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Title: Membrane bioreactor treatment of pulp and paper wastewater:
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Keywords: Membrane Bioreactor; Pulp Wastewater; Capillary Suction Time;
Black Liquor

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Abstract: Pulp and paper wastewaters are challenging to treat due to their variability, complexity and toxicity. Membrane Bioreactor's (MBR's) provide high-quality filtrates but are subject to periods of low filterability / high capillary suction time (CST) which potentially limit their use in this application. There is a paucity of published literature concerning long-term operational issues in full-scale, industrial MBR's. We hypothesised that either changes of bacterial population, alteration/reduction of bacterial metabolism or bacterial production of extra-cellular polymers (ESPs) increased CST and reduced filterability. This study, conducted over two years at the 16.4MLD Gippsland Water Factory, was structured to inform this bacterial centric hypothesis. However, no shifts in bacterial population were seen as causative of high CST and microbial stress levels and biological activity were observed to be low during high CST and no sign of toxicity was found. The strongest correlator with CST was the concentration of colloidal Total Organic Carbon of the mixed liquor suspended solids (MLSS) supernatant (following centrifugation). The carbonaceous colloids accumulated in the supernatant were named 'Non-Centrifugable Total Organic Carbon' (NCTOC). Their accumulation in the MLSS was found to be due to their biologically refractory nature and their retention by the MBR membrane. The accumulation of NCTOC typically followed a period where the influent had a high colour and a high sodium to calcium ratio, which is consistent with a pulping by-product called 'Black Liquor'. Additional experiments with Black Liquor allowed us to conclude that high CST can be produced by addition of Black Liquor.

Colloidal carbon interference in the treatability of pulp and paper wastewater by MBR

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Abstract

Pulp and paper wastewaters are challenging to treat due to their variability, complexity and toxicity. Membrane Bioreactor's (MBR's) provide high-quality filtrates but are subject to periods of low filterability / high capillary suction time (CST) which potentially limit their use in this application. There is a paucity of published literature concerning long-term operational issues in full-scale, industrial MBR's. We hypothesised that either changes of bacterial population, alteration/reduction of bacterial metabolism or bacterial production of extra-cellular polymers (ESPs) increased CST and reduced filterability. This study, conducted over two years at the 16.4MLD Gippsland Water Factory, was structured to inform this bacterial centric hypothesis. However, no shifts in bacterial population were seen as causative of high CST and microbial stress levels and biological activity were observed to be low during high CST and no sign of toxicity was found. The strongest correlator with CST was the concentration of colloidal Total Organic Carbon of the mixed liquor suspended solids (MLSS) supernatant (following centrifugation). The carbonaceous colloids accumulated in the supernatant were named 'Non-Centrifugable Total Organic Carbon' (NCTOC). Their accumulation in the MLSS was found to be due to their biologically refractory nature and their retention by the MBR membrane. The accumulation of NCTOC typically followed a period where the influent had a high colour and a high sodium to calcium ratio, which is consistent with a pulping by-product called 'Black Liquor'. Additional experiments with Black Liquor allowed us to conclude that high CST can be produced by addition of Black Liquor.

1.0 Introduction

Pulp and paper production is a water-intensive manufacturing process, which can result in the release of difficult to treat, highly coloured, complex and odorous wastewaters [1-3].

Available Literature

The use of MBRs in the treatment of PPMW effluent is not widespread although the use of membrane systems in mills has been reported in the literature [4-7].

Literature available on the use of MBR's to treat PPMW is largely based on laboratory or pilot scale operations [8-10] or is simply theoretical [11]. Some use of anaerobic MBR's to treat PPMW has been reported in the literature [12].

MBR's are utilised for recycling single water streams in pulp or paper manufacturing [13]. Currently there are 10 such MBR based effluent treatment plants in Europe [14-16]. For example, one of these operating at 55°C, enables the recycling of much of the 290m³/d of hot bleaching wastewaters [17]. Frustratingly little research has been published relating to the operation of these plants and a recent review of developments in PPMW treatment identified no long term, large scale MBR studies [3].

Pilot scale research (incorporating an MBR) to evaluate options for 'closing water loops' at mills, did reveal that an MBR was a viable alternative to regular activated sludge. However issues with silica scale were experienced [18]. Similar scaling problems were experienced by Lerner and colleges who experienced both calcium carbonate and organic fouling [9].

