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Two-dimensional chromatography for the analysis of valorisable biowaste: a review

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Abstract:

Various everyday areas such as agriculture, wood industry, and wastewater treatment yield residual biowastes in large amounts that can be utilised for the purpose of sustainability and circular economy. Depending on the type of biowaste, they can be used to extract valuable chemicals or converted into alternative fuels. However, for efficient valorisation, these processes need to be monitored, for which thorough chemical characterisation can be highly beneficial. For this aim, two-dimensional (2D) chromatography can be favourable, as it has a higher peak capacity and sensitivity than one-dimensional (1D) chromatography. Therefore, here we review the studies published since 2010 involving gas chromatography (GC) or liquid chromatography (LC) as one of the dimensions.

For the first time, we present the 2D chromatographic characterisation of various biowastes valorised for different purposes (chemical, fuels), together with future prospects and challenges. The aspects related to the 2D chromatographic analysis of polar, poorly volatile, and thermally unstable compounds are highlighted. In addition, it is demonstrated how different 2D setups can be applied for monitoring the biowaste conversion processes.

Keywords:

two-dimensional chromatography; liquid chromatography; gas chromatography; biowaste; 2D plots; valorisation

Abbreviations:

¹D, ²D: first and second dimension, respectively; 1D, 2D: one-dimensional and two-dimensional chromatography, respectively; GC: gas chromatography; RPLC: reversed-phase liquid chromatography; HTL: hydrothermal liquefaction; HILIC: hydrophilic interaction liquid chromatography; LLE: liquid-liquid extraction; BSTFA: N,O-bis(trimethylsilyl)trifluoroacetamide; MSTFA: N-methyl-N-(trimethylsilyl)-trifluoroacetamide; CPC: centrifugal partition chromatography; FAMES: fatty acid methyl esters; TAGs: triglycerides; NCD: nitrogen chemiluminescence detector; NPLC: normal-phase liquid chromatography; SEC: size exclusion chromatography; GPC: gel permeation chromatography; SFC: supercritical fluid chromatography; VUV: vacuum ultraviolet absorbance detector.

1. Introduction

Biowaste is generally a by-product or non-edible organic residue from a plant or animal resource. Some examples of biowaste include sludge from sewage treatment plants, by-product animal fat, processed algae, and agricultural by-products like seeds, leaves, shells, and stalks. Although these materials are considered as waste, they still contain high amounts of valuable compounds, which can be valorised by extraction or converted into liquid transportation fuels in favour of a circular economy. Biofuels are an alternative to fossil fuels, and the recovered biochemicals can be used in various products, such as bioactive compounds for pharmaceuticals, hydrocarbons for the production of polymers, phenols to produce pesticides, furans in adhesives, and numerous other applications. The benefit of valorising biowaste instead of biomass lays in the aspect that biowaste does not compete with the food market and does not require additional resources - e.g., land, nourishment - for cultivation. Additionally, this rational utilisation helps with the problem of disposing environmentally toxic wastes (e.g., crambe seed and tobacco residues¹⁻³), decreasing landfill space, and increasing pollution caused by the direct burning of the material.⁴⁻⁹

