

1 **Wastewater characterisation by combining size fractionation, chemical composition and**
2 **biodegradability**

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4 Kristin T. Ravndal^{a,b}, Eystein Opsahl^a, Andrea Bagi^{a,c} and Roald Kommedal^{a,*}

5 ^a University of Stavanger, Department of Mathematics and Natural Science, 4036 Stavanger,
6 Norway

7 ^b Cranfield Water Science Institute, Cranfield University, Bedfordshire, MK43 0AL, UK

8 ^c Marine Environment Group, International Research Institute of Stavanger, Mekjarvik 12,
9 4070 Randaberg, Norway

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13 * Corresponding author: roald.kommedal@uis.no

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17 Abstract

18 The potential for resource recovery from wastewater can be evaluated based on a detailed
19 characterisation of wastewater. In this paper, results from fractionation and characterisation of
20 two distinct wastewaters are reported. Using tangential flow filtration, the wastewater was
21 fractionated into 10 size fractions ranging from 1 kDa to 1 mm, wherein the chemical
22 composition and biodegradability were determined. Carbohydrates were dominant in
23 particulate size fractions larger than 100 μm , indicating a potential of cellulose recovery from
24 these fractions. While the particulate size fractions between 0.65-100 μm show a potential as
25 a source for biofuel production due to an abundance of saturated C16 and C18 lipids. Both
26 wastewaters were dominated by particulate ($>0.65 \mu\text{m}$), and oligo- and monomeric ($<1 \text{ kDa}$)
27 COD. Polymeric (1-1000 kDa) and colloidal (1000 kDa-0.65 μm) fractions had a low COD
28 content, expected due to degradation in the sewer system upstream of the wastewater
29 treatment plant. Biodegradation rates of particulate fractions increase with decreasing size.
30 However, this was not seen in polymeric fractions where degradation rate was governed by
31 chemical composition. Analytical validation of molecular weight and particle size distribution
32 showed below filter cut-off retention of particles and polymers close to nominal cut-off,
33 shifting the actual size distribution.

34 **Keywords**

35 Wastewater fractionation

36 Wastewater characterization

37 Resource recovery

38 Biodegradability

39

40 **Abbreviations**

41 BOD: Biological oxygen demand

42 COD: Chemical oxygen demand

43 COD/TN-ratio: COD to total nitrogen ratio

44 COD/TP-ratio: COD to total phosphorous ratio

45 MALS: Multi angle light scattering detector

46 OM: Organic matter

47 p.e: Person equivalents

48 PSD: Particle size distribution

49 SEC: Size exclusion chromatography

50 TSS: Total suspended solids

51 VSS: Volatile suspended solids

52 WWTP: Wastewater treatment plant

53 1 Introduction

54 Wastewater treatment has over the last century effectively improved recipient water quality,
55 reduced water pathogenicity and improved human health (van Loosdrecht and Brdjanovic,
56 2014). Being primarily concerned with water and sludge quality prior to disposal, attention
57 has been on removal mechanisms and unit processes for that purpose (Wilsenach et al., 2003).
58 Freshwater shortage in arid regions stimulated research and development of technologies for
59 reuse of treated wastewater (Asano and Levine, 1998). Energy and nutrient recovery has
60 gradually been integrated with treatment, and the last decades have seen increased attention
61 on both improved recovery and energy efficiency (Verstraete et al., 2009). More recently,
62 wastewater is seen as an important resource for bio-based production in circular waste
63 management (Puyol et al., 2017; Wang et al., 2015). This calls for future wastewater
64 technologies that recover wastewater valuables, in addition to nutrient, energy and water
65 reclamation (Puchongkawarin et al., 2015). Among putative technologies includes membrane
66 based up-concentration (Verstraete et al., 2009), intensified and integrated distillation
67 (Harmsen, 2007), advanced biological conversion and absorption (Matassa et al., 2015; Puyol
68 et al., 2017) and mixed culture solvent extraction (Wahlen et al., 2011).

69 Characterisation of physical and chemical components in wastewater is a prerequisite for
70 design and operation of wastewater treatment processes. Molecular and particulate size
71 distributions of components are important for evaluation of degradation and removal
72 mechanisms, and was first combined by Levine et al. (1991). As novel process technologies
73 and formulation of next generation treatment objectives for resource recovery will target more
74 specific compounds in raw wastewater, more detailed characterization is required (Choubert
75 et al., 2013; Tran et al., 2015). Compared to current characterisation strategies (e.g. the
76 STOWA approach; see review by Roeleveld and Van Loosdrecht, 2002), details on chemical
77 composition and biodegradability of size classes is necessary.

78 Traditionally, organic matter (OM) in wastewater has been fractionated into four size
79 fractions (Levine et al., 1991), however, the use of micro- and ultrafiltration allowed for
80 further separation of dissolved and colloidal size fractions in later studies (Dulekgurgen et al.,
81 2006; Karahan et al., 2008; Sophonsiri and Morgenroth, 2004). A few researchers have
82 combined size fractionation either with chemical composition (Sophonsiri and Morgenroth,
83 2004) or biodegradability (Karahan et al., 2008). However, these studies do not combine all
84 three factors. In addition, none of these studies validated the actual size distribution of the
85 obtained fractions, a concern related to known bias effects in ultra- and nanofiltration
86 (Cheryan, 1998). Hence, there is a need to evaluate wastewater components by the
87 combination of a validated size fractionation, with both chemical composition and
88 biodegradability of all size fractions.

89 The objectives of this study were to i) separate wastewater into several size fractions from
90 below 1 kDa to above 1 mm and validate the size distribution of the fractions analytically, ii)
91 quantitatively characterize the fractions in terms of nutritional and macromolecular
92 composition, iii) determine the biodegradability and evaluate which factors affect
93 biodegradation in each size range, and iv) evaluate the potential for resource recovery based
94 on the detailed composition analysis performed.

95 2 Materials and Methods

96 Wastewater samples were collected from two wastewater treatment plants (WWTP) located in
97 south-west Norway. Vik WWTP (approx. 50000 p.e.) receives wastewater from households in
98 the Jæren region, with significant contributions from agriculture and food processing
99 industries. Mekjarvik WWTP (approx. 250000 p.e) is located downstream of the urban areas
100 of Stavanger and Sandnes, and receive mainly domestic wastewater with some contribution
101 from small-scale service industries. Hence, the two wastewaters investigated here represent

102 (1) a high strength (especially in terms of COD) wastewater with dominant industrial loading
103 (Vik) and (2) a typical low to intermediate strength municipal wastewater (Mekjarvik).

104 2.1 Wastewater sampling and fractionation

105 At Vik WWTP, a 50 L screened (6 mm) raw wastewater grab-sample was collected in the
106 morning. At Mekjarvik WWTP, 60 L of wastewater was collected by a flow proportional
107 automatic composite sampler over 20 hours (Contronics PSW 2000). Immediately upon
108 arrival to the lab, pH of the wastewater sample was lowered to below 2.3 with concentrated
109 hydrochloric acid and kept refrigerated at 2 °C to prevent biodegradation during storage. Size
110 fractionation was carried out upon arrival by serial sieving, and cross flow micro- and
111 ultrafiltration as described in the supplementary information. Sieving was performed using
112 stainless steel sieves (VWR collection) with nominal sieve mesh sizes of 1 mm, 100 µm and
113 25 µm. Followed by serial microfiltration and ultrafiltration using a Cogent M1 tangential
114 flow filtering system (Merck Millipore). Microfiltration modules used were 0.65 µm and 0.1
115 µm Pellicon 2 Durapore C-screen filters 0.1 m² (Merck Millipore), while ultrafiltration were
116 performed using 1000 kDa, 100 kDa and 10 kDa Pellicon 2 Ultracel C-screen filter cassettes
117 0.1 m² (Merck Millipore), and 1 kDa Pellicon 2 Mini Ultrafiltration Module C-screen 0.1 m²
118 (Merck Millipore).

119 2.2 Validation of size distribution

120 2.2.1 Particle size distribution

121 Particle size distributions (PSD) of the 100-1000 µm, 25-100 µm and 0.65-25 µm fractions
122 were analysed by a Multisizer 4 coulter counter (Beckman Coulter) using a 1000 µm aperture
123 tube (measuring range 25-600 µm), a 200 µm aperture tube (measurement range 4-120 µm)
124 and a 100 µm aperture tube (2-60 µm) for the respective size fractions. For 200 and 100 µm
125 aperture tubes, 0.9 M% NaCl was used as electrolyte, while for the largest fraction, a mixture
126 of 0.9 M% NaCl (60 V%) and glycerol (40 V%) was used. Depending on the particle

127 concentration, 0.1-1 mL sample was diluted to 200 mL with corresponding electrolyte for all
128 analysis. Electrolyte blanks were measured for background noise subtraction.

129 2.2.2 Molecular weight and rms-radius

130 Molecular weight and rms-radius distribution of polymeric and colloidal fractions were
131 measured using an Agilent 1260 Infinity HPLC connected to multi angle light scattering
132 (MALS) detector (Dawn Heleos II 18, Wyatt Technology) and a differential refractive index
133 detector (Waters 2410). The HPLC system was equipped with a Shodex OHpak SB-807 HQ
134 column, a SB-805 HQ column (with additional SB-803 HQ column for the lower fractions)
135 and a OHpak SB-807G guard column, all kept at 20 °C throughout the experiment. Sodium
136 azide (0.1 g L^{-1}) fixated concentrated fraction samples were filtered through either $1.2 \text{ }\mu\text{m}$ or
137 $0.45 \text{ }\mu\text{m}$ cellulose nitrate syringe filters, depending on fraction size, and $100 \text{ }\mu\text{L}$ was injected
138 into an isocratic mobile phase flow of 0.5 mL min^{-1} . The mobile phase was prepared by
139 filtering an aqueous buffer solution containing 13.4 g L^{-1} Na-HEPES (Sigma-Aldrich), 0.625
140 g L^{-1} HNO_3 (Sigma-Aldrich), 8.5 g L^{-1} NaNO_3 (Sigma-Aldrich), and 0.1 g L^{-1} sodium azide
141 (Sigma-Aldrich) through a $0.1 \text{ }\mu\text{m}$ filter (Millipore - Durapore). Sample run time was set to
142 90 minutes with total elution time of roughly 45 minutes, to allow re-equilibration of the
143 columns. The injection needle was flushed between each injection to limit cross
144 contamination. A bovine serum albumin (BSA) standard (0.5 g L^{-1} , 0.185 mL g^{-1} , 66 kDa) was
145 used as a control with regards to normalization, alignment, band broadening and for
146 verification of the method in general. A dn/dc value of 0.165 was used as it approximate the
147 average value for heterogeneous biopolymers (Cheong et al., 2015; Zhao et al., 2011). The
148 ASTRA 6.0 software (Wyatt Technology, 2017) was used for data interpretation using a
149 Berry 1st degree fit for all fractions. Detectors 8 through 18 were enabled for molecular
150 weight analysis, while detectors 3 through 18 were enabled for the determination of rms
151 radius of fractions larger than 100 kDa. MALS data was extrapolated at low peak-end of

152 fractions smaller than 10 kDa, while dRI data was extrapolated on high peak-end of fractions
153 larger than 100 kDa.