Lerner *et al* did note the possibility of an "accumulation of non-biodegradable organic and inorganic compounds in the bioreactor which could be harmful to the microbial population or to the membrane structure" [9]. An early study on the plant investigated in this research detailed the unique bioreactor and its sulfide oxidising capabilities [19]. The research presented here is a significant addition to the literature as it addresses fundamental questions of treatability of this complex wastewater using an MBR. In addition, this study directly addresses the issue raised by Lerner and discussed briefly by [19] and significantly adds to the body of literature concerning industrial scale plant operation.

The Gippsland Water Factory

The pulp and paper mill wastewater in this study originates from a manufacturing facility with numerous paper machines, a continuous Eucalypt Kraft mill, a batch Pine Kraft mill, a continuous Neutral Sulfite Semi-Chemical (NSSC) mill, a bleaching plant as well as a de-inking plant. Gippsland Water Factory (GWF) is a major wastewater treatment facility located in the Latrobe Valley in Victoria, Australia. The plant, commissioned in 2010, has two distinct wastewater treatment process trains: An industrial train for pulp and paper manufacturing wastewater (PPMW) and a domestic train for approximately 16MLD of municipal sewage (see Figure One). Both treatment trains contain Membrane Bioreactors (MBRs) with submerged Memcor® hollow fibre membrane modules. This study focuses on GWF's industrial MBR which treats 16.4MLD of mostly pulping and bleaching waters. To our knowledge, the GWF was the first plant to apply a combination of anaerobic lagoons with subsequent MBR treatment of mixed PPMW on this scale.

The GWF industrial processing train (fig 1) starts with an anaerobic digestion phase (approx. 24hrs retention) effluent from the anaerobic digesters is fed directly into an anoxic/aerobic bioreactor before final filtration by submerged membranes. The anoxic/aerobic bioreactor consists of two individual bioreactors each with three aeration zones (Z1-Z3) [19]. See supplementary material for more detail.

Fig. 1: Schematic of the Gippsland Water Factory Highlighting Industrial Treatment
PPMW enters the two anaerobic reactors and is combined with WAS and primary sludge. Anaerobic Effluent enters the two MBR reactors. In the MBR Zone One (Z1) comprises anoxic sulfide conversion, zones 2 and 3 (Z2, Z3) are increasingly aerobic. The GWF has a total of twelve filtration cells (generally allocated as eight industrial and four domestic).

The GWF's use of MBRs is designed to produce treated effluent with the lowest possible Biochemical Oxygen Demand (BOD) [20]. In this regard, GWF is highly effective, with the BOD of the ultrafiltrate typically at or below the limit of detection. However, there are periods of reduced filterability of the MLSS. Subsequently, dewatering, chemical and other operational costs increase but most notably is the loss of capacity to treat the required volume of PPMW. Reduced MBR performance is associated with an increase in the MLSS Capillary Suction Time (CST), the principal operational measurement of treatability (see Fig. S1 for details on CST). The CST during the two-year period studied here ranged from 7 to 742 seconds (and from 11 to 507 seconds on the days sampled). This is in stark contrast to the domestic treatment stream where CST was rarely above 50 seconds.

The primary objective of this study was to understand the causes of high CST values and associated reduced MBR filterability during these events. We hypothesised that factors in the PPMW adversely affected the biomass (ie were toxic), resulting in either the death of microbial species or the production of extra-cellular polymers (ESPs) due to stress or nutrient limitation. We reasoned that the resultant biological material (dead cell debris, nucleic acids & proteins) or extra-cellular polymers (of unknown composition) were ultimately responsible for the reduced membrane filterability. To address this hypothesis, we applied a wide range of analytical techniques. The methodology involved a detailed temporal analysis of microbiological, physicochemical, and operational data from sources as summarized in Fig. 2.

Fig. 2: The six possible contributors to poor filterability / high CST. Areas investigated to inform the hypothesis included biological aspects (in yellow) and non-biological aspects (in blue). Each of these possible contributors was investigated using methods detailed below.

2.0 Materials and Methods

2.1 Sample Selection:

The GWF industrial train was sampled on 28 separate occasions between October 2014 and September 2016 (nominally every four weeks). On these days, three MLSS samples were gathered from the MBR (10 minutes apart) at a location just before the feed to the filtration cells (see figure one). The MLSS samples were subjected to a wide range of chemical and biological tests (detailed below). The GWF data recording system (Historian) provided additional monitoring data (flows, temperatures, and membrane parameters) obtained from the SCADA system. Samples of the plant influent, anaerobic effluent, zone one supernatant and MBR ultrafiltrate were also gathered and chemically analysed.