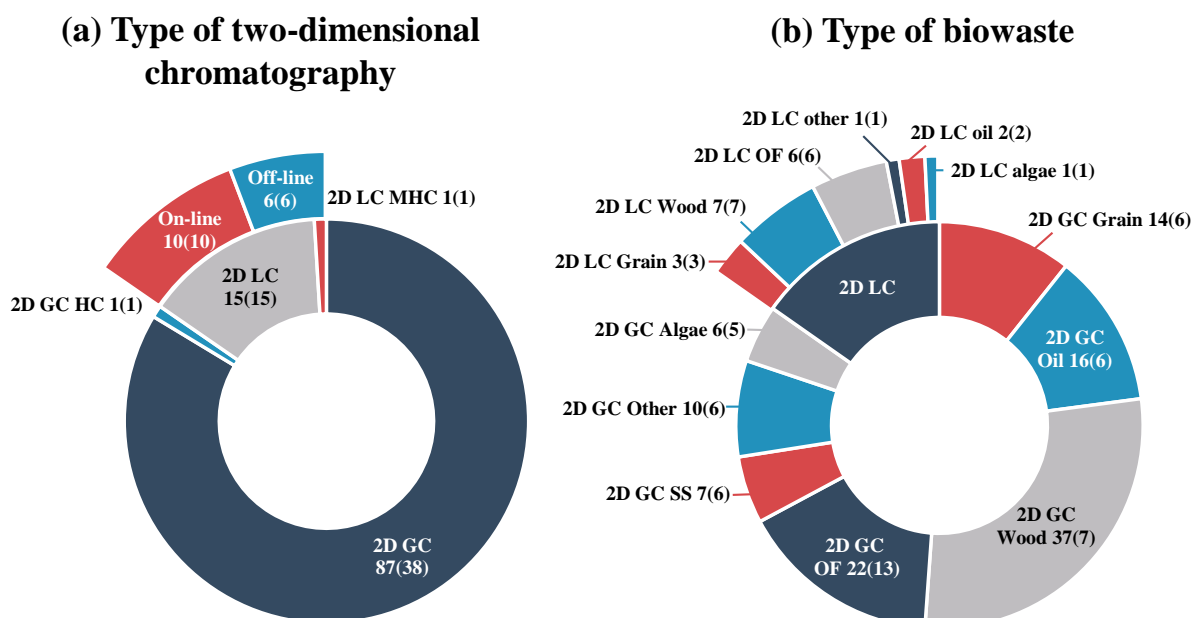
Although some valuable compounds can be directly extracted from biowastes (e.g., carotenoids from overripe fruits¹⁰), more often thermo- or biochemical conversion is needed to obtain the desired product. For example, polymeric lignin (a waste of the paper industry) has to be properly depolymerised for the production of monomeric compounds such as vanillin¹¹ or thermochemically converted into a liquid that can be used as biofuel. In both cases, comprehensive chemical characterisation can be highly beneficial for developing efficient upgrading processes. For example, the liquid obtained from the thermochemical conversion of lignin contains harmful compounds, such as acids that are corrosive for engines.^{12,13} For the production of high-quality biofuel, these compounds have to be removed by applying additional treatment, which effectiveness can be monitored by studying the chemical composition of the product. However, this is not an easy task as both converted and unconverted biowaste are generally highly complex mixtures of hundreds or even thousands of molecules originating from the degradation of various larger compounds, e.g., carbohydrates, lipids, lignin, and proteins. Therefore, the mixture can contain analytes having a wide range of polarities and molecular weights but at the same time, also analytes that are structurally highly similar.¹²

For a comprehensive characterisation of such complex mixtures, advanced multidimensional methods can be highly beneficial. One powerful technique is two-dimensional (2D) chromatography, which compared to the classical one-dimensional (1D) chromatography has demonstrated a higher separation power (in other words: higher peak capacity) and consequently fewer co-elutions, which aids to detect more compounds in complex samples.^{5,9,14} This can be helpful even when powerful detection such as high-resolution mass spectrometry (HRMS) is used, as an efficient separation can yield cleaner spectra and facilitate the identification. In some cases, 2D chromatography has shown to have less matrix effects and lower limit of detection, thus also enabling the identification of minor components.^{13,15,16} In 2D chromatography, two chromatographic dimensions with different separation selectivities are performed in series by *i*) collecting the fractions of the first dimension before subsequent injection into the second dimension (off-line); or *ii*) connecting the two dimensions via an interface/modulator (on-line).¹⁷ In this review, a dimension is defined as a chromatographic technique, and we do not consider liquid-liquid extraction or other sample pre-treatment steps as one of the separation dimensions. Therefore, the words “off-line” and “on-line” indicate how the two chromatographic dimensions are connected, not how the sampling is performed. The number of dimensions is written in the normal script (e.g., 2D), and the number that represents the order of the dimension is in superscript (e.g., ¹D). This review focuses on separation methods for which one of the dimensions is gas chromatography (GC) or high-performance liquid chromatography (HPLC) and where the separation is performed in a comprehensive (noted with “x”, e.g., GC×GC) or heart-cutting (noted with “-“, e.g., LC-LC) mode. In the case of multiple heart-cutting, then “m” is added to the symbols: mLc-Lc. Often comprehensive techniques are needed to characterise the sample thoroughly. However, when the aim is to additionally separate only some specific co-eluting areas on the chromatogram, then (multiple) heart-cutting technique can be the suitable method.