154 2.3 Solids and chemical composition analysis

155 2.3.1 Solids

156 Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according
157 to standard method 2540 (APHA et al., 2012) whereby five replicates of raw wastewater
158 samples (50 mL) were filtered with 1 μm pore size GF/C filters (Whatman). Five blank
159 samples were prepared with 50 mL distilled water.

160 2.3.2 COD, nitrogen and phosphorous

161 Chemical oxygen demand (COD), total nitrogen (total-N), ammonium, nitrate, total
162 phosphorous (total-P) and phosphate were determined using range appropriate commercially
163 available spectrophotometric kits (Spectroquant, Merck). Samples were analysed in
164 triplicates. COD, total-N, and total-P was determined in all wastewater fractions, while
165 ammonium, nitrate, and phosphate were only measured in the TSS-filtrate and in the final
166 permeate.

167 2.3.3 Carbohydrate

168 Carbohydrate concentration was analysed using the anthrone-sulfuric assay with
169 recommended modifications (Leyva et al., 2008). Anthrone reagent (Sigma-Aldrich) was
170 prepared at 0.2 % concentration in concentrated sulphuric acid (Merck Millipore). A dilution
171 series of sucrose (5-1500 mg L^{-1} , VWR) was used as calibration standards. Samples (50 μL)
172 were loaded onto transparent flat-bottom 96-well microtiter plates in quadruplicates before
173 adding 200 μL Anthrone-sulphuric reagent. The contents in the loaded plate were mixed in an
174 ultrasonic bath (10 min), followed by incubation at 95 $^{\circ}\text{C}$ (5 min) and cooling to room
175 temperature. Absorbance was read at 485 $\text{nm} \pm 10 \text{ nm}$ with a Tecan Infinite f200 PRO plate
176 reader. Carbohydrate concentrations were transformed to COD equivalents based on an

177 assumed typical composition of $C_{10}H_{18}O_9$ giving $1.12 \text{ g COD g}^{-1}$ carbohydrate (Henze et al.,
178 2002).

179 2.3.4 Proteinaceous material

180 Total protein, in this work referred to as proteinaceous material, was determined with the
181 NanoOrange Protein Quantitation Kit (Invitrogen N6666). A calibration standard dilution
182 series was prepared using bovine serum albumin (>98 % heat shock fraction, Sigma-Aldrich)
183 in Milli-Q water. The entire assay and preparative steps was performed in a darkroom with
184 deep red illumination. Wastewater samples (10 % of total reagent volume) and reagent diluent
185 were prepared and loaded onto transparent flat bottom 96-well microtiter plates in
186 quadruplicates. Fluorometry was performed with a Tecan Infinite f200 PRO plate reader at
187 excitation wavelength of $465 \text{ nm} \pm 20 \text{ nm}$ and emission wavelength of $590 \text{ nm} \pm 10 \text{ nm}$.
188 Concentrations were transformed to COD equivalents based on a typical average composition
189 of proteinaceous material, $C_{14}H_{12}O_7N_2$, giving $1.20 \text{ g COD g}^{-1}$ proteinaceous material (Henze
190 et al., 2002).

191 2.3.5 Lipids

192 Lipid content was determined according to a procedure described in detail in the
193 supplementary information. Total oils and fats were extracted in a 1:1 mixture of chloroform
194 and methanol, and determined gravimetrically. Total lipids and fatty acids composition was
195 determined by GC-FID (Agilent Technologies) following transesterification and methylation
196 of lipids into fatty acid methyl esters (FAME) according to Ryckebosch et al. (2012). Total
197 lipids and free fatty acids concentrations were transformed to COD equivalents based on a
198 typical ThOD of 2.8 g COD g^{-1} lipid, for remaining total oils and fats a reported average of
199 $2.03 \text{ g COD g}^{-1}$ oils and fats was used (Henze et al., 2002).

200 2.4 Biodegradability

201 Biological oxygen demand (BOD) was measured by static respirometry (OxiTop®-C, WTW,
202 Germany). Test samples were diluted to 200 mg COD L⁻¹ final concentration for Vik
203 wastewater, and to 100 mg COD L⁻¹ for Mekjarvik wastewater. Some fractions had lower
204 COD concentration than 100 mg COD L⁻¹, and for these fractions the highest achievable
205 concentration based on the concentration after fractionation and upconcentration was used.
206 Inorganic nutrients, amino acids and vitamins were added as described earlier (Bagi et al.,
207 2014) and pH was adjusted to ~7. Three replicate test flasks were prepared for each fraction,
208 and each flask was spiked with sludge: (1) fresh sludge (250 µL) from Vik WWTP for Vik
209 wastewater fractions, (2) sludge (250 µL) from a fed-batch reactor (initially seeded by the
210 same Vik sludge) maintained in our laboratory for Mekjarvik wastewater fractions. Test
211 blanks were prepared by treating autoclaved tap water the same way as wastewater samples.
212 Oxygen consumption was monitored for 7, 14 or 21 days at 20 °C incubation temperature
213 under continuous mixing. Oxygen utilisation rate (OUR) was calculated as rate of change in
214 BOD over six measurement points (gliding average). Both BOD and OUR data were
215 normalized against initial COD concentration in the test bottles. Biodegradable and inert COD
216 content in wastewater were estimated as described in supplementary information based on the
217 method proposed by (Roeleveld and Van Loosdrecht, 2002).

218 3 Results and discussion

219 3.1 Wastewater composition

220 Wastewater composition (COD, Total-N, Total-P, carbohydrate, proteinaceous material, total
221 oils and fats, total lipids and free fatty acids, biodegradable COD (BCOD) and inert COD) are
222 summarised in table 1 and 2 for Vik and Mekjarvik wastewater, respectively. Overall
223 recovery of COD was 78 ± 3 % for Vik wastewater, and 98 ± 4 % for Mekjarvik wastewater
224 fractionations, respectively. Unidentified COD was determined as the difference between the

225 measured total COD and the sum of identified COD fractions. As expected from upstream
226 sources, a higher total COD ($1421 \pm 58 \text{ mg COD L}^{-1}$) was measured in raw Vik wastewater,
227 compared to Mekjarvik ($690 \pm 30 \text{ mg COD L}^{-1}$). Carbohydrate content as a percentage of total
228 COD of $11.6 \pm 0.8 \%$ and $57 \pm 3 \%$ was found for Vik and Mekjarvik wastewater,
229 respectively. Compared to measured carbohydrate content in raw municipal wastewater from
230 earlier studies (15-36 %; Gorini et al., 2011; Huang et al., 2010; Raunkjær et al., 1994), Vik
231 wastewater had a low carbohydrate content, while Mekjarvik wastewater contained
232 substantially more. The total oils and fats content was $27 \pm 2 \%$ and $30 \pm 2 \%$, with a total
233 lipids and free fatty acids content of $21.8 \pm 0.9 \%$ and $15.4 \pm 0.7 \%$ in Vik and Mekjarvik
234 wastewater, respectively. Lipid content were comparable to literature values (1-38 %; Gorini
235 et al., 2011; Huang et al., 2010; Raunkjær et al., 1994), and represented 51 to 81 % of the total
236 oils and fats. A low content of proteinaceous material was found in both Vik ($0.89 \pm 0.06 \%$
237 of total COD) and Mekjarvik wastewater ($5.8 \pm 0.3 \%$ of total COD) compared to literature
238 values of 16-28 % (Gorini et al., 2011; Huang et al., 2010; Raunkjær et al., 1994). Vik
239 wastewater has a high lipid concentration (table 1) which is known to interfere with the
240 NanoOrange Protein assay (Jones et al., 2003), hence, it is not unlikely that protein content
241 was underestimated. An underestimation of protein content could also explain the high
242 amount of unidentified COD in Vik wastewater ($61 \pm 5 \%$).

243 Particle size distribution has in earlier studies been found to directly affect wastewater
244 biodegradability (Dulekgurgen et al., 2006). The size distribution of COD, Total-N, Total-P,
245 macromolecules, BCOD and inert COD among particulate ($> 0.65 \mu\text{m}$), colloidal (1000 kDa –
246 $0.65 \mu\text{m}$), polymeric (1-1000 kDa), and oligomeric and monomeric ($< 1 \text{ kDa}$) size fractions is
247 presented in figure 1. Relative abundance in each fraction is presented as a percentage
248 (fraction %) of raw wastewater concentrations, except for size fractions with over 100 %
249 recovery (table 1 and table 2). There, the sum of recovered material was used as 100 %

250 instead of the raw wastewater concentration in order to allow for relative comparisons. The
251 majority of COD in Vik wastewater was found in the particulate (36 ± 2 %), and oligomeric
252 and monomeric fractions (40 ± 2 %). In addition, 22 ± 2 % of total COD was unaccounted for
253 during fractionation. Similarly, particulate (44 ± 2 %), and oligomeric and monomeric (53 ± 2
254 %) COD dominated in Mekjarvik wastewater. Low COD concentrations found in polymeric
255 and colloidal size fractions, between 1 kDa and $0.65 \mu\text{m}$, corresponds well with the low COD
256 concentrations measured in polymeric size range of municipal wastewater in earlier studies
257 (Doğruel, 2012; Dulekgurgen et al., 2006). Low COD within colloidal and polymeric ranges
258 can be either due to degradation in the sewer system upstream of the WWTP, coagulation and
259 flocculation of polymers and colloids, or degradation of colloids and polymers during the
260 filtration process due to shear stress and enzymatic degradation. Degradation upstream of the
261 WWTP would lead to: (1) an increase of bacterial biomass, which would subsequently appear
262 as high COD in the $0.65\text{-}25 \mu\text{m}$ fraction, and (2) an increase of oligomeric and monomeric
263 partly recalcitrant degradation products, which would appear as high inert COD in the < 1
264 kDa fraction. In addition, if the system has a low active biomass concentration or the biomass
265 is predominantly flocculated or in a biofilm, depolymerisation of colloidal and polymeric
266 COD would lead to increased concentrations of biodegradable COD in the oligomeric and
267 monomeric size fraction (Ravndal and Kommedal, 2017). In our study, both wastewaters had
268 high COD concentrations in these two fractions, and high concentration of inert material was
269 found in the oligomeric and monomeric size fraction (table 1 and table 2). This suggests that
270 significant degradation of COD in the wastewater occurred upstream of the WWTP, and that
271 sewer system hydraulic retention may shift COD size distributions. The latter has been
272 observed by several authors (Hvitved-Jacobsen et al., 2002).