2.2 Chemical Methods

Total Organic Carbon (TOC) and Total Bound Nitrogen (TNb) were measured according to Std. Meth. 5310 B[21] on an Analytik Jena Multi N/C 3100 fitted with a CLD Plus nitrogen analyser. Simultaneous cation and anion chromatography were performed on twin Metrohm 881 Compact IC Pro's. The soluble cations (Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+}) were determined using Metrohm C4 150/4 columns with 1.7mmol HNO_3 and 0.7mmol/L Dipicolinic acid. The soluble anions (F^- , Cl^- , NO_3^- , PO_4^{2-} , SO_4^{2-}) analysis method utilized a suppressed Metrosep A Supp 5 150/4 1.0mmol/L NaHCO_3 and 3.2mmol/L Na_2CO_3 . Chemical Oxygen Demand (COD) was determined using a Merck test kit (1.145410.001) as per Std. Meth. 5220D [21]. Total Phosphate was determined with Merck test kit (1.14543.001) as per Std. Meth. 4500-P E [21] and using a Merck Nova60 Spectrophotometer.

2.3 Physico-Chemical Methods

Total and Volatile Suspended Solids were analysed in triplicate using a 90mm GFC filter-paper as per Standard Methods 2540 B & E [21]. The CST was determined in triplicate using a Triton Electronics 304M CST apparatus with Whatman® Chr 17 (70mm x 100mm) paper according to Std. Meth. 2710G [21]. A diagram of the CST apparatus is provided in Figure S1 of the Supplementary Material.

The Relative Hydrophobicity of the total MLSS was determined using the MATH test [22] where the hydrocarbon was hexane. The MLSS was first diluted to a concentration of 2500mg/l using a buffer (with the salt content of average ultrafiltrate). Before and after the extraction step, the absorbance of the water phase at 650nm was measured using a Hach DR5000/DR6000 Spectrophotometer, rather than the conventional TSS/VSS measurements [23].

Viscosity was measured using a spindle style Brookfield viscosity meter model LVTD. Bound Water was measured using the centrifugal method [24] at 1510 x g for three minutes. Results were normalized to grams per gram of TSS.

True Colour of the influent and ultrafiltrate samples was determined on 0.45µm filtrate. The samples were analysed without pH correction at a wavelength of 465nm on a Hach DR5000 /DR6000 Spectrophotometer. The VFA and Alkalinity ratio of the anaerobic reactor effluent was determined via dual end-point titration as per [25].

2.4 Biological Monitoring

Cellular and total ATP content were analysed using LuminUltra QG21W™ kits [26]. Calculations incorporating this data gave Biomass Stress Index and the Active Biomass Ratio as per the manufacturer's instruction.

Specific Oxygen Uptake Rate (SOUR) was performed using a Hach HQ40d LDO probe on 200ml of biomass as per Standard Methods 2710 B. Biomass was diluted with two parts of prewarmed buffer (with a salt content of average ultrafiltrate) and kept aerated at 32°C before the test. The Maximum Specific Oxygen Uptake Rate (SOUR_{MAX}) was established by adding 5ml of 10g/l acetic acid [27].

A Resazurin dye (7-hydroxy-3H-phenoxazin-3-one-10-oxide) assay was also used as an indicator of cellular dehydrogenase enzyme activity [28]. Triplicate vials of were inoculated with MLSS and 50mg /l of resazurin and incubated in a shaking water bath at 32°C for 30 minutes. A Hach DR5000/DR6000 spectrophotometer was used to measure the amount of remaining resazurin (not yet converted to resofuran) at 600nm.

2.5 Biological Molecular Analysis

200ul of MBR biomass was processed in triplicate using the PowerSoil® DNA Isolation Kit (Mobio Laboratories Carlsbad, CA, USA). The DNA was sent to the Australian Genome Research Facility (AGRF) where 300bp Paired End Illumina MiSeq was carried out on 16S DNA amplicons generated with universal bacterial primers 27F (AGAGTTTGATCMTGGCTCAG) and 519R (GWATTACCGCGGCKGCTG).

Sequence reads (300bp) were assembled using PEAR (version 0.9.5) [29]. Primer sequences were removed using Seqtk (version 1.0). These trimmed sequences were then processed using Quantitative Insights into Microbial Ecology (QIIME 1.8) [30] using tools USEARCH v8.0.1623 [31, 32] and UPARSE. Sequences were clustered and chimera filtered using “rdp_gold” database as a reference. To obtain the number of reads in each Operational Taxonomic Unit (OTU, which is roughly equivalent to a species), reads were mapped back to OTUs with a minimum identity of 97%. Using QIIME, taxonomy was assigned using Greengenes database5 (Version 13_8, Aug 2013) [33]. Post Biological Observation Matrix (BIOM) metagenomics analysis and graphing were performed in Calypso [34] and MetagenAssist [35].