101 Several characteristics can be used to describe how powerful and hence, suitable, is the developed 2D
 102 chromatography technique for the separation of analytes under question. One option is to use
 103 orthogonality, which is the highest (100 %) when the retention data of the two dimensions have no
 104 correlation.^{18,19} This can be achieved when the two dimensions are based on different molecular
 105 interactions between the analyte and the stationary phase. However, one must take care when making
 106 conclusions based on the orthogonality value as various approaches have been used for the calculations.
 107 Often the coverage of the area containing the sample components, also called the separation space, is
 108 studied, where clustering of peaks reduces the orthogonality value.²⁰ Therefore, even when the selected
 109 separations are non-correlated, the orthogonality values are generally still below 100 % and sample
 110 dependant. Another option is to use the peak capacity, which indicates the number of peaks that
 111 theoretically could be separated by the 2D setup. Further insight into these values can be found in the
 112 publications of Camenzuli and Schoenmakers,²⁰ Ryan *et al.*²¹ (GC×GC), and Li *et al.*²² (2D LC). A more
 113 detailed overview of the basic theoretical concepts of the 2D chromatographic methods can be found in
 114 previously published works.^{23–28}

115
 116
 117 Figure 1 represents the papers published since 2010 on the 2D chromatography of valorisable biowaste.
 118 A part of these publications – GC×GC analysis of pyrolysed bio-oils – has been discussed in a
 119 comprehensive review by Staš *et al.*²⁹ Therefore, the publications discussed there that mostly included
 120 the 2D GC analysis of pyrolysed wood-derived biomass will not be addressed in our review. For readers
 121 interested more in the chromatographic and other analytical analysis of lignocellulosic biomass, we
 122 suggest the reviews by Grams³⁰ and Rodrigues *et al.*,³¹ in which 2D chromatography is mentioned only
 123 briefly. Also, Wang *et al.*³² reviewed the usage of different analytical methods for the analysis of
 124 pyrolysed biomass and discussed 2D GC only briefly without mentioning 2D LC. The 1D and 2D GC
 125 analysis of biomass has been discussed by Beccaria *et al.*³³; however, their review does not include 2D
 126 LC methods. Conversely, recent developments of 2D LC, including the analysis of complex samples,
 127 can be found in a review by Pirok *et al.*²⁴ Therefore, in the present review, we focus on the analysis of
 128 complex biowaste of any origin (see Table 1 for summary) and, based on this, highlight the technical
 129 aspects of the 2D chromatographic methods involving GC or LC.

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133 *Figure 1.* Studies published since 2010 involving the 2D chromatographic analysis of biowaste, including the
 134 papers reviewed by Staš *et al.*²⁹ In parentheses () are shown the number of papers that are discussed more
 135 thoroughly in our review but were not included in the review by Staš *et al.*²⁹ (a) Distribution of the 104 papers
 136 based on the applied 2D technique. All techniques are comprehensive if not stated otherwise (“HC” represents
 137 heart-cutting and “MHC” multiple heart-cutting). (b) Distribution of the publications based on 2D GC or 2D LC

138 method and the type of the analysed biowaste. One paper can be under several groups as some studies analysed
139 more than one type of biowaste. “**Wood**” – wood-derived biowaste, “**Grain**” – residues of grain processing,
140 “**Oil**” – used oil or residues of oil production, “**OF**” – other food-related waste, “**SS**” – sewage sludge, “**Algae**” –
141 microalgae waste, “**Other**” – wastes/residues of tobacco, bioethanol production, energy grass, and yeast.

144 **2. Analysis of different type of biowastes**

145
146 As can be seen from Figure 1, the more widely used technique for the analysis of biowastes is GC×GC.
147 This is likely caused by the easier implementation compared to 2D LC (discussed more thoroughly
148 under the following paragraphs) and the suitability for the analysis of volatile organic compounds found
149 in biofuels. Generally, the publications including GC×GC analysis apply the method to characterise
150 (mainly qualitatively but also quantitatively) the sample issued from conversion or further upgrading
151 processes, whereas 2D LC publications reviewed here are more focused on developing and/or improving
152 the 2D separation. Still, with both methods a wide variety of biowastes have been analysed, where
153 agricultural wastes such as residues related to grain, oil, or other food production/use and wood-derived
154 wastes (e.g., lignin, sawdust) have been investigated the most. In the case of biowaste, commonly the
155 composition of the whole sample is of interest, not just a few targeted compounds. Therefore,
156 comprehensive 2D methods are more widely applied than heart-cutting approaches. Even though the
157 following paragraphs are largely sectioned by the type of the biowaste, the main conclusions can be
158 extrapolated for other types of samples containing similar compounds.