273 The distribution of inert material between size fractions varied between the two studied
274 wastewaters (table 1 and 2). Inert material was estimated by modelling the BOD data using

275 first order kinetics (supplementary information). Modelling BOD curves using first order
276 kinetics generally has its limitations due to the models inability to follow complex
277 degradation patterns. Especially for large particulate fractions with slower initial degradation
278 and a distinct bimodal growth phase, the first order model gave a poor fit (supplementary
279 information figure E1). However, for most fractions the first order model reached a
280 representable ultimate BOD level, thus, a representable inert fraction could be estimated. Vik
281 wastewater had a higher inert content in the particulate fractions ($18 \pm 1 \%$) than Mekjarvik
282 wastewater ($9 \pm 1 \%$). Mekjarvik wastewater was highly dominated by oligomeric and
283 monomeric inert matter ($90 \pm 3 \%$). In Vik wastewater only $32 \pm 2 \%$ of inert matter was in
284 this fraction, however a large fraction of the inerts were not recovered. Compared to Vik
285 WWTP, Mekjarvik WWTP has a larger inflow of petroleum industry wastewater and urban
286 runoff. These sources contain a higher fraction of slowly biodegradable priority organic
287 pollutants and inert pollutants (Kommedal et al., 2016). In addition, high concentrations of
288 recalcitrant matter in the oligomeric and monomeric size fraction could be a result of
289 degradation of OM upstream of the WWTP. Mekjarvik WWTP has a longer sewer system
290 retention time compared to Vik WWTP, allowing for a more complete biodegradation.
291 Finally, concentrations of inert and unknown COD in the oligomeric and monomeric fraction
292 in Mekjarvik wastewater are comparable (table 2), further supporting the hypothesis that inert
293 matter are due to the combination of degradation upstream of the WWTP, and urban runoff
294 and inflow from the petroleum industry.

295 Both COD/TN-ratio and COD/TP-ratio decreased with decreasing size fraction for particulate
296 and colloidal size fractions (figure 2) confirming an earlier study by van Nieuwenhuijzen et
297 al. (2004). Hence, nitrogen and phosphorous containing substances (e.g. proteinaceous
298 material and phospholipids) were expected to be more abundant in lower size fractions in
299 wastewater, while macromolecules not containing nitrogen or phosphorous (e.g. cellulose,

300 starch and lignin) were expected to be more abundant in the large particulate fractions. This
301 corresponds with the analysis of carbohydrates, proteinaceous material and lipids in the two
302 wastewaters. Carbohydrates were abundant in the largest particulate fractions (figure 4),
303 expected due to toilet paper residues (Ruiken et al., 2013). Proteinaceous material content was
304 found to increase with decreasing size for particulate and colloidal size fractions (figure 4),
305 and there was a significant correlation between nitrogen content and content of proteinaceous
306 material (p-value < 0.05, SI). Total lipids and free fatty acids content increase with decreasing
307 size in the particulate fractions (figure 4) and were positively correlated with total-P content
308 in both wastewaters (p-value < 0.05, SI), supporting the theory of phospholipids affecting the
309 trend seen for COD/TP-ratio.

310 3.2 Factors affecting biodegradation of OM in different size fractions

311 Maximum OUR (figure 3) and estimated first order rate constants (k_1 , supplementary
312 information table D1) increased with decreasing particle size for both Vik and Mekjarvik
313 wastewater, for the latter the increase continued also with decreasing size for colloids. This
314 confirms earlier results were degradation rate increased with decreasing particle size for
315 faecal particles (Ravndal et al., 2015), egg white particles (Dimock and Morgenroth, 2006)
316 and casein particles (Aldin et al., 2011). Increasing degradation rate with decreasing size can
317 be explained by the particle breakup model proposed by (Dimock and Morgenroth, 2006).
318 Surface area to volume ratio of particles increases with decreasing size, hence substrate
319 availability will increase with decreasing size leading to a faster degradation for smaller
320 particles. In addition, the time it took to reach maximum substrate specific OUR, was
321 significantly higher for the two largest particle fractions of both wastewaters in comparison
322 with the smaller size fractions (figure 3), further supporting a slower degradation of large
323 particulate fractions.

324 Besides OUR and k_1 , extent of degradation increased with decreasing particle size
325 (supplementary information, figure D1). A similar increase was also observed for colloids.
326 Extent of degradation should be the same between fractions if chemical composition was the
327 same, hence, we interpret that as being due to a difference in chemical composition between
328 the size fractions (table 1 and table 2). Size dependency on extent of degradation was not
329 observed in earlier studies where extent of biodegradation was independent of particle size
330 (Aldin et al., 2011; Ravndal et al., 2015).

331 Polymeric fractions on the other hand, do not show the same size dependency (figure 3). In
332 fact, Vik wastewater polymeric fractions show the opposite trend with decreasing rate with
333 decreasing size, however, no trend was observed for Mekjarvik wastewater. For polymers
334 with identical chemical composition, such as dextran standards, degradation rate increase with
335 decreasing size (Kommedal et al., 2006). The polymeric wastewater fractions differed in
336 chemical composition (table 1 and table 2), and we suggest differences in chemical
337 composition to be more important for biodegradation rate than differences in polymer size in
338 the polymeric fractions.

339 3.3 Analytical validation of size fractionation

340 In earlier studies, validation of size fractionation has not been performed (Dulekgurgen et al.,
341 2006; Karahan et al., 2008; Sophonsiri and Morgenroth, 2004). When serial filtering
342 wastewater, actual size distribution of OM in the different size fractions can differ from the
343 nominal membrane cut-off due to filter cake retention, induced shear stress, shape of
344 molecules, concentration polarization and membrane rejection (Logan and Jiang, 1990).
345 These effects result in an underestimation of low molecular weight compounds. On the
346 contrary, induced shear stress may degrade particulate and large polymeric materials,
347 increasing the lower molecular weight fractions. In earlier studies, such as the studies by
348 Dulekgurgen et al. (2006) and Huang et al. (2010) this was not considered. Sophonsiri and

349 Morgenroth (2004) used parallel filtration when possible to overcome these problems,
350 however, filter clogging led to the need to serially filter some samples. To minimise cake
351 formation and concentration polarisation, and to control the mass balance and estimate
352 recovery, serial filtration using tangential flow filtration was selected in this study.

353 Particle size distribution (PSD), rms radius, and molecular weight distributions for Mekjarvik
354 wastewater are presented in figure 5. Based on PSD data, the 100-1000 μm fraction contained
355 a significant amount of particles smaller than nominal sieve cut-off (approximately 70 % of
356 particle volume). The same was observed in the 25-100 μm fractions, but to a lesser extent
357 (36 %). Retention of particles smaller than nominal sieve cut-off probably occurred due to
358 filter cake retention. Observed PSD in the 0.65-25 μm fraction seem to fall well within the
359 defined limits for the macroscopic particles ($> 2 \mu\text{m}$). However, we observed severe
360 membrane fouling of the 0.65 μm membrane which most likely led to increased retention of
361 colloids and polymers below the 0.65 μm nominal cut-off. Evidently, the fractions
362 immediately below 0.65 μm had much lower COD concentrations (table 1 and 2). In spite of
363 this, chemical composition and biodegradability of the immediately lower size fractions are
364 expected to be representative due to non-selective retention. Cumulative weight average
365 molecular weight and particle diameter of colloidal and polymeric size fractions (figure 5)
366 show a decrease in diameter or molecular weight with descending nominal filter cut-off,
367 however, molecules and particles larger than membrane cut-off was present. For polymeric
368 fractions, a larger shift towards higher size fractions was seen for size fractions with
369 decreasing molecular weight. This could be an effect of the shape of the molecules, or
370 alternatively, a result of overrepresentation of larger molecules in the MALS technique, since
371 bigger molecules scatter light exponentially more than small ones (Wyatt, 1993).

372 3.4 Implications for wastewater treatment processes and resource recovery
373 Detailed analysis of wastewater composition is a prerequisite for potential resource recovery
374 from wastewater. Size distributions are important for development and selection of adequate
375 unit processes, and both elemental and macromolecular information are necessary for proper
376 recovery estimations with respect to the aforementioned unit processes. In addition, both
377 design and operation of novel and complex recovery plants would benefit greatly from the use
378 of advanced bioprocess models able to predict and systematically analyse system
379 performance. Compared to the existing modelling frameworks used in the field (ASM and
380 ADM models), this would probably mean higher model resolution (several fractional state
381 variables), and several stoichiometric and kinetic parameters. Consequently, compositional
382 analysis as presented in this work is required, both in terms of size fractionation and chemical
383 species composition. By combining particle size distribution, chemical composition, and
384 biodegradability analysis, an example of such an analysis is given of two wastewaters
385 representing two very different scenarios for recovery: The classical household and diverse
386 industrial and the food-industrial dominated municipal wastewaters. In both wastewaters, the
387 two largest particulate fractions (> 1 mm and $100\ \mu\text{m} - 1$ mm) contained mainly
388 carbohydrates (table 1 and table 2), likely due to toilet paper residuals. Earlier research has
389 shown that sieving raw wastewater with 0.35 mm has great potential to recover slowly
390 biodegradable cellulose from toilet paper, recovered cellulose could be used as feedstock for
391 biofuel production or for production of new toilet paper (Ruiken et al., 2013). Based on the
392 results of this study sieves down to 0.1 mm might be applicable for extraction of cellulose
393 from raw wastewater. Removing the large particulate fraction mainly composed of slowly
394 degradable carbohydrates early, could lead to more efficient biological treatment stages
395 downstream of the sieves.