2.6 Statistical Analyses

The MLSS for the Physical, Chemical and Biological analysis was collected in triplicate. The individual samples were measured in duplicate or triplicate. Mean values for each of the 28 rounds including the relative abundance of the top 50 Bacterial Identity groups (OTU’s) (some 165 results) were input into a spreadsheet. Using PAST 3.10 [36], a correlation matrix of the linear (Pearson) correlation r and p values data was produced. In excel the r values were squared, and the significant values highlighted using conditional formatting.

The 28 datasets were ranked according to their CST and split into quarters with each quarter representing seven separate sampling dates. Thus, quarter one (Q1) clusters seven dates with the lowest CST and quarter four (Q4), the seven dates with the highest CST. For a range of parameters, the means of each quarter were calculated for subsequent simplicity of statistical analysis.

2.7 Colloidal Measurements of MLSS Supernatant

Assessing the accumulation of colloidal materials in the MLSS supernatant is an essential aspect of this study. Firstly, the MLSS is centrifuged at 1510 x g for 3 minutes at room temperature. The supernatant is removed for TOC analysis. As the values determined comprises both colloidal and dissolved fractions, the TOC of the dissolved portion was subtracted to calculate the value of the colloidal fraction only. This colloidal fraction was denoted as Non-Centrifugable Total Organic Carbon (NCTOC).

Typical practice for wastewater analysis is to define anything that passes through a 0.45 μ m filter as ‘dissolved.’ The TOC of the 0.45 μ m filtered MLSS sample was therefore determined and defined as the soluble portion. While technically correct, the use of a 0.45 μ m filter to divide colloidal from soluble is arbitrary and not necessarily operationally relevant. In the ultrafiltration membranes used at GWF, the membrane nominal pore size is much smaller (0.05 μ m), and a portion of the technically soluble material (i.e. it would pass through a 0.45 μ m filter) is still too large to exit the MBR in the ultrafiltrate. Therefore, the second method of calculation of the colloidal material was performed using the MBR ultrafiltrate as the soluble fraction.

Calculation 1: Colloidal TOC = Centrifuged Supernatant TOC – Dissolved TOC (0.45 μ m filtered)
Calculation 2: Colloidal TOC = Centrifuged Supernatant TOC – MBR ultrafiltrate TOC (0.05 μ m)

2.8 Addition of Black Liquor to MLSS

Samples of Weak Black Liquor from three pulping processes were added to volumetric flasks and topped up with low CST biomass. The low CST MLSS sample was taken from the MBR treating the domestic wastewater at GWF. The CST of the resulting supplemented MLSS was analysed for CST in triplicate.

3.0 Results and Discussion

3.1 General Plant Performance

This research is primarily concerned with deducing the cause of capacity limitations in an MBR treating PPW. However, for completeness and to increase the publicly available performance data on full scale MBR's, the chemical characterisations have been supplied. The wastewater from the pulp and papermill is detailed in supplementary Table One, the anaerobic reactor effluent is in supplementary Table Two and the ultrafiltrate is in supplementary Table Three.

This study documents the performance of an MBR at a full scale over a period of 708 days. During this time GWF achieved an 86.3% reduction in mean COD concentration and an 85.0% reduction in mean TOC.

Continuous long-term data collection is vital to understanding an operational wastewater treatment plant as the PPMW is highly variable. This is shown by the data in supplementary table one; where the influent pH ranges from 6.7 to 10.8, the EC from 0.96 to 6.06 mS/cm and the colour from 208 to 11450PtCo units. Despite influent variation to the anaerobic reactors, their effluent (MBR influent) remained relatively stable as indicated by a maximum VFA/Alkalinity ratio of 0.329 indicating a stable process [37, 38]. Variation in biogas volume and quality was observed and was most often associated with sudden drops in influent temperature.

The high degree of individuality of pulp and paper wastewaters makes direct comparison between studies difficult. The average COD removal is similar however to that found by Spanish researchers (80%) [39] using a lab scale MBR. A slightly higher COD removal (85.6%) was achieved in a study of a pilot size anaerobic/aerobic (MBR) treating recycled paper manufacturing wastewater [18].

3.2 GWF parameters that correlate with CST values

Of the 165 operational, physico-chemical and biological parameters for which correlations with CST were calculated, 49 parameters were found to correlate with a p-value of below 0.05, 29 parameters below 0.005, and 14 at below 0.0005. The top 20 discriminating correlations are detailed in Table One.