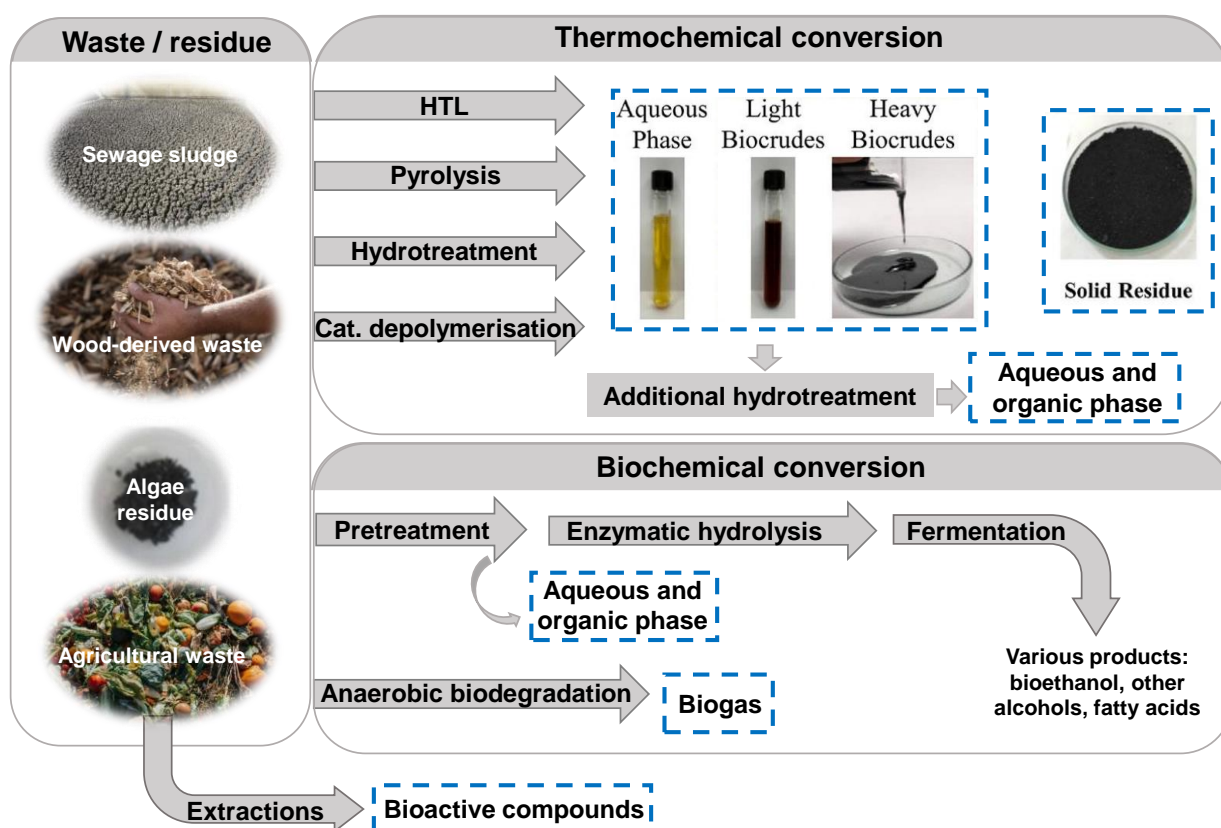
161 **2.1. Some general considerations for biowaste conversion and sample preparation**

162
163 As it was mentioned before, different thermo- and biochemical processes involving numerous types of
164 chemical reactions are used to convert biowaste into smaller or more suitable compounds. In the topic
165 of this review, the most common thermochemical processing methods are pyrolysis, which takes place
166 in the range of 400-700°C without oxygen,^{7,18} and hydrothermal liquefaction (HTL) performed under
167 milder conditions (250-370°C) on wet biomass.^{2,34} The conversion processes can yield one or several of
168 the following phases: liquid, gas, and solid. Mainly the liquid part is used further and fractionated by
169 solvent extraction, yielding an organic phase (containing compounds with low polarity, also called the
170 bio-oil or biocrude) and the aqueous wastewater phase (containing highly polar compounds).^{6,15,35} The
171 organic and aqueous phase can also spontaneously form two layers, which can be separated by simple
172 decantation.^{12,36,37} The bio-oil has to be further upgraded by additional hydrotreatments (these have also
173 been applied directly on biowastes^{38,39}) to have a product with higher heating value by reducing the
174 content of oxygenated compounds.^{18,40} One type of hydrotreatment is hydrodeoxygenation, which is
175 performed under hydrogen pressure using a robust catalyst and mild reaction temperatures around 350-
176 450°C.^{34,38} For economical sustainability, the residual aqueous wastewater likewise containing
177 significant amounts of organic compounds should also be valorised.⁴¹ In the case of biochemical
178 conversions reviewed here, just two types were used: 1) anaerobic biodegradation and 2) enzymatic
179 hydrolysis for the production of bioethanol. The last conversion consists of three main steps that have
180 to be monitored for an efficient conversion: pretreatment, enzymatic hydrolysis, and fermentation. A
181 more detailed overview of various biomass processing methods can be found in the review by Huber *et*
182 *al.*⁴² and Awasthi *et al.*⁴³ In Figure 2, a general scheme focusing on the conversion processes applied in
183 the publications reviewed here is presented. The products that have been analysed with a 2D technique
184 are highlighted with blue dashed lines. As can be see, the converted products may originate from various
185 stages and types of the conversion processes; therefore, different analytes can be found even when the
186 same biowaste has been analysed. In addition, in the case of some agricultural wastes, only extractions
187 have been used before the 2D analysis (see section 2.4.2.). Thus, it is vital to consider possible analytes
188 in the (converted) sample before selecting the 2D technique. In Table 1, the phase and main conversion
189 or sample preparation technique applied before the 2D chromatographic analysis of the biowastes are
190 presented.