396 The particulate size fractions between 0.65-100 μm has a high lipids content in both
397 wastewaters. The reason for this is unclear, however, in the dairy dominated Vik wastewater
398 we suspect this to be lipid micelles from milk fat, and presence of general fat globules (both
399 known to be in the lower μm range; Fox and McSweeney, 2006). The most abundant lipids in
400 these size fraction were C16 (relative concentration 38 % in Vik wastewater and 31 % in
401 Mekjarvik wastewater, SI) and C18 lipids (relative concentration 43 % in Vik wastewater and
402 54 % in Mekjarvik wastewater, SI). In addition, a high saturation levels of C16 and C18 was
403 found in both wastewaters (combined saturation level of 62 % in Vik wastewater and 58 % in
404 Mekjarvik wastewater, SI). Abundance of highly saturated C16 and C18 lipids is ideal for
405 biodiesel production (Zhu et al., 2017), hence, the two size fractions between 0.65-100 μm are
406 appropriate fractions for direct biofuel recovery.

407 Enhanced energy recovery by biogas production and treatment process energy efficiency are
408 both dependent on the ability of wastewater treatment unit processes to separate COD
409 containing components before bacterial oxidation (Sutton et al., 2011). This is a key argument
410 used for anaerobic wastewater treatment, however, residual effluent COD and lack of nutrient
411 removal mechanisms limit its use as stand-alone processes. Another strategy is to maximize
412 separation of COD containing components from the raw wastewater upstream of
413 bioconversion processes by enhanced primary treatment (Verstraete et al., 2009). Our results
414 indicate that the particulate fraction that normally do not settle in the primary settler, the 0.65
415 – 25 μm fraction contain 15-22 % of the raw COD (almost all biodegradable), would upon
416 adequate separation significantly enhance energy performance and recovery. Furthermore, the
417 high lipid content in this fraction would stimulate biogas production potential, as the specific
418 COD of lipids are high. Contrary to energy, nutrient recovery potential in the particulate
419 fractions are limited. About 80 % of the size class specific nutrients are found in the low M_w
420 dissolved fraction ($> 1 \text{ kDa}$), and unit operations for direct recovery should focus on these.

421 While this paper focus on wastewater characterization and potential for resource recovery,
422 recovery also rely on the technological and economic feasibility. Ruiken et al. (2013)
423 presented an economic and technical evaluation of cellulose recovery from municipal
424 wastewater, and concluded sieving of the $> 350 \mu\text{m}$ fraction to be a feasible energy recovery
425 and efficiency measure. Lipids recovery, as highlighted here as a potential resource, has been
426 technically and economically evaluated using algal bioreactors, but full-scale implementation
427 is still a challenge due to unfeasible technical or economical limitations (Zhu et al, 2017). Of
428 the several challenges summarized, efficient and economically favourable extraction and
429 purification unit processes needs to be developed for wastewater lipids recovery.

430 4 Conclusions

431 We investigated biodegradability, COD and nutrient composition on detailed size fractionated
432 wastewaters. To our knowledge, this is the most detailed and comprehensive compositional
433 analysis published and allow for novel strategies for modelling and resource recovery. Key
434 findings are:

- 435 • Particulate size fractions larger than $100 \mu\text{m}$ are highly abundant in carbohydrates,
436 possibly from toilet paper, that can be recovered by sieving. The particulate size
437 fractions between $0.65\text{-}100 \mu\text{m}$ are highly abundant in highly saturated C16 and C18
438 lipids, making these size fractions a good source for lipids extraction for biodiesel
439 production.
- 440 • Biodegradation rates of particles in wastewater were controlled by particle size, i.e.,
441 surface area to volume ratio of the organic matter, resulting in smaller particles having
442 higher degradation rates. In the polymeric fraction, degradation rates of soluble
443 polymeric organic matter were governed by chemical composition and not by polymer
444 size.

- 445 • The two municipal wastewaters analysed here were dominated by particulate and
446 oligomeric and monomeric size organic matter. Very little COD was found in
447 polymeric and colloidal fractions, possibly due to degradation in the sewer upstream
448 from WWTP.
- 449 • Overall recovery of organic material was relatively high in both fractionation
450 experiments. Analytical validation of actual PSD in our size-fractions showed below
451 filter cut-off retention of particles close to nominal cut-off in particulate size ranges,
452 leading to PSD shifting to lower sizes. An opposite trend was observed for polymeric
453 fractions, where a shift towards higher than the nominal membrane pore sizes was
454 seen.

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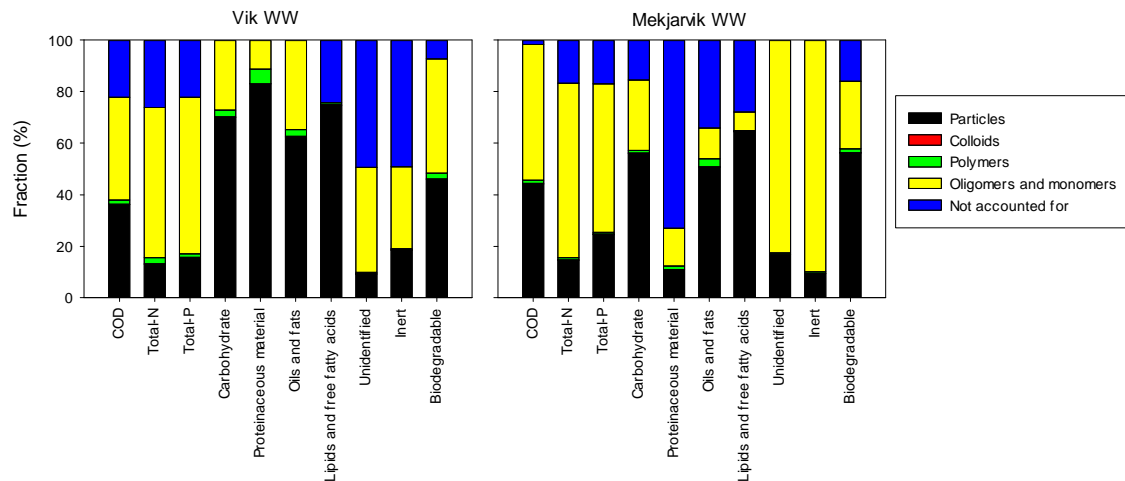
Table 1: Concentration of COD, total-N, total-P, carbohydrate, proteinaceous material, total oils and fats, and total lipids and free fatty acids in raw wastewater (WW), TSS filtrate and all size fractions from Vik wastewater (\pm standard error)

Fraction	COD (mg L ⁻¹)	Total-N (mg N L ⁻¹)	Total-P (mg P L ⁻¹)	Carbohydrate (mg COD L ⁻¹)	Proteinaceous material (mg COD L ⁻¹)	Oils and fats (mg COD L ⁻¹)	Lipids and free fatty acids (mg COD L ⁻¹)	Unidentified (mg COD L ⁻¹)	Inert (mg COD L ⁻¹)	Biodegradable (mg COD L ⁻¹)
Raw WW	1421 \pm 58	62 \pm 6	10.7 \pm 0.6	165 \pm 8	12.6 \pm 0.6	382 \pm 16	310 \pm 1	862 \pm 61	506 \pm 21	915 \pm 38
TSS filtrate	590 \pm 7	43 \pm 1	6.62 \pm 0.04	n/a	n/a	n/a	n/a	n/a	n/a	n/a
> 1 mm	113 \pm 4	1.13 \pm 0.05	0.30 \pm 0.07	109 \pm 45	0.72 \pm 0.04	18.4 \pm 0.6	14.74 \pm 0.04	0	17 \pm 1	97 \pm 7
100 μ m – 1 mm	51 \pm 4	0.82 \pm 0.05	0.19 \pm 0.01	39 \pm 18	1.4 \pm 0.5	8.9 \pm 0.4	5.69 \pm 0.06	1 \pm 18	15 \pm 2	36 \pm 4
25 – 100 μ m	32.2 \pm 0.6	0.76 \pm 0.02	0.144 \pm 0.003	5.9 \pm 0.4	0.9 \pm 0.2	15.1 \pm 0.5	10.3 \pm 0.5	10 \pm 1	7.6 \pm 0.5	25 \pm 2
0.65 – 25 μ m	318 \pm 2	5.4 \pm 0.4	1.03 \pm 0.05	16 \pm 2	9.7 \pm 0.4	225 \pm 3	200 \pm 1	67 \pm 5	54 \pm 4	263 \pm 17
0.1 – 0.65 μ m	3.14 \pm 0.08	0.150 \pm 0.002	0.025 \pm 0.002	0.41 \pm 0.05	< 0.04	n/a	n/a	2.7 \pm 0.1	1.66 \pm 0.06	1.49 \pm 0.05
1000 kDa – 0.1 μ m	0.38 \pm 0.04	0.016 \pm 0.002	0.0039 \pm 0.0004	< 0.15	< 0.04	1.4 \pm 0.1	1.2 \pm 0.9	0	0.20 \pm 0.02	0.18 \pm 0.02
100 – 1000 kDa	0.83 \pm 0.02	0.050 \pm 0.002	0.0082 \pm 0.0003	0.23 \pm 0.03	< 0.04	1.6 \pm 0.1	1 \pm 1	0	0.29 \pm 0.03	0.54 \pm 0.06
10 – 100 kDa	4.30 \pm 0.02	0.317 \pm 0.02	0.040 \pm 0.002	0.9 \pm 0.1	0.26 \pm 0.02	3.6 \pm 0.2	1 \pm 1	0	0.54 \pm 0.04	3.8 \pm 0.3
1 – 10 kDa	16.4 \pm 0.1	1.07 \pm 0.02	0.093 \pm 0.002	5.2 \pm 0.6	0.63 \pm 0.08	6.2 \pm 0.2	0.152 \pm 0.002	4.4 \pm 0.7	0.5 \pm 0.3	16 \pm 12
< 1 kDa	567 \pm 5	36.3 \pm 1	6.5 \pm 0.2	66 \pm 8	1.72 \pm 0.08	149 \pm 9	0.00 \pm 0.00	351 \pm 13	161 \pm 5	406 \pm 13
Recovery (%)	78 \pm 3	74 \pm 8	78 \pm 5	147 \pm 30	122 \pm 8	112 \pm 5	75.7 \pm 0.8	50 \pm 4	51 \pm 2	93 \pm 5