Table One: The Top 20 Factors Correlating with CST

Rank	Data Point that CST Correlates with	Grouping	Pearson's r with CST	CST R ² Correlation	p value
1	Z4 Supernatant TOC minus 0.45um TOC (NCTOC)	Carbon	0.9829	0.9661	1.3E-20

2	Z4 Supernatant TOC minus ultrafiltrate TOC (NCTOC*)	Carbon	0.9823	0.9649	2.0E-20
3	Z4 Supernatant TOC	Carbon	0.9782	0.9569	2.8E-19
4	Z4 Supernatant Total Nitrogen	-	0.8827	0.7792	1.1E-09
5	Ave SRF 3days prior @15LMH & 25°C	Membrane	0.7542	0.5688	3.6E-06
6	Ave SRF 3days prior @15LMH	Membrane	0.7366	0.5425	7.9E-06
7	3 days prior mean resistance	Membrane	0.7077	0.5008	2.5E-05
8	Z4 dissolved monovalent to divalent cation ratio	Salt Balance	0.7070	0.4998	3.7E-05
9	Average daily SRF @15LMH	Membrane	0.6870	0.4720	5.4E-05
10	Average daily mean resistance	Membrane	0.6852	0.4695	5.7E-05
11	Reactor 4 Zone 1 dissolved TOC	Carbon	0.6779	0.4595	7.4E-05
12	Average daily SRF @15LMH & 25°C	Membrane	0.6778	0.4594	7.4E-05
13	Bound Water (specific g/g TSS)	-	-0.6634	0.4400	0.00012
14	Z4 Total Phosphorous	Phosphorous	0.6308	0.3979	0.00032
15	Z4 Divalent	Salt Balance	-0.6180	0.3819	0.00059
16	Reactor 3 Zone 1 dissolved TOC	Carbon	0.6131	0.3759	0.00067
17	Anaerobic Reactor Effluent VFA/ALK ratio	-	0.6089	0.3708	0.00058
18	10 days prior average colour (at influent point)	-	0.6029	0.3635	0.00068
19	Z4 dissolved calcium	Salt Balance	-0.5999	0.3599	0.00094
20	Reactor 3 Zone 1 dissolved phosphorous	Phosphorous	0.5983	0.3579	0.00098

The various factors that were found to correlate with CST are generally to do with Carbon, Salt Balance (ratio) or Membrane Factors. These Groupings are used to indicate instances where multiple related correlations exist. Note: SRF is specific resistance to filtration.

3.3 Microbial Species

The bacterial species (contributor 4 in figure two) were expected to be a major factor driving CST increases. However, the evidence gathered does not indicate a causal link. An examination of four of the most widely used microbial population diversity metrics (figure three) also shows minimal definitive variation with CST. Chao1 [40] and Richness (number of OTU's per sample) suggest a slight increase in average Bacterial population diversity from quarters 1 through 3, and then a decrease in the highest quarter. The Shannon and Simpson Indices show a decrease in the mean diversity from Q1-Q4. However, the spread of values in each group (mainly for the Simpson Index) were quite broad reducing confidence of its significance.

Fig 3: Four commonly used Population Diversity Measures. The dataset is split over the same four quarters with increasing CST as discussed in section 2.6 Chao1 and Richness suggest a slight increase in diversity from 1-3 and then a decrease in the highest quarter. The Shannon and Simpson Indexes show a decrease in the mean diversity from Q1-Q4. However, the spread of values (particularly for the Simpson Index) diminish the confidence of this finding.

No bacterial genera correlated with CST sufficiently to be included in the Top 20 correlations (Table One). Several genera were however, differentially abundant (Anova $p=0.0005$) between Q1 (low CST) and Q4 (high CST).

- An unidentified genera of the uncultured order HOC36, which comprised three main OTU's. These Gammaproteobacteria are much more prevalent in high CST samples.
- An unidentified genera from the family Sinobacteraceae. Comprising eight main OTU's These Gammaproteobacteria are also more prevalent in the high CST samples.
- Chlorobi of the Class SJA28, which are more prevalent in low CST samples.