191
192 Both 2D GC and 2D LC methods have been applied for the analysis of the organic bio-oil and the

193 aqueous wastewater phases of a converted biowaste. In the case of single-phase liquid, extraction with
 194 water has been used to analyse the water-soluble compounds with LC.¹⁵ For LC analysis, the aqueous
 195 wastewater can be just centrifuged and filtered (and diluted, if necessary) before the 2D LC analysis.<sup>7,15–
 196 18,37,44</sup> However, generally the wastewater cannot be directly injected into the GC because of the high
 197 water content (see section 2.6.1. for an exception). To overcome this issue, different sample preparation
 198 techniques have been applied to extract the compounds from the aqueous wastewater. One of the most
 199 common procedures is liquid-liquid extraction (LLE) with an organic solvent, often
 200 dichloromethane.^{5,6,35,45} Although LLE is a simple and quick procedure, the qualitative and quantitative
 201 analysis can be affected by the fact that typically the analytes are from various chemical families with
 202 different affinities for the extraction solvent. Therefore, the complete extraction of all compounds
 203 (especially highly polar compounds) from the aqueous phase is rarely achievable, causing lower analyte
 204 concentrations or total absence in the analysable solution.^{6,7,36} Also, the evaporation of water and
 205 redissolving of the residue in methanol has been applied,^{2,46} but with this approach, some volatile
 206 compounds may evaporate and/or the whole sample might not be dissolved. The organic bio-oil (or the
 207 whole single-phase liquid) is too concentrated to be injected directly into the GC; therefore, it is typically
 208 dissolved in an organic solvent or solvent mixture,^{3,12,40,46–50} or LLE is performed.^{2,9,35,51,52} The selection
 209 of a suitable diluent is a crucial step to ensure that the sample is fully solubilised, especially in the case
 210 of quantitative analysis. At the same time, it is important to ensure that during the GC×GC run, the first
 211 eluting compounds do not elute simultaneously with the diluent(s). If the converted (diluted) biowaste
 212 contains also undissolved parts, then those are removed by filtration or centrifugation.^{2,34,46,53,54} In that
 213 case, it must be kept in mind that only a fraction of the converted product is analysed with the 2D
 214 technique.

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Figure 2. General scheme demonstrating the first steps of sample preparation techniques that were applied in the publications reviewed here – thermochemical conversion, biochemical conversion, and direct extractions. The products surrounded by boxes with blue dashed lines represent samples that were analysed in the reviewed papers. Catalytic depolymerisation is noted as cat. depolymerisation. Images of conversion products were adapted from Saengsuriwong *et al.*⁴⁶

Table 1. Overview of the biowaste analysis by 2D GC and 2D LC techniques. The main classification corresponds to the sections 2.2.-2.6. and the subclass “Fig 1” shows the classification used in Figure 1. For each publication, the characteristics of the applied or best performing 2D technique are shown. ¹The usage of conventional column setup is noted as “C” and reversed setup as “R”. ²Here the main conversion or sample preparation technique is presented, where ”O” represents the organic phase and “A” represents the aqueous phase of the converted biowaste.