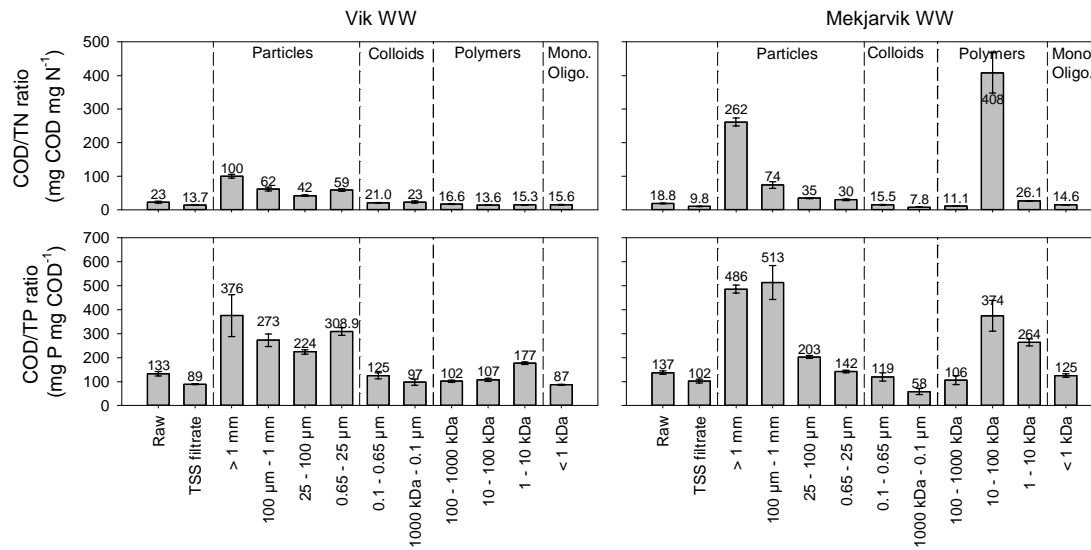
1 **Table 2:** Concentration of COD, total-N, total-P, carbohydrate, proteinaceous material, total oils and fats, and total lipids and free fatty acids in raw wastewater (WW),
 2 TSS filtrate and all size fractions from Mekjarvik wastewater (\pm standard error)

Fraction	COD (mg L ⁻¹)	Total-N (mg N L ⁻¹)	Total-P (mg P L ⁻¹)	Carbohydrate (mg COD L ⁻¹)	Proteinaceous material (mg COD L ⁻¹)	Oils and fats (mg COD L ⁻¹)	Lipids and free fatty acids (mg COD L ⁻¹)	Unidentified (mg COD L ⁻¹)	Inert (mg COD L ⁻¹)	Biodegradable (mg COD L ⁻¹)
Raw WW	690 \pm 30	37 \pm 1	5.0 \pm 0.2	395 \pm 16	39.9 \pm 0.8	209 \pm 12	106 \pm 2	47 \pm 36	191 \pm 10	499 \pm 27
TSS filtrate	262 \pm 10	27 \pm 1	2.6 \pm 0.2	n/a	n/a	n/a	n/a	n/a	121 \pm 6	142 \pm 7
> 1 mm	139 \pm 5	0.53 \pm 0.02	0.286 \pm 0.003	149 \pm 23	1.09 \pm 0.02	37.3 \pm 0.6	21.4 \pm 0.1	0	11 \pm 3	128 \pm 37
100 μ m – 1 mm	27 \pm 4	0.37 \pm 0.02	0.053 \pm 0.002	45 \pm 5	0.31 \pm 0.04	5.5 \pm 0.2	4.5 \pm 0.5	0	5.6 \pm 0.8	22 \pm 3
25 – 100 μ m	37.2 \pm 0.8	1.07 \pm 0.03	0.184 \pm 0.004	11.1 \pm 0.3	1.04 \pm 0.05	17.9 \pm 0.5	11.7 \pm 0.1	7 \pm 1	5.3 \pm 0.5	32 \pm 3
0.65 – 25 μ m	101.6 \pm 0.9	3.4 \pm 0.2	0.71 \pm 0.03	15.4 \pm 0.3	1.9 \pm 0.1	45.4 \pm 0.4	30.9 \pm 0.4	39 \pm 1	2.5 \pm 0.5	99 \pm 18
0.1 – 0.65 μ m	0.64 \pm 0.02	0.0412 \pm 0.0005	0.0053 \pm 0.0007	0.47 \pm 0.01	0.022 \pm 0.002	0.00 \pm 0.00	0.044 \pm 0.001	0.13 \pm 0.03	0.06 \pm 0.04	0.6 \pm 0.4
1000 kDa – 0.1 μ m	0.20 \pm 0.02	0.0264 \pm 0.0005	0.0035 \pm 0.0007	0.54 \pm 0.03	0.023 \pm 0.002	0.00 \pm 0.00	0.08 \pm 0.04	0	0.035 \pm 0.003	0.17 \pm 0.02
100 – 1000 kDa	0.382 \pm 0.009	0.0345 \pm 0.0004	0.0036 \pm 0.0006	0.52 \pm 0.03	0.026 \pm 0.002	0.00 \pm 0.00	0.04 \pm 0.07	0	0	0.382 \pm 0.009
10 – 100 kDa	2.6 \pm 0.3	0.0065 \pm 0.0006	0.0071 \pm 0.0009	1.36 \pm 0.05	0.080 \pm 0.003	0.00 \pm 0.00	0.021 \pm 0.002	1.2 \pm 0.3	0.71 \pm 0.09	1.9 \pm 0.3
1 – 10 kDa	6.36 \pm 0.06	0.244 \pm 0.009	0.024 \pm 0.001	1.7 \pm 0.2	0.42 \pm 0.01	6.4 \pm 0.2	0.299 \pm 0.001	0	1.2 \pm 0.2	5.2 \pm 0.8
< 1 kDa	363 \pm 5	25 \pm 1	2.9 \pm 0.2	108 \pm 2	5.9 \pm 0.4	25 \pm 3	7.68 \pm 0.09	224 \pm 6	233 \pm 6	131 \pm 3
Recovery (%)	98 \pm 4	83 \pm 4	83 \pm 4	84 \pm 7	27 \pm 1	66 \pm 4	72 \pm 2	580 \pm 448	136 \pm 8	84 \pm 9

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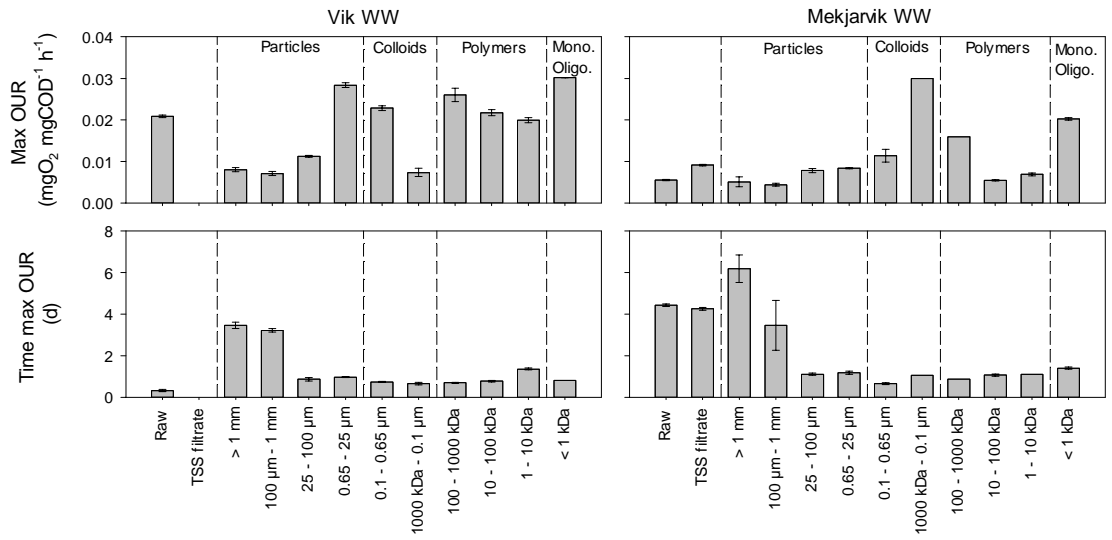
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 2 **Figure 1:** Size distribution of OM in particulate ($>0.65 \mu\text{m}$), colloidal ($1000 \text{ kDa} - 0.65 \mu\text{m}$), polymeric (1-1000
 3 kDa) and truly dissolved ($< 1 \text{ kDa}$) size ranges. Calculations are based on raw wastewater measurement except
 4 for carbohydrate, proteinaceous material, and oils and fats for Vik wastewater, and unidentified and inert
 5 material for Mekjarvik wastewater, where the sum of material in the size fractions is used due to a recovery
 6 higher than 100 %
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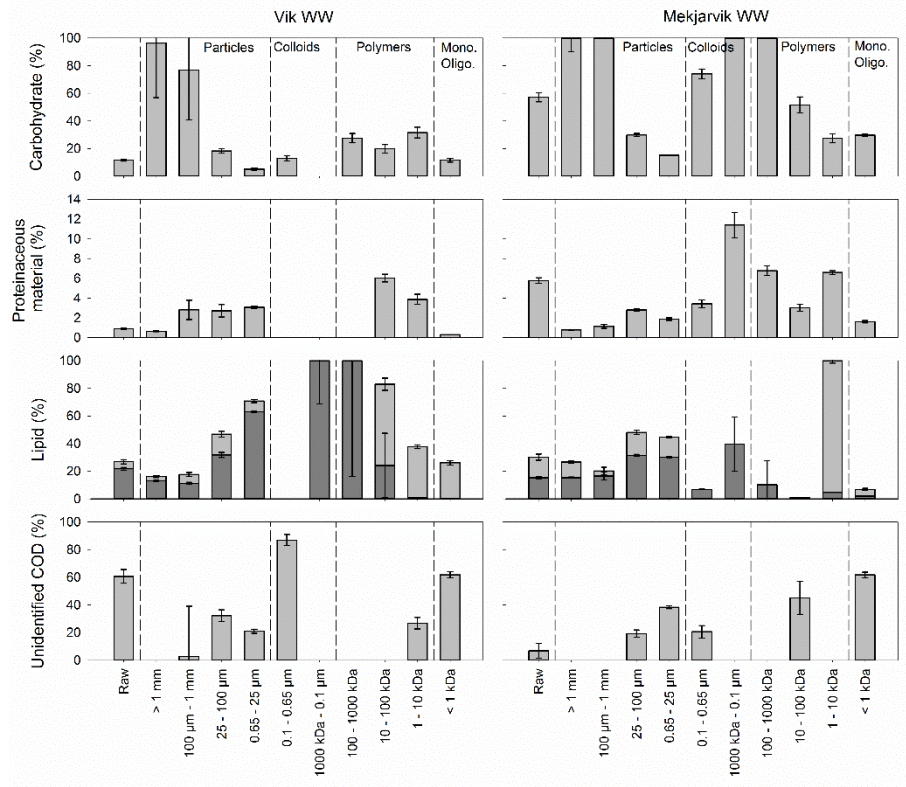
2 **Figure 2:** COD/TN and COD/TP in raw wastewater and all size fractions for Vik and Mekjarvik wastewater.

