated sludge system — the effect of physiological states of activated sludge on membrane fouling." Desalination **120**(3): 221-233.

Chen, G. W., *et al.* (1996). "Capillary suction time (CST) as a measure of sludge dewaterability." Water Science and Technology **34**(3-4): 443-448.

Choi, H., *et al.* (2006). "Effect of activated sludge properties and membrane operation conditions on fouling characteristics in membrane bioreactors." Chemosphere **63**(10): 1699-1708.

Costa, J. C., *et al.* (2013). "Quantitative image analysis for the characterization of microbial aggregates in biological wastewater treatment: a review." Environmental Science and Pollution Research International **20**(9): 5887-5912.

da Silva, A. M. E. V., *et al.* (2011). "Optimization of the determination of chemical oxygen demand in wastewaters." Analytica Chimica Acta **699**(2): 161-169.

Dalzell, D. J. B. and N. Christofi (2002). "An ATP luminescence method for direct toxicity assessment of pollutants impacting on the activated sewage sludge process." Water Research **36**(6): 1493-1502.

Davey, H. M. (2011). "Life, Death, and In-Between: Meanings and Methods in Microbiology." Applied and Environmental Microbiology **77**(16): 5571-5576.

Díaz, M., *et al.* (2010). "Application of flow cytometry to industrial microbial bioprocesses." Biochemical Engineering Journal **48**(3): 385-407.

Domínguez, L., *et al.* (2010a). "Effect of different extraction methods on bound EPS from MBR sludges. Part I: Influence of extraction methods over three-dimensional EEM fluorescence spectroscopy fingerprint." Desalination **261**(1-2): 19-26.

Domínguez, L., *et al.* (2010b). "Effect of different extraction methods on bound EPS from MBR sludges: Part II: Influence of extraction methods over molecular weight distribution." Desalination **262**(1-3): 106-109.

Drews, A. (2010). "Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures." Journal of Membrane Science **363**(1-2): 1-28.

Drews, A., *et al.* (2008). "Does fouling in MBRs depend on SMP?" Desalination **231**(1-3): 141-149.

Dubois, M., *et al.* (1956). "Colorimetric Method for Determination of Sugars and Related Substances." Analytical Chemistry **28**(3): 350-356.

Eikelboom, D. H. (1975). "Filamentous organisms observed in activated sludge." Water Research **9**(4): 365-388.

FAO (2012). AQUASTAT, Global information system on water and agriculture. FAO. <http://www.fao.org/nr/water/aquastat/wastewater/index.stm>.

Farabegoli, G., *et al.* (2003). "Study on the use of NADH fluorescence measurements for monitoring wastewater treatment systems." Water Research **37**(11): 2732-2738.

Foladori, P., *et al.* (2010). "Direct quantification of bacterial biomass in influent, effluent and activated sludge of wastewater treatment plants by using flow cytometry." Water Research **44**(13): 3807-3818.

Frimmel, F. H. and G. Abbt-Braun (2011). 3.01 - Sum Parameters: Potential and Limitations. Treatise on Water Science. P. Wilderer. Oxford, Elsevier: 3-29.

Frølund, B., *et al.* (1995). "Enzymatic activity in the activated-sludge floc matrix." Applied Microbiology and Biotechnology **43**(4): 755-761.

Frølund, B., *et al.* (1996). "Extraction of extracellular polymers from activated sludge using a cation exchange resin." Water Research **30**(8): 1749-1758.

Gale, R. S. and R. C. Baskerville (1967). "Capillary Suction Method for determination of the filtration properties of solid-liquid suspension." Chem, Ind (London) **9**: 355-356.

Gernaey, A. K., *et al.* (2001). "Activated sludge monitoring with combined respirometric–titrimetric measurements." Water Research **35**(5): 1280-1294.

Gray, N. F. (2010). Chapter 5 - Micro-organisms and Pollution Control. Water Technology (Third Edition). N. F. Gray. Oxford, Butterworth-Heinemann: 93-116.

Hammes, F., *et al.* (2010). "Measurement and interpretation of microbial adenosine tri-phosphate (ATP) in aquatic environments." Water Research **44**(13): 3915-3923.

Henze, M. and Y. Comeau (2008). Wastewater Characterisation. Biological Wastewater Treatment: Principles, Modeling and Design. M. Henze, M. C. M. van Loosdrecht, G. A. Eckma and D. Brdjanovic. London, UK., IWA Publishing.