<i>Type of biowaste</i>	<i>Analysis method¹</i>	<i>First dimension</i>	<i>Second dimension</i>	<i>Sample prep²</i>	<i>Ref</i>
Sewage sludge					
Fig 1: Sewage sludge (SS)					
Sewage sludge	GC×GC(C)-TOF-MS	Rx-5 ms (29 m)	BPX-50 (1.5 m)	O from microwave assisted pyrolysis	13
Sewage sludge	GC×GC(C)-TOF-MS	Non-polar column	Polar column	O from pyrolysis	51
Sewage sludge	GC×GC(C)-TOF-MS	Rxi-1 (60 m)	Rxi-17 (1.0 m)	O from HTL and additional hydrotreatment	55,56
Primary, secondary, and digested sludge	GC×GC(R)-TOF-MS	Presumably: Restek stabilwax (60 m)	Presumably: Rxi 5MS (3.3 m)	A from HTL	41
Municipal solid waste landfills, sludge from wastewater treatment plant	GC×GC(C)-MS	DB-5MS (30 m)	DB-17 (1.35 m)	Treated and untreated biogas	57
Biowaste containing fatty acids / triglycerides					
Fig 1: Oil					
Soursop and bocaiuva seed cakes	GC×GC(C)-TOF-MS	DB-5 (30 m)	DB-17 (1.2 m)	O from slow pyrolysis	12
Crambe seed cakes	GC×GC(C)-TOF-MS	DB-5MS (60 m)	DB-17MS (2.15 m)	O from pyrolysis	1
Crambe seed, coconut fibres	RPLC×RPLC-DAD-MS	X-Bridge amide (A) H ₂ O/FA (B) ACN/FA	Poroshell EC-C18 (A) H ₂ O/FA (B) ACN/FA	A from fast pyrolysis	17
Coconut fibres	GC×GC(C)-TOF-MS	DB-5 (60 m)	DB-17 (2.1 m)	O and A from pyrolysis	9
Coconut fibres	GC×GC(C)-TOF-MS	DB-5 (60 m)	DB-17MS (2.15 m)	O and A from fast pyrolysis	6
Coconut fibres	RPLC×RPLC-PDA-MS	Ascentis RP-Amide (A) H ₂ O (B) ACN	Ascentis Express C8 (A) H ₂ O (B) ACN	A from fast pyrolysis	7
Used cooking oil	GC×GC(C)-MS/FID	HP-5 (30 m)	SLB-IL61 (1.0 m)	Derivatisation	58
Oils, fats, grease	GC×GC(C)-TOF-MS	Rxi-1 (60 m)	Rxi-17 (1.0 m)	O from HTL and additional hydrotreatment	55
Fig 1: Other Food (OF)					
Animal fat waste	GC×GC(C)-TOF-MS	Rxi-5MS (30 m)	Rxi-17Sil MS (1.3 m)	Column chromatography, derivatisation	59
Agricultural waste					
Fig 1: Grain					

Wheat straw	RPLC×RPLC(-IMS)- timsTOF	Hypercarb (A) H ₂ O/FA (B) IPA/FA	Zorbax Eclipse Plus C18 (A) H ₂ O/FA (B) ACN/FA	A from sulphuric acid-based pretreatment	16
Wheat straw	Off-line SEC×RPLC- PDA/TOF-MS	PolySep-GFC-P2000 ACN/H ₂ O/FA	Kinetex C18 (A) H ₂ O/FA (B) ACN/FA	A from 1) fast pyrolysis; 2) sulphuric acid-based pretreatment	15
Wheat straw lignin	GC×GC(C)-MS	ZB1 (30 m)	ZB50 (2.0 m)	O from hydroconversion	38
Wheat straw lignin	GC×GC(R)-MS/FID	VF1701 (30 m)	DB1 (2.0 m)	O from hydroconversion	39
Wheat straw lignin	GC×GC(R)-FID	Rtx-1701 (30 m)	Rxi-5Sil MS (1.2 m)	O from catalytic depolymerisation	60
Wheat straw lignin	GC×GC(R)-TOF-MS	SLB-IL111 (30 m)	BPX-50 (1.6 m)	O from fast pyrolysis	50
Rice husk	GC×GC(C)-MS	DB-5 (60 m)	DB-17MS (2.15 m)	O from pyrolysis	36
Rice husk	RPLC×RPLC-DAD-MS	X-Bridge amide (A) H ₂ O/FA (B) ACN/FA	Poroshell EC-C18 (A) H ₂ O/FA (B) ACN/FA	A from pyrolysis	37
Grain residue	GC×GC(R)-TOF-MS	Presumably: Restek stabilwax (60 m)	Presumably: Rxi 5MS (3.3 m)	A from HTL	41
Fig 1: Other Food (OF)					
Sugarcane bagasse	GC×GC(C)-TOF-MS	DB-5 (30 m)	DB-17 (1.2 m)	O from HTL	40
Sugarcane straw and bagasse	GC×GC(C)-MS	DB-5 (60 m)	DB-17 (2.15 m)	O from fast pyrolysis	5
Sugarcane straw and bagasse	RPLC×RPLC-PDA-MS	Ascentis RP-Amide (A) H ₂ O (B) ACN	Ascentis Express C8 (A) H ₂ O (B) ACN	A from fast pyrolysis	7
Sugarcane bagasse lignin	GC×GC(R)-TOF-MS	SLB-IL111 (30 m)	BPX-50 (1.6 m)	O from fast pyrolysis	50
Sugarcane bagasse, mango seed almond, pineapple leaves, sugarcane bagasse, cottonseed, coffee silverskin, cassava peel	RPLC×RPLC-DAD-MS	X-Bridge amide (A) H ₂ O/FA (B) ACN/FA	Poroshell EC-C18 (A) H ₂ O/FA (B) ACN/FA	A from fast pyrolysis	17
Mango seed tegument and almond	GC×GC(C)-TOF-MS	DB-5 (60 m)	DB-17MS (1.2 m)	O from pyrolysis	45
Orange pulp	GC×GC(C) FID/TOF- MS	DB-5 (30 m)	DB-17MS (1.3 m)	O from fast pyrolysis	61
Bark of acuri fruit, endocarp of baru fruit	GC×GC(C)-TOF-MS	DB-5 (60 m)	DB-17MS (2.15 m)	O from pyrolysis	8
Grape pomace, sugar beet tailings	GC×GC(R)-TOF-MS	Presumably: Restek stabilwax (60 m)	Presumably: Rxi 5MS (3.3 m)	A from HTL	41
Overripe fruits: hybrid persimmon-apple, banana pulp, banana peel, nectarine	NPLC×RPLC-PDA-MS	Ascentis ES Cyano (A) Hex (B) Hex/butyl- acetate/acetone	Ascentis Express C18 (A) ACN/H ₂ O (B) IPA	Multiple extractions	10
Black chokeberry pomace	HILIC×RPLC-DAD- MS	Hypersil GOLD amino (A) ACN/FA (B) H ₂ O/FA	Ascentis Express C18 (A) H ₂ O/FA (B) ACN	Multiple extractions	4
Grapevine canes	HILIC×RPLC-DAD-	Lichrospher diol-5	Ascentis Express	Extraction	19