3 Error bars show standard deviations calculated for relative values.



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Figure 3: Max OUR (mg O₂ mg COD⁻¹ h⁻¹) and time to reach max OUR (d). Error bars show standard error. *)
1000 kDa – 0.1 μm and 100-1000 kDa fraction Mekjarvik wastewater data from only one parallel, the rest
average of data from three parallels.

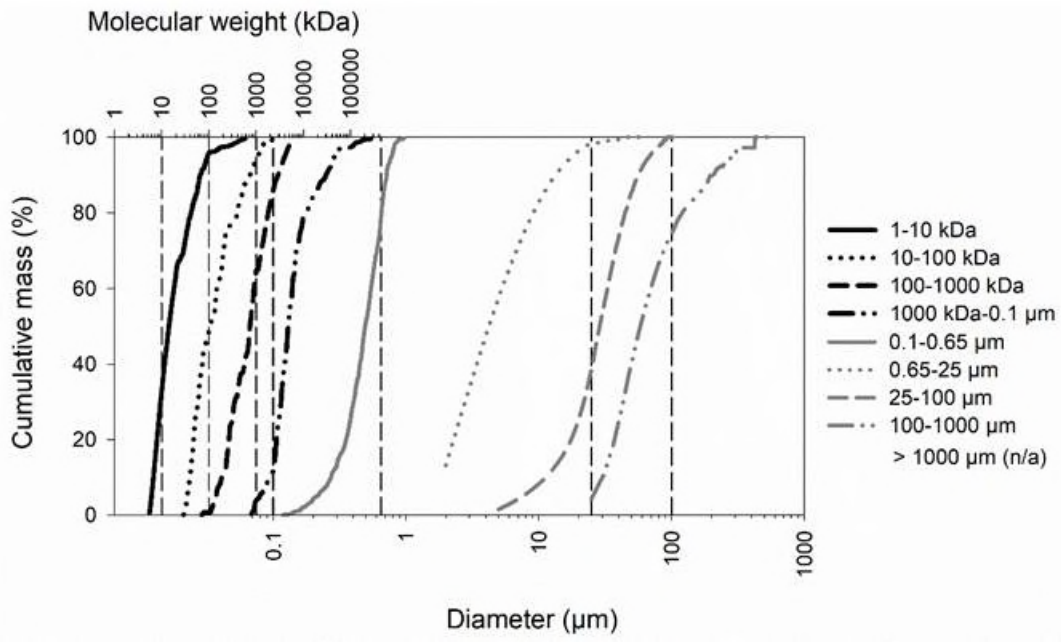


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Figure 4: Content of carbohydrate, proteinaceous material, lipids as total oils and fats (light grey) and total lipids and fatty acids (dark grey), and unidentified COD in raw wastewater and all size fractions for Vik and Mekjarvik wastewater. Percentage was calculated as percentage of measured COD in each individual fraction. Error bars show standard deviations calculated for relative values.

6



1

2 **Figure 5:** Analysed size distribution in size fractionated wastewater from Mekjarvik WWTP. Particulate

3 fractions are based on volume measurements and density was assumed constant between fractions.

4

Supplementary information

A. Serial filtration of wastewater

Figure A1 shows the WW-fractionation setup, the left section shows the initial sieving while the right section shows the tangential flow filtration (TFF) stages. During the sieving process, raw wastewater was fed with a peristaltic pump onto a column holding the three sieves with consecutively smaller cut-offs. A small amount of washing water (Milli-Q pH 2.3 adjusted with HCl) was used to remove trapped sub-fraction particles. The amount of washing water was recorded and the water itself was pooled together with the rest of filtered WW. The sieving-filtrate was then transferred to the TFF feed tank (first pass). During the TFF, the system was run in a continuously fed batch mode until all the water had passed through each filter. The first TFF run was carried out with the 0.65 μm cutoff filter. At the end of the run, a small amount of washing water was added to the concentrate to wash out salts and trapped sub-fraction particles. Again, the amount of the washing water was measured and the water itself was pooled together with the filtrate. This washing step was carried out at each filtration stage. The fraction concentrate was drained from the lowest point of the TFF system unassisted until it stopped flowing. Finally, the filtrate was returned to the clean and emptied TFF feed tank and the process was repeated using the next filter (0.1 μm cutoff). Similarly, four more rounds were carried out with to complete the size fractionation. Throughout the entire TFF process, temperatures were logged externally for the concentrate tank, feed tank, membrane filter and filtrate tanks. Feed, transmembrane and delta pressures were also logged in order to keep track of filter performance. The weight of concentrate was set as a trigger to stop the process preventing running the system dry. In addition, coolant loops and heat

exchangers where either submerged in tanks or placed in line to keep the wastewater cool. The WW were also continuously mixed at 200 rpm or circulated to prevent settling.

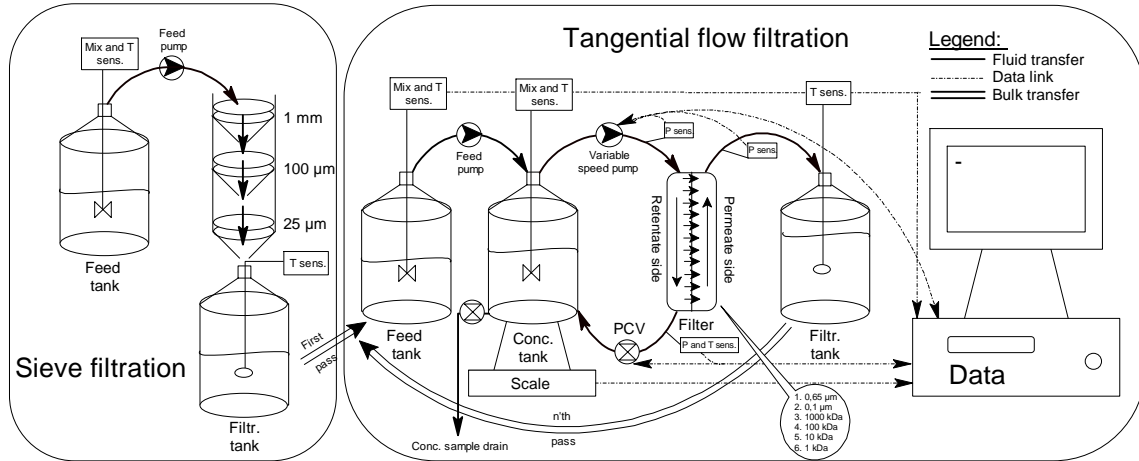


Figure A1: Experimental setup of the serial filtration process. The wastewater was first sieved, followed by micro and ultrafiltration with a tangential flow filtration setup.

B. Determination of lipids content in wastewater

Total oils and fats extraction

All glassware and volumetric equipment were rinsed using a 1:1 mixture of analytical grade Methanol (NOMAPURE, VWR Int.) and Chloroform (EMSURE, Merck GmbH). Volumetric aliquots (about 10 ml) of concentrated fractionated wastewater was quantitatively transferred to Teflon sealed 200 ml Schott bottles (Duran Group GmbH) and mixed with 20 ml Chloroform and 20 ml Methanol. Lipids were extracted for a minimum of 2 hours on a shaker, intermittently sonicated for 2x8 min on a Branson 2510 sonicator at 50°C/100 W (Branson Ultrasonics, Danbury CT, USA). Following extraction, the mixture was quantitatively transferred to a 250 ml separation funnel and left overnight for phase separation. The lower fraction was collected, and the liquid re-extracted two times by shaking using 20 ml Chloroform and 10 ml deionized water. The

combined extract was transferred to a Syncore analyst (BÜCHI Labortechnik AG, CH), vacuum distiller and concentrated to < 1 ml (at 55°C and 250 mbar A). The concentrate was quantitatively transferred to a Teflon lined 10 ml vial using an additional 3-4 ml Chloroform and stored in the dark at 4°C.

Clean up

Extracted samples were transferred to 15 ml conical Duran glass centrifuge tubes and added 1 ml distilled water, vortexed and centrifuged at 1000 g for 5 min. The upper layer was removed using a Pasteur pipette, while the lower Chloroform layer was transferred to an anhydrous Na₂SO₄ packed glass column for residual water removal. The removed water layer was transferred back to the centrifuge tube, and re-extracted using 5 ml of 1:1 Chloroform-Methanol and dried over the same column (same procedure as above). The combined extracts were evaporated to dryness by a gentle stream of Argon inside a 50°C heat block, and resuspended in 2 ml Toluene:Methanol (2:1). 1 ml was transferred to a 2 ml glass vial for gravimetric determination of total oil and fats, while the remaining sample (1.00 ml) was used for total lipids determination.

Gravimetric determination of total oils and fats

The 1.00 ml oil and fats extract was evaporated to dryness in a Visiprep SPE Vacuum manifold (Supelco Inc) to dryness by a gentle stream of Argon at 250 mbar A. Following vacuum drying, the sample was weighed using a high precision analytical balance (0.01 mg resolution, Mettler Toledo model XSE). The vial was dried for another 15 min until constant weight. Total oils and fats was removed using repeated flushing using a 1:1 Chloroform – Methanol mixture, and the vials were vacuum dried until constant weight.

Total oils and fats was determined as the differential weight before and after oil and fats removal.