That the HOC36 and Sinobacteraceae are significantly associated with high CST samples and that Chlorobi with low CST samples is supported by the LEfSE analysis (supplementary S2) [41]. Very little information is available on the Bacterial genera (HOC36, SJA28). The unidentified, Sinobacteraceae was first described and named as a Genus in 2008 [42]. It has subsequently been upgraded to its own family, which has at least ten different genera many of which can degrade xenobiotic compounds. Sinobacteraceae are considered part of the core microbiome of domestic wastewater treatment systems [43]. The HOC36 and Sinobacteraceae genes may be able to utilize the NCTOC as a carbon source hence their

relative abundance during high CST events. This does not seem likely given the prolonged duration of the CST events which suggest that the NCTOC is rather refractory. The other possibility is that these taxa flourish because of a higher affinity for nutrients often dosed into the MLSS during high CST events (discussed below).

3.4 Microbial Viability and Vitality

Investigation into the potential CST impact of changes in microbial viability (Contributor 5 Fig. 2) revealed little data to support the hypothesis the established bacterial population of the MBR was perturbed by high CST events. Neither the biomass stress index (from ATP testing), the mitochondrial metabolic activity (from Resazurin testing) nor the specific oxygen uptake rate (Fig. 4) correlated with CST ($R^2=0.0023$, $R^2=0.0361$ & $R^2=0.014$ respectively).

Fig. 4: The oxygen uptake dataset is split over four quarters according to the CST. SOUR and SOUR_{max} averages at various CSTs (n=7 events sampled in triplicate for each quarter). The Specific Oxygen Uptake Rate (blue) is slightly reduced at high CST's despite the presence of several thousand mg/l of TOC in the surrounding supernatant. However, when fed acetate for the SOUR_{max} test it was found to be capable of respiration above average levels (red).

The specific oxygen uptake rate (SOUR) was slightly reduced (Fig. 4) during high CST events. This is despite the presence of over 1000 mg/l of fine organic carbon in the MLSS supernatant. It could be argued that NCTOC materials in the supernatant exert an inhibitory effect on bacterial activity, however, when acetate was introduced for determination of the SOUR_{max} the respiration rate of the MLSS was above average. This indicates that the microbial population was not inhibited by NCTOC but were largely or totally incapable of using it as a carbon source.

3.4 MLSS Properties

The physical properties of the MLSS in the MBR (Contributor 3 in Fig. 2) presented stronger evidence of correlations with CST. The data presented in Table Two (below) mainly concern physical properties but also dependent on the plant influent (Contributor 1 in Fig. 2).

Table Two: Average Physical Characteristics of the MLSS quartered by CST value.

Parameter	Units	Q1 Ave	Q2 Ave	Q3 Ave	Q4 Ave
CST	seconds	16.1	26.2	64.5	267.6
pH	SU	7.17	7.15	7.28	7.41
EC	mS/cm	2.88	2.92	2.80	3.12
MBR temp (online)	Ave C	33.21	32.84	31.28	30.41
Colour Average 10 d prior	PtCo units	1013	1243	1322	2298
Specific Resistance to Filtration (norm).	$m^{-1}/10^{12}$	2.312	2.626	3.449	5.404
TSS	mg/l	8307	8431	8535	10407
VSS	mg/l	6826	7001	7153	8379
Specific Bound Water	g/gTSS	1.61	1.46	1.39	1.24
Relative Hydrophobicity	%	22.9	19.3	16.2	9.4
Viscosity	d	48.1	33.2	27.3	26.0
ABR	%	31.24	35.14	40.60	36.66
SOUR	O ₂ /h/gMLSS	5.70	8.02	7.77	6.41
SOURMAX	O ₂ /h/gMLSS	27.19	28.55	37.43	30.83
MonoValent to DiValent	ratio	2.83	3.54	4.45	6.35
Z4 (supnt) TOC	mg/l_C	148	217	404	1289
ARE VFA/ALK	mgVFA/mg Alk as CaCO ₃	0.142	0.143	0.168	0.200

Average Physical Characteristics of the MLSS split over four groups each with progressively higher average CST. (n=7 events sampled in triplicate for each quarter).

As can be seen in Table 2, there are some physico-chemical MLSS parameters which have vary in line with changes in CST. In general, higher CST's correlated with less relative hydrophobicity (Q1 22.9%, Q2 19.3%, Q3 16.2%, Q4 9.4%). The favourable properties of **more** hydrophobic sludges have previously been noted in conventional activated sludge treatment of PPW treatment [44]. Other studies of MBR MLSS are typically confined to domestic MLSS with CST's below 30 seconds [45, 46]. The CST's involved in the MBR treatment of pulp and paper wastewater can be more than an order of magnitude higher. The amount of bound water per gram of TSS reduced with increasing CST (Q1 1.61 g/g TSS, Q2 1.46 g/g TSS, Q3 1.39 g/g TSS, Q4 1.24 g/g TSS). Somewhat counterintuitively, the viscosity reduced as the CST increased which may be related to the decrease in bound water. This finding that high CST in MBR MLSS is not correlated with increased viscosity is supported by Moreau and co-workers [47].