	MS	(A) ACN/FA (B) MeOH/AmAc/AA	C18 (A) H ₂ O/FA (B) ACN/FA		
Coffee bean tegument	GC×GC(C)-MS	OV-5 (60 m)	DB-17MS (2.15 m)	O from pyrolysis	35
Peanut shell, spent coffee grounds, peach core	RPLC×RPLC-DAD-MS	X-Bridge amide (A) H ₂ O/FA (B) ACN/FA	Poroshell EC-C18 (A) H ₂ O/FA (B) ACN/FA	A from pyrolysis	37
Food waste from canteens	GC×GC(C)-TOF-MS	Rxi-5MS (30 m)	Rxi-17silMS (1.0 m)	O and A from HTL	46
Food waste	GC×GC(C)-TOF-MS	Rxi-1 (60 m)	Rxi-17 (1.0 m)	O from HTL and additional hydrotreatment	55,56
Agricultural biogas plant	GC×GC(C)-MS	DB-5MS (30 m)	DB-17 (1.35 m)	Treated and untreated biogas	57
Fig 1: Other					
Tobacco residues	GC×GC(R)-TOF-MS	Rxi-5 MS (30 m)	Rxi-17MS (1.0 m)	O and A from HTL	2
Tobacco residues	GC×GC(R)-TOF-MS	DB-5 (60 m)	DB-17MS (2.15 m)	O from fast pyrolysis	3
Waste from wood industry or energy crops					
Fig 1: Wood (W)					
Aspen wood	GC×GC(C)-MS/FID	DB-5 (60 m)	DB-17 (1.0 m)	O from HTL	62
Aspen wood	GC×GC(C)-MS	DB-5 (60 m)	DB-17 (1.0 m)	O from HTL	63
Aspen wood lignin, Birch wood, Kraft lignin	GC×GC(R)-TOF-MS	SLB-IL111 (30 m)	BPX-50 (1.6 m)	O from fast pyrolysis	50
Pine woodchips	GC×GC(R)-MS/FID	HP-INNOWAX (30 m)	DB-5 (5.0 m)	O from HTL (and additional hydrotreatment)	64
Pine woodchips	GC×GC(R)-MS/FID	INNOWAX (30 m)	DB-5 (5.0 m)	O from HTL and additional hydrotreatment	65
Pine lignin	GC×GC(R)-FID	RTX-1701 (30 m)	Rxi-5Sil MS (1.20 m)	O from catalytic hydrotreatment	66
Softwood sawdust	Off-line CPC×RPLC-UV-MS	MTBE/H ₂ O/NaOH	Kinetex C18 (A) H ₂ O/FA (B) ACN/FA	A from fast pyrolysis	44
Softwood sawdust	Off-line CPC×RPLC-DAD-MS	Heptane/Ethyl acetate/MeOH/H ₂ O	Kinetex XB C18 (A) H ₂ O/FA (B) ACN/FA	O from fast pyrolysis	53
Softwood sawdust	Off-line SEC×RPLC-PDA/TOF-MS	PolySep-GFC-P2000 ACN/H ₂ O/FA	Kinetex C18 (A) H ₂ O/FA (B) ACN/FA	A from 1) fast pyrolysis; 2) sulphuric acid-based pretreatment	15
Eucalyptus sawdust	RPLC×RPLC-DAD-MS	X-Bridge amide (A) H ₂ O/FA (B) ACN/FA	Poroshell EC-C18 (A) H ₂ O/FA (B) ACN/FA	A from pyrolysis	37
Lignin	GC-GC(C)-MS/FID	HP-5MS (30 m)	DB-Wax (30 m)	O from pyrolysis and hydrodeoxygenation	67
Birch wood lignin	RPLC×SFC-DAD	Agilent Zorbax Eclipse	Torus Diol	Monomeric fraction from catalytic	68