Derivatisation and analysis of total lipids

Total lipids and fatty acids composition was determined by GC-FID following transesterification and methylation of lipids into fatty acid methyl esters (FAME) according to Ryckebosch et al. (2012). 1 ml of fresh 2% (v/v) sulfuric acid (pro analysis, VWR Int.) in methanol (NOMAPURE, VWR Int.) was added to the remaining 1.00 ml total oils and fats extract (above). The sample was vortexed and left for lipids methylation for 1 hour at 80°C. After methylation and cooling to room temperature, 0.5 ml 5 M aqueous NaCl solution was added and sample vortexed, followed by addition of 2 ml Hexane (HiPerSolv CHROMANORM® for HPLC, VWR chemicals) and vortexing. The samples were centrifuged for 2 min at 1000 g, and the upper layer transferred to a glass column packed with anhydrous Na₂SO₄ for drying. Samples were re-extracted by another 2 ml Hexane and 1 ml water, centrifuged and the hexane layer dried and combined with the initial upper layer. The methylated extract was dried under a gentle stream of Argon at 50°C (in heat block), and upon drying, resuspended in 1000 µl Hexane:Chloroform (2:1) mixture for GC analysis.

FAME distribution and quantification was determined using an Agilent 6890 gas chromatograph equipped with an Agilent J&W DB-225MS capillary column (PN 122-2932) operated in constant pressure mode, using Helium as carrier gas. 1 µl sample was injected using a Gerstel MPS liquid mode sampler onto a 220°C pulsed split inlet (50:1 split ratio, at 350 kPa for initial 0.5 min, followed by 93 kPa run pressure). The initial oven temperature was 50°C, followed by an immediate ramp of 26°C/min to 200°C, and a final ramping to 230°C at 2.7°C/min (hold for 11.12 min) to give a total runtime of 28

min. FAME's were detected by a flame ionization detector kept at 240°C operated in constant makeup mode. Quantification and retention time identification was achieved by external standard calibration using a five point dilution series of certified Supelco 37 component FAME mix standard (Supelco, Sigma-Aldrich) containing 37 FAME standards from C4:0 to C24:6 methylated fatty acids. Retention time confirmation and unknown peak identification were performed under the same capillary column and GC conditions, but using a quadropole mass selective detector (Agilent 5975) operated in SCAN EI mode (scan range 35 to 400 amu at 70 eV) and a source and quad temperature of 180 and 150°C, respectively. Unknown peaks and standard peak elution order verification were done by identification using NIST library comparison (Agilent NIST bundle version 05).

Recovery and quality control

Matrix recovery was determined by addition of a known amount of commercial olive oil (31.86 mg) to three replicates of SNJ 25-100 µm fraction sample. Results showed a stable sample matrix recovery of 70 ± 2 % for FAME determination, and 86 ± 1 % for gravimetric determination of oil and fats. All sample analytical results were compensated using these recovery factors. A quality standard of 2 mg lipids sample using commercial olive oil, was prepared and used for analytical stability and recovery checks. External calibration standards were co-analyzed with samples to correct for retention time drifting and FID response factor compensation.

C. Solids and nutrient content in wastewater

Concentrations of total COD, TSS, VSS, ammonia, nitrate and phosphate in Vik and Mekjarvik WW are provided in table C1.

Table C1: Concentrations of total COD, TSS, VSS, ammonia, nitrate and phosphate in Vik and Mekjarvik WW (\pm standard error)

	Vik WW	Mekjarvik WW
COD	1421 \pm 58	690 \pm 30
TSS (mg L ⁻¹)	371 \pm 4	262 \pm 13
VSS (mg L ⁻¹)	343 \pm 4	222 \pm 10
NH ₄ ⁺ (mg N L ⁻¹)	30.3 \pm 0.3	22.7 \pm 0.5
NO ₃ ⁻ (mg N L ⁻¹)	< 0.5	< 0.5
PO ₄ ³⁻ (mg P L ⁻¹)	5.70 \pm 0.06	2.53 \pm 0.03

D. Correlations of total-N, total-P and chemical components

Nitrogen and phosphorous content of COD was compared with carbohydrate, proteinaceous material, total oils and fats, total lipids and free fatty acids, and unidentified COD by testing for correlations based on Pearson product-moment correlation coefficients (table D1). For this analysis, ammonia and phosphate concentrations was subtracted from total-N and total-P data for raw WW and for the < 1 kDa fraction.

Table D1: Correlation between proteinaceous material, carbohydrate, total oils and fats, total lipids and free fatty acids and unidentified COD content (%) with total-N (TN/COD) and total-P (TP/COD) content. Correlations based on Pearson product-moment correlation coefficients, correlations are considered significant if P-values < 0.05

	Vik WW			Mekjarvik WW		
	Correlation Coefficient	P-value	Significant	Correlation Coefficient	P-value	Significant
Carbohydrate vs total-N	-0.28	0.4	No	0.66	0.03	Yes
Proteinaceous material vs total-N	0.84	0.009	Yes	0.84	0.001	Yes
Total oils and fats vs total-N	0.38	0.3	No	-0.16	0.6	No
Total lipids and free fatty acids vs total-N	0.24	0.5	No	0.46	0.2	No
Unidentified vs total-N	-0.18	0.6	No	-0.42	0.2	No
Carbohydrate vs total-P	-0.22	0.5	No	0.64	0.03	Yes
Proteinaceous material vs total-P	0.92	0.001	Yes	0.79	0.004	Yes
Total oils and fats vs total-P	0.78	0.008	Yes	-0.20	0.6	No
Total lipids and free fatty acids vs total-P	0.69	0.03	Yes	0.60	0.05	Yes
Unidentified vs total-P	-0.30	0.4	No	-0.33	0.3	No

E. First order simulation of BOD

For Vik WW, biological oxygen demand (BOD) was measured over 7 days for raw WW and 14 days for all size fractions (figure E1). For Mekjarvik WW, BOD was measured over 21 days for all samples (figure E1). Modelling BOD curves using first order kinetics generally has its limitations due to the models inability to follow complex degradation patterns. Especially for large particulate fractions with slower initial degradation and a distinct bimodal growth phase, the first order model gave a bad fit (figure E1). Bimodal growth was a result of initial fast growth of readily degradable particles, followed by degradation of slowly degradable particles. Bimodal growth was also observed in the smaller size fractions, however these had a faster initial growth, followed by a slower second growth period. However, for most fractions the first order model will reach a representable ultimate BOD level, thus a representable inert fraction was estimated.

The second growth phase observed in several fractions after 12 days for Vik WW and 15 days for Mekjarvik WW (figure E1) were most likely due to nitrification. No nitrification inhibitor was used during BOD tests due to possible degradation of the inhibitor itself, which would lead to interference with the BOD measurements. This is normally checked using parallel controls, but limited instrumental capacity did not allow for this in this experiment. The smaller size fractions have a late second growth phase, and in general has a lower COD/TN ratio than the particulate fractions (figure 2). On the contrary, the 10-100 kDa fraction of Mekjarvik WW had a very high COD/TN ratio and does not have a second growth phase further supporting this to be an effect of nitrification.

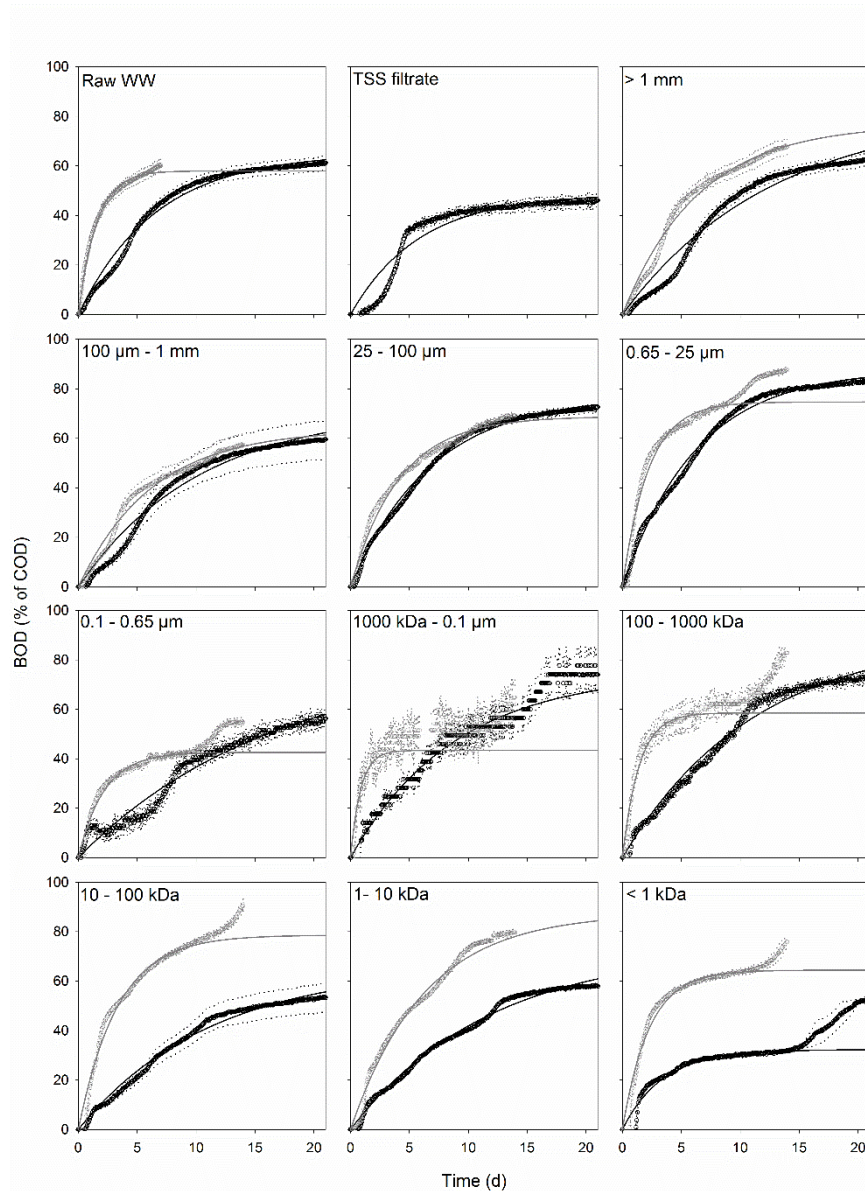


Figure E1: BOD as % of initial COD value for Vik WW (grey) and Mekjarvik WW (black). Dotted lines show standard deviations calculated for relative values, and solid lines show 1.order simulation of BOD data modelled after Roeleveld and van Loosdrecht (2002). *) 1000 kDa – 0.1 µm and 100-1000 kDa fraction data from Mekjarvik WW from one parallel only.

Inert COD fractions was estimated based on the method proposed by Roeleveld and van Loosdrecht (2002). First order kinetics was used to estimate ultimate biodegradable COD (BCOD). Inert COD was then calculated by subtracting BCOD from initial COD.