Higgins, J., *et al.* (2004). A review of Water Quality Criteria in Australian Reclaimed Water Guidelines and Sewage Effluent Discharge Licences. Q. for Environmental Protection Agency.

Higgins, M. J. and J. T. Novak (1997). "The effect of cations on the settling and dewatering of activated sludges: Laboratory results." Water Environment Research **69**: 215.

771 Hwang, B. K., *et al.* (2008). "Correlating TMP increases with microbial characteristics in the bio-cake on the membrane
772 surface in a membrane bioreactor." Environmental Science and Technology **42**(11): 3963-3968.

773 Janga, N., *et al.* (2007). "Characteristics of soluble microbial products and extracellular polymeric substances in the
774 membrane bioreactor for water reuse." Desalination **202**(1-3): 90-98.

775 Jefferies, M. and D. Comstock (2001). "Predicting calculating scaling tendency in membrane plants." Desalination
776 **139**(1-3): 341-344.

777 Jenkins, D., *et al.* (2004). Manual on the Causes and Control of Activated Sludge Bulking and Foaming and other solids
778 separation problems, Lewis Publishers.

779 Jordan, M. A., *et al.* (2014). "Ubiquity of activated sludge ferricyanide-mediated BOD methods: A comparison of sludge
780 seeds across wastewater treatment plants." Talanta **125**(0): 293-300.

781 Jørgensen, P. E., *et al.* (1992). "Estimation of viable biomass in wastewater and activated sludge by determination of
782 ATP, oxygen utilization rate and FDA hydrolysis." Water Research **26**(11): 1495-1501.

783 Jouanneau, S., *et al.* (2014). "Methods for assessing biochemical oxygen demand (BOD): A review." Water Research
784 **49**(0): 62-82.

785 Judd, S. and C. Judd (2011). Chapter 1 - Introduction. The MBR Book (Second Edition). Oxford, Butterworth-Heinemann:
786 1-54.

787 Judd, S. and C. Judd (2011). Chapter 2 - Fundamentals. The MBR Book (Second Edition) Principles and Applications of
788 Membrane Bioreactors for Water and Wastewater Treatment. Oxford, Butterworth-Heinemann: 55-207.

789 Keasler, V., *et al.* (2012). "Expanding the microbial monitoring toolkit: Evaluation of traditional and molecular
790 monitoring methods." International Biodeterioration & Biodegradation(0).

791 Kimura, K., *et al.* (2012). "Further examination of polysaccharides causing membrane fouling in membrane bioreactors
792 (MBRs): Application of lectin affinity chromatography and MALDI-TOF/MS." Water Research **46**(17): 5725-5734.

793 König, K., *et al.* (1997). "Time-resolved and steady-state fluorescence measurements of β -nicotinamide adenine
794 dinucleotide-alcohol dehydrogenase complex during UVA exposure." Journal of Photochemistry and Photobiology B:
795 Biology **37**(1-2): 91-95.

796 Kraemer, J. T., *et al.* (2012). "A practitioner's perspective on the application and research needs of membrane
797 bioreactors for municipal wastewater treatment." Bioresource Technology **122**(0): 2-10.

798 Le-Clech, P. (2010). "Membrane bioreactors and their uses in wastewater treatments." Applied Microbiology and
799 Biotechnology **88**(6): 1253-1260.

800 Le-Clech, P., *et al.* (2006). "Fouling in membrane bioreactors used in wastewater treatment." Journal of Membrane
801 Science **284**(1-2): 17-53.

802 Lebegue, J., *et al.* (2008). "MBR functioning under steady and unsteady state conditions. Impact on performances and
803 membrane fouling dynamics." Desalination **231**(1-3): 209-218.

804 Li, J., *et al.* (2012). "Microbial community and biomass characteristics associated severe membrane fouling during
805 start-up of a hybrid anoxic-oxic membrane bioreactor." Bioresource Technology **103**(1): 43-47.

806 Life Technologies (2010). The Molecular Probes® Handbook—A Guide to Fluorescent Probes and Labeling Technologies,
807 Life Technologies.

808 Liu, D. (1986). "Resazurin reduction method for toxicity assessment of water soluble and insoluble chemicals." Toxicity
809 Assessment **1**(2): 253-258.

810 Liwarska-Bizukojc, E. and S. Ledakowicz (2003). "Estimation of viable biomass in aerobic biodegradation processes of
811 organic fraction of municipal solid waste (MSW)." Journal of Biotechnology **101**(2): 165-172.

812 Lofrano, G. and J. Brown (2010). "Wastewater management through the ages: A history of mankind." Science of The
813 Total Environment **408**(22): 5254-5264.