3.5 Nitrogen and Phosphorous

Key MBR Plant influent properties (Contributor 1 of Fig. 2) such as nutrient levels, impact on its treatment efficacy and hence CST. Pulp and paper wastewater is well known for its low nutrient values which is problematic for biological treatment [48]. To achieve a more balanced C:N:P ratio in the MLSS and thus increase biological function, it is operational practice to increase aqua ammonia and phosphoric acid dosing during a high CST events. As nutrient dosing is an operational setting, examining this covers contributor 2 of Fig. 2 (GWF process). Overall the mean-ratio of TOC: N: P in MLSS achieved was 100 : 12.5 : 4.9. The operational control strategy for aqua ammonia dosing typically targets an excess of 0.1mg/L of nitrate/ammonia in the ultrafiltrate. Total nitrogen in the centrifuged supernatant strongly correlated with the CST ($R^2=0.779$) and ranked 4th in correlation Table 1. As

ammonia is taken up cellular biomass it would be expected to have little to do with this colloidal accumulation. The correlation is most likely due to the relatively constant C:N ratio of the organic materials from the paper mill (eg lignin).

The strategy for phosphorous dosing is less straightforward because a reliable ortho p residual could not be obtained by simply by measuring the ortho-P in the ultrafiltrate (as is the strategy for ammonia); i.e. volume of phosphoric acid required to record a residual ortho P in the ultrafiltrate intermittently fluctuated several-fold. In these cases, the excess phosphate was absorbed by the biomass itself in concentrations above normal cellular requirements. The ability to take up phosphorous is a feature of phosphate accumulating organisms (PAO's), suggesting that these are present in the MLSS at GWF. The presence of PAO's supported by the findings of ortho-P at levels (up to 28.5mg/L_P during this study) in the supernatant of the anoxic zones. It seems likely that the dosing of phosphoric acid during high CST events is the cause of the CST and phosphorous correlations (Table 2).

No conventional PAO's were identified in the top 50 bacterial sequences detected in the GWF system (data not shown). This lack of PAO's is not surprising as they are reported to be sensitive to sulfide [49]. PAO's that perform phosphorous removal in conjunction with the sulfur cycle have been discovered in treatment plants with the SANI process [50, 51]. Although the concentration of sulfur compounds is much more significant in PPW than in seawater, this data suggests that a similar phenomenon may be occurring at the GWF plant. Additional evidence of PAO's are the varying amounts of total phosphorous in the MLSS on a dry weight basis. Over this study, the amount of total phosphorous, per gram of TSS varied from 12.78 – 47.76 mg/g TSS. A fluctuating proportion of PAO's could account for the variability of these figures.

3.6 Non-Centrifugable Total Organic Carbon (NCTOC)

In domestic wastewater colloidal materials in the MLSS supernatant would normally be termed Extra-Cellular Polymers (Contributor 6 in Fig. 2). In the MBR literature, extracellular polymeric substances (EPS) are widely discussed (Drews 2010, Jiang et al. 2010) and categorised as either 'Bound EPS' (meaning attached to the floc structure) or 'Soluble EPS.' Here in the processing of pulp and paper waste waters, the colloids are extracellular but are neither bound nor soluble. Given the evident lack of microbial stress, there is nothing to suggest that the colloids are due to microbial death and decay. The high nutrient dosing during high CST's ensures the extra cellular materials are not a product of nutrient deficiency. Indeed, material of microbial origin would be expected to provide a readily degradable carbon source for other microbial species. This colloidal NCTOC material, however, appears highly refractory to biological treatment.

High CST values were found to correlate strongly with an increase in the concentration of total organic carbon (TOC) in the MLSS supernatant. The two highest ranked correlations (in Table 1) were obtained with data regarding the TOC colloidal portion of the MLSS measured in two different ways (Section 2.7; Calculation 1 and Calculation 2).

The colloidal result using the 0.45um filtered supernatant as the soluble portion (Calculation 1) gave the highest correlation ($R^2=0.966$, $p=1.25 \times 10^{-20}$). The method using the MBR

ultrafiltrate as the soluble portion (* in Table 1 using Calculation 2) showed a correlation of $R^2=0.965$, $p=1.97 \times 10^{-20}$ (Fig. 5).

Fig. 5: Correlation of CST with the NCTOC of the centrifuged MLSS supernatant. Calculated using the MBR ultrafiltrate as the soluble portion. This represents the 23 sample days which range between 11 and 507seconds.