Estimated first order rate constants (k_1) and estimated fraction of inert COD (f_{inert}) are presented in table E1.

Table E1: Estimated first order rate constants (k_1) and estimated fraction of inert COD in Vik and Mekjarvik WW. Estimations based on method proposed by Roeleveld and van Loosdrecht (2002).

Fraction	Vik WW		Mekjarvik WW	
	k_1	f_{inert}	k_1	f_{inert}
	(h ⁻¹ mg COD ⁻¹)	(mg COD mg COD ⁻¹)	(h ⁻¹ mg COD ⁻¹)	(mg COD mg COD ⁻¹)
Raw WW	0.0033 ± 0.008	0.356 ± 0.002	0.00148 ± 0.00007	0.277 ± 0.009
TSS filtrate	n.a.	n.a.	0.0034 ± 0.0002	0.46 ± 0.01
> 1 mm	0.00078 ± 0.00005	0.146 ± 0.009	0.00075 ± 0.00006	0.08 ± 0.02
100 µm – 1 mm	0.00080 ± 0.00006	0.29 ± 0.02	0.0010 ± 0.0001	0.204 ± 0.008
25 – 100 µm	0.00155 ± 0.00006	0.24 ± 0.01	0.00146 ± 0.00002	0.14 ± 0.01
0.65 – 25 µm	0.00238 ± 0.00006	0.17 ± 0.01	0.00159 ± 0.00001	0.025 ± 0.004
0.1 – 0.65 µm	0.00304 ± 0.00007	0.53 ± 0.01	0.0028 ± 0.0003	0.10 ± 0.06
1000 kDa – 0.1 µm	0.065 ± 0.006	0.52 ± 0.03	0.0026*	0.17*
100 – 1000 kDa	0.0172 ± 0.0007	0.35 ± 0.04	0.0011*	0.00*
10 – 100 kDa	0.00154 ± 0.00004	0.127 ± 0.009	0.0009 ± 0.0001	0.27 ± 0.02
1 – 10 kDa	0.00080 ± 0.00001	0.03 ± 0.02	0.00084 ± 0.00004	0.18 ± 0.03
< 1 kDa	0.00229 ± 0.00004	0.284 ± 0.009	0.0029 ± 0.0002	0.64 ± 0.01

*) data from one parallel

F. Lipids concentrations

Relative concentrations of lipids in Vik wastewater (table F1) and Mekjarvik wastewater (table F2) are calculated as percentage of total lipid concentration in the size fraction.

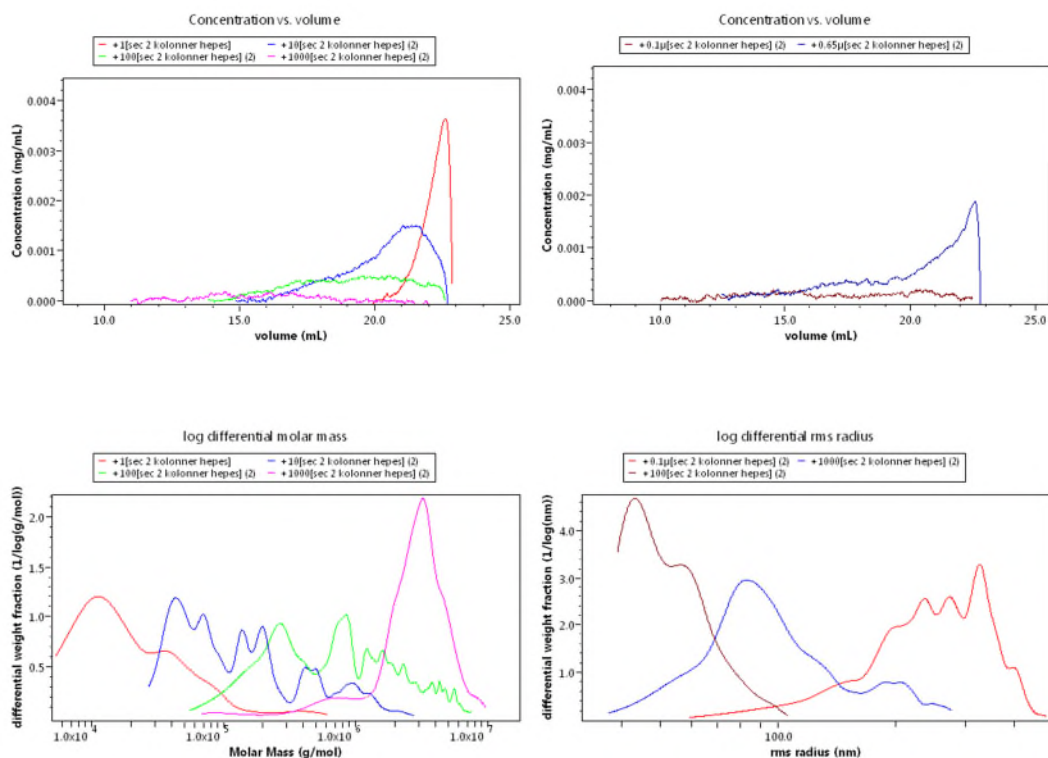
Table F1: Relative concentration of lipids in Vik wastewater (%)

	Raw	> 1 mm	100 µm – 1 mm	25 – 100 µm	0.65 – 25 µm	0.1 – 0.65 µm	1000 kDa – 0.1 µm	100 – 1000 kDa	10 – 100 kDa	1 – 10 kDa	< 1 kDa
C6:0	0.00	0.00	0.00	0.00	0.96		0.00	0.00	0.00	0.00	0.00
C8:0	0.00	0.00	0.00	0.00	0.89		0.00	0.00	0.00	0.00	0.00
C9:0	0.00	0.00	0.00	0.00	0.10		0.00	0.00	0.00	0.00	0.00
C10:0	0.00	0.00	0.00	0.00	2.25		0.00	0.00	0.00	0.00	0.00
C11:0	0.00	0.14	0.00	0.00	0.05		0.00	0.00	0.00	0.00	0.00
C12:0	0.42	1.92	0.00	0.85	4.21		0.00	0.00	0.00	0.00	0.00
C13:0	0.00	0.00	0.00	0.00	0.10		0.00	0.00	0.00	0.00	0.00
C14:0	6.93	5.78	3.43	6.55	12.37		0.00	0.00	0.00	0.00	0.00
C14:1	0.82	1.38	0.82	0.96	0.99		0.00	0.00	0.00	0.00	0.00
C15:0	1.02	0.61	0.70	0.88	0.06		0.00	0.00	0.00	0.00	0.00
C15:1	0.00	0.24	0.00	0.26	0.28		0.00	0.00	0.00	0.00	0.00
C16:0	39.52	29.75	27.99	31.91	36.91		11.73	5.63	10.42	30.63	0.00
C16:1	2.34	2.84	4.17	4.28	1.91		0.00	0.00	2.39	37.10	0.00
C17:0	0.52	0.55	0.92	1.32	0.70		8.46	0.00	0.00	0.00	0.00
C17:1	0.00	0.00	0.00	0.00	0.31		0.00	0.00	0.00	0.00	0.00
C18:0	17.19	19.44	16.35	17.30	13.04		25.06	15.72	17.91	9.62	0.00
C18:1n9	30.21	24.15	32.40	28.06	23.10		3.38	1.46	0.37	0.00	0.00
C18:2n6	0.47	6.83	8.59	2.84	0.08		0.00	0.00	0.00	0.00	0.00
C18:3n6	0.00	0.22	0.00	0.50	0.03		0.00	0.00	0.00	0.00	0.00
C18:3n3	0.55	2.98	1.67	0.57	0.17		0.00	0.00	0.00	0.00	0.00
C20:0	0.00	0.56	0.95	0.71	0.29		5.92	38.19	35.90	22.65	0.00
C20:1	0.00	0.23	0.00	0.17	0.20		2.68	35.61	33.00	0.00	0.00
C20:2	0.00	0.00	0.00	0.00	0.03		0.00	0.00	0.00	0.00	0.00
C20:3n6	0.00	0.00	0.00	0.00	0.08		0.00	0.00	0.00	0.00	0.00
C20:4n6	0.00	0.00	0.00	0.00	0.04		0.00	0.00	0.00	0.00	0.00
C21:0	0.00	0.00	0.00	0.00	0.04		0.00	0.00	0.00	0.00	0.00
C20:3n3	0.00	0.00	0.00	0.00	0.07		0.00	0.00	0.00	0.00	0.00
C20:5n3	0.00	0.00	0.00	0.84	0.06		0.00	0.00	0.00	0.00	0.00
C22:0	0.00	0.49	0.00	0.59	0.16		0.00	0.00	0.00	0.00	0.00
C22:1n9	0.00	0.00	0.00	0.00	0.02		0.00	0.00	0.00	0.00	0.00
C22:2	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
C23:0	0.00	1.90	2.01	1.41	0.38		0.00	3.39	0.00	0.00	0.00
C22:6n3	0.00	0.00	0.00	0.00	0.00		42.77	0.00	0.00	0.00	0.00
C24:0	0.00	0.00	0.00	0.00	0.11		0.00	0.00	0.00	0.00	0.00
C24:1	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00

G. SEC-MALS-dRI data

This sections contains additional SEC-MALS-dRI data for comparison. Fractions $> 0.65 \mu\text{m}$ not included. Note the wide distributions and lack of adequate separation (complete exclusion) for fractions larger than $\sim 1000\text{kDa}$. Note the unexpected peak shape of the $> 0.65 \mu\text{m}$ fraction, indicating a cut-off due to the filtration of sample prior to injection. SEC-MALS-dRI data for $> 0.65 \mu\text{m}$ fraction is therefore not included in the dataset.

Fraction	Mw (kDa)	Uncertainty Mw	Rz (nm)	Uncertainty Rz	Conc. from dRI (mg L^{-1})
$> 1\text{kDa}$	35	35 %	-	-	35
$> 10\text{kDa}$	260	22 %	-	-	48
$> 100\text{kDa}$	1164	19 %	47	72 %	27
$> 1000\text{kDa}$	13203	57 %	162	22 %	5
$> 0.1 \mu\text{m}$	-	-	335	7 %	13
$> 0.65 \mu\text{m}$	-	-	-	-	-



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Wastewater characterisation by combining size fractionation, chemical composition and biodegradability

Ravndal, Kristin T.

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