814 Lowry, O. H., *et al.* (1951). "Protein measurement with the Folin phenol reagent
815 " J. Biol. Chem., **193**: 265-275.

816 Lyko, S., *et al.* (2008). "Long-term monitoring of a full-scale municipal membrane bioreactor—Characterisation of
817 foulants and operational performance." Journal of Membrane Science **317**(1-2): 78-87.

818 Ma, Z., *et al.* (2013). "Effect of temperature variation on membrane fouling and microbial community structure in
819 membrane bioreactor." Bioresource Technology **133**(0): 462-468.

820 Maeng, S. K., *et al.* (2013). "Influences of solid retention time, nitrification and microbial activity on the attenuation
821 of pharmaceuticals and estrogens in membrane bioreactors." Water Research **47**(9): 3151-3162.

822 McNicholl, B. P., *et al.* (2007). "Development and application of a resazurin-based biomass activity test for activated
823 sludge plant management." Water Research **41**(1): 127-133.

824 Metcalf and Eddy (2003). Wastewater Engineering—Treatment and Reuse. New York, Macgraw Hill.

825 Miyoshi, T., *et al.* (2012). "Identification of proteins involved in membrane fouling in membrane bioreactors (MBRs)
826 treating municipal wastewater." International Biodeterioration and Biodegradation **75**: 15-22.

827 Müller, E., *et al.* (2007). "Filamentous Scum Bacteria in Activated Sludge Plants: Detection and Identification Quality
828 by Conventional Activated Sludge Microscopy versus Fluorescence In Situ Hybridization." Water Environment Research
829 **79**(11): 2274-2286.

830 Namour, P. and N. Jaffrezic-Renault (2010). "Sensors for measuring biodegradable and total organic matter in water."
831 TrAC Trends in Analytical Chemistry **29**(8): 848-857.

832 Ng, H. Y. and S. W. Hermanowicz (2005). "Specific Resistance to Filtration of Biomass from Membrane Bioreactor
833 Reactor and Activated Sludge: Effects of Exocellular Polymeric Substances and Dispersed Microorganisms." Water
834 Environment Research **77**(2): 187-192.

835 OECD (2010). Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation), Method 209. O. G. f.
836 T. o. C. o. A. Toxicity. Paris, OECD.

837 Pan, J. R., *et al.* (2010). "Effect of sludge characteristics on membrane fouling in membrane bioreactors." Journal of
838 Membrane Science **349**(1–2): 287-294.

839 Parikh, C., *et al.* (2011). A decade of simultaneous nitrification and denitrification experience in over 60 conventional
840 and MBR applications – lessons learned. WEFTEC. Los Angeles Water Environment Federation.

841 Pascaud, A., *et al.* (2009). "A fluorescence-based assay for measuring the viable cell concentration of mixed microbial
842 communities in soil." Journal of Microbiological Methods **76**(1): 81-87.

843 Patterson, J. W., *et al.* (1970). "Measurement and significance of adenosine triphosphate in activated sludge."
844 Environmental Science & Technology **4**(7): 569-575.

845 Peck, M. W. and D. P. Chynoweth (1992). "On-line fluorescence-monitoring of the methanogenic fermentation."
846 Biotechnology and Bioengineering **39**(11): 1151-1160.

847 Pollice, A., *et al.* (2005). "Sub-critical flux fouling in membrane bioreactors — a review of recent literature."
848 Desalination **174**(3): 221-230.

849 Powitz, R. W. (2007). "ATP Systems Help Put Clean to the Test." Food Safety Magazine **June/July**.

850 Ramothokang, T. R., *et al.* (2006). "'Morphological shifts' in filamentous bacteria isolated from activated sludge
851 processes." World Journal of Microbiology and Biotechnology **22**(8): 845-850.

852 Raszka, A., *et al.* (2006). "The role and significance of extracellular polymers in activated sludge. Part I: Literature
853 review." Acta hydrochimica et hydrobiologica **34**(5): 411-424.

854 Rosenberger, S., *et al.* (2005). "The importance of liquid phase analyses to understand fouling in membrane assisted
855 activated sludge processes—six case studies of different European research groups." Journal of Membrane Science
856 **263**(4): 113-126.

857 Rosenberger, S. and M. Kraume (2002). Filterability of activated sludge in membrane bioreactors, Elsevier Science B.V.,
858 P.O. Box 211 Amsterdam 1000 AE Netherlands.