The NCTOC is the key factor identified here as responsible for the MBR performance issues. It is operationally challenging for three reasons;

1. Firstly, the bulk of it is retained by the membranes (0.05um nominal) due to the effective size of its colloidal particles. While some of the highly coloured discharge (the soluble portion) exits the MBR ultrafiltrate several days after entering the plant, the retained portion appears to accumulate.
2. The inability (by definition) of the standard WWTP dewatering practice (centrifugation) to remove the NCTOC. Centrifuge supernatant (centrate) carrying the NCTOC is generally of low quality and is returned to the anaerobic digesters (see Figure One). Due to its colloidal nature, the NCTOC does not settle significantly in the anaerobic digesters and therefore has limited contact with the anaerobic biomass. It thus exits the anaerobic reactor in the supernatant and once again goes to the MBR and repeatedly cycles in this manner.
3. The apparent inability of the microbial populations of the MBR biomass to degrade the NCTOC as evidenced by low oxygen uptake rates.

The NCTOC material is also associated with an increase in sodium and a decrease in calcium (hence a change in the monovalent : divalent cation ratio) as shown by positions 8, 15 and 19 in table One. The start of high CST events is often observed to be preceded by influent which is darkly coloured. Due to these reasons, the compounds comprising the NCTOC are hypothesized to be chemically altered lignins from 'Black Liquor,'. This pulping by-product is notable for its high colour and low degradability [52, 53].

3.7 Addition of Black Liquor to MLSS

To confirm that high CST can be caused by Black Liquor, three neat "Weak Black Liquor" (WBL) samples were obtained. WBL is so named as they are later concentrated to a semisolid material to be burnt for energy production at which point they are called 'Black Liquor'. WBL's are an extremely concentrated salt and degraded lignin liquor. Tests on the black liquor revealed their organic carbon to be almost exclusively non-centrifugable at original pH (data not shown). WBL's originating from all three pulping processes were added to a low CST MLSS sample. Fig 6 shows that CST increases with the amount of WBL added for the two Kraft Pulp Mill samples.

Fig. 6: Dose Response Curves for three types of Black Liquor dosed into MLSS vs CST. Three types of Black Liquor were added in various volumetric percentages. Cont Kraft (CKM) is from the Continuous Kraft Pulp Mill, Batch Kraft (BKM) from the Batch Kraft Pulp Mill and NSSC is from the Neutral Sulfite Semi Chemical Pulp Mill. R^2 values displayed on the right for the line of best fit (cubic). The two Kraft Black Liquors demonstrate an ability to markedly increase the CST, while the NSSC Black Liquor does not.

The results in Fig. 6 suggest that Black liquor derived from the two Kraft processes have an impact on CST. The results may be partly due to the different concentrations of the 'Weak Black Liquor' used in this experiment (CKM = 60.6g/l Total Carbon, BKM = 57.2g/l Total Carbon and NSSC = 34.8g/l Total Carbon). However, if the concentration were the only factor some increase would be expected particularly in the 2.5 and 5.0% samples. The differences in their ability to cause CST increase are possibly explained by particle size and charge produced by the different pulping processes. The NSSC process utilises a partly mechanical rather than wholly chemical lignin degradation. The NSSC WBL has a more neutral pH and the sulfur is mostly in the form of sulphite. The WBL's produce by the Kraft process are extremely alkaline and the added sulfur is mostly as sulfide (USEPA 1990). Kraft WBL subsequently contains appreciable lignosulfonate concentrations.

4.0 Conclusions

This study undertook extensive chemical and biological and genomic testing to identify and understand the causes of high CST and provide operational solutions to maintain MBR

capacity. The hypothesis that high CST was a result of bacterial population shifts or stress linked EPS production was disproved.

Little evidence was found to support the theory that established bacterial MBR populations are perturbed by high CST events. Reduced biomass activity appears related to a lack of a readily degradable carbon source rather than bacterial toxicity. High CST MLSS exhibited several other tendencies; an increased hydrophilicity, less bound water and lower viscosity.

This study identified a significant and thus far uncharacterised correlator with MBR treatability i.e. a high level of supernatant TOC that is neither particulate nor soluble and did not settle upon centrifugation. It was thus named 'Non-Centrifugable Total Organic Carbon' (NCTOC). This substance is notable for its lack of degradability. Evidence at this time indicates that the NCTOC is consistent with 'Black Liquor' from pulping. That Black Liquor can cause high CST was experimentally confirmed.

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