859 Sanz, J. L. and T. Köchling (2007). "Molecular biology techniques used in wastewater treatment: An overview." Process
860 Biochemistry **42**(2): 119-133.

861 Sawalha, O. (2010). Capillary Suction Time (CST) Test: Developments in testing methodology and reliability of results,
862 The University of Edinburgh.

863 Scholz, M. (2005). "Review of recent trends in capillary suction time (CST) dewaterability testing research." Ind. Eng.
864 Chem. Res., **44**: 8157.

865 Sekiguchi, Y., *et al.* (2015). "First genomic insights into members of a candidate bacterial phylum responsible for
866 wastewater bulking." PeerJ **3**: e740.

867 Seviour, E. M., *et al.* (1997). "The filamentous morphotype Eikelboom Type 1863 is not a single genetic entity." Journal
868 of Applied Microbiology **82**(4): 411-421.

869 Seviour, R. J. and P. H. Nielsen (2010). Microbial Ecology of Activated Sludge. London, IWA Publishing.

870 Sheng, G.-P., *et al.* (2010). "Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater
871 treatment systems: A review." Biotechnology Advances **28**(6): 882-894.

872 Silva, A. F., *et al.* (2012). "Step-by-step strategy for protein enrichment and proteome characterisation of extracellular
873 polymeric substances in wastewater treatment systems." Applied Microbiology and Biotechnology **95**(3): 767-776.

874 Strotmann, U. J., *et al.* (1993). "The Dehydrogenase Assay with Resazurin: Practical Performance as a Monitoring
875 System and Ph-Dependent Toxicity of Phenolic Compounds." Ecotoxicology and Environmental Safety **25**(1): 79-89.

876 Strotmann, U. J., *et al.* (1995). "Biological test systems for monitoring the operation of wastewater treatment plants."
877 Chemosphere **30**(2): 327-338.

878 Thomas, O., *et al.* (1999). "TOC versus UV spectrophotometry for wastewater quality monitoring." Talanta **50**(4): 743-
879 749.

880 Tian, Y., *et al.* (2011). "Correlating membrane fouling with sludge characteristics in membrane bioreactors: An especial
881 interest in EPS and sludge morphology analysis." Bioresource Technology **102**(19): 8820-8827.

882 Tilley, D. F. (2011). Aerobic Wastewater Treatment Processes; History and Development. London, IWA publishing.

van den Brink, P., *et. al.* (2011). "Effect of temperature shocks on membrane fouling in membrane bioreactors." Water Research **45**(15): 4491-4500.

Vanrolleghem, P. A. and D. S. Lee (2003). "On-line monitoring equipment for wastewater treatment processes: state of the art." Water Science & Technology **47**(2): 1-34.

Visco, G., *et. al.* (2005). "Organic carbons and TOC in waters: an overview of the international norm for its measurements." Microchemical Journal **79**(1-2): 185-191.

Vollertsen, J., *et. al.* (2001). "Comparison of methods for determination of microbial biomass in wastewater." Water Research **35**(7): 1649-1658.

Wang, Z., *et. al.* (2013). "Recent advances in membrane fouling caused by extracellular polymeric substances: a mini-review." Desalination and Water Treatment **51**(25-27): 5121-5131.

Wang, Z., *et. al.* (2013). "Potential Foulants and Fouling Indicators in MBRs: A Critical Review." Separation Science and Technology (Philadelphia) **48**(1): 22-50.

Wanner, J. and P. Grau (1989). "Identification of filamentous microorganisms from activated sludge: A compromise between wishes, needs and possibilities." Water Research **23**(7): 883-891.

Water Environment Federation (2012). Membrane Bioreactors: WEF Manual of Practice No. 36 McGraw-Hill Education.

Weddle, C. L. and D. Jenkins (1971). "The viability and activity of activated sludge." Water Research **5**(8): 621-640.

Wos, M. and P. Pollard (2006). "Sensitive and meaningful measures of bacterial metabolic activity using NADH fluorescence." Water Research **40**(10): 2084-2092.

Xiao, Y., *et. al.* (2015). "Toxicity measurement in biological wastewater treatment processes: A review." Journal of Hazardous Materials **286**(0): 15-29.

Zhou, Z., *et. al.* (2015). "Metaproteomic analysis of biocake proteins to understand membrane fouling in a submerged membrane bioreactor." Environmental Science and Technology **49**(2): 1068-1077.

Ziglio, G., *et. al.* (2002). "Assessment of activated sludge viability with flow cytometry." Water Research **36**(2): 460-468.