		Plus C18 (A) H ₂ O (B) ACN	(A) CO ₂ (B) ACN	depolymerisation	
Mixture of red oak, white oak, ash, maple	RPLC×RPLC-DAD	Hypercarb (A) H ₂ O/FA (B) ACN/FA	Kinetex Phenyl-Hexyl (A) H ₂ O/FA (B) MeOH/FA	A from hydrotreatment	18
Presumably wood	RPLC×SFC-UV	Hypercarb (A) H ₂ O (B) ACN	Acquity BEH 2-EP (A) CO ₂ (B) MeOH/ACN	A of bio-oil	69
Fig 1: Other					
Napier grass lignin	GC×GC(R)-TOF-MS	SLB-IL111 (30 m)	BPX-50 (1.6 m)	O from fast pyrolysis	50
Miscanthus lignin	Off-line mGPC-RPLC-UV-Vis	Polargel-L DMSO/LiBr	Zorbax 300SB-CN (A) H ₂ O/AA (B) ACN	Catalytic depolymerisation	54
<i>Residues from algae processing</i>					
Fig 1: Algae					
Microalgae	GC×GC(R)-TOF-MS	Restek stabilwax (60 m)	Rxi 5MS (3.3 m)	A from HTL	70
Microalgae	GC×GC(R)-TOF-MS	A polar column (60 m)	A non-polar column (2.3 m)	A from HTL	71
Microalgae	GC×GC(R)-TOF-MS	Presumably: Restek stabilwax (60 m)	Presumably: Rxi 5MS (3.3 m)	A from HTL	41
Microalgae	Off-line RPLC×SFC-TOF-MS	CSH Phenyl-Hexyl (A) H ₂ O (B) ACN	Torus Diol (A) CO ₂ (B) EtOH	A from HTL	72
Microalgae residue	GC×GC(R)-MS/FID	ZB-35 (30 m)	DB-1 (2.0 m)	O and A from HTL	48
Microalgae residue	GC×GC(R)-MS/FID	ZB-35 (30 m)	DB-1 (2.0 m)	O from HTL	49
Fig 1: Other					
Oleaginous yeast	GC×GC(R)-TOF-MS	Presumably: Restek stabilwax (60 m)	Presumably: Rxi 5MS (3.3 m)	A from HTL	41
Bioethanol production residue	GC×GC(C)-MS	Rxi-5Sil MS (60 m)	Rtx-200MS (1.0 m)	O from HTL	47
Bioethanol production residue	GC×GC(R)-FID	RTX-1701 (30 m)	Rxi-5Sil MS (1.20 m)	O from HTL and/or hydrodeoxygenation	34

2.2. Sewage sludge

Sewage sludge is a semi-solid residue resulting from the treatment of industrial or municipal wastewater. Although a part of this waste can be used in agricultural applications, still a substantial amount ends up as landfills. Alternatively, the sludge could be processed into chemicals or fuels.¹³ Due to its high water content, HTL can be directly applied as an intermediate step in the valorisation process. The conversion yields an organic bio-oil, which has been analysed with GC×GC using the conventional non-polar × polar (or semi-polar) column setup, where the separation is performed based on boiling points (non-polar column) followed by additional separation based on polarity (polar or semi-polar column). In Figure 3, a GC×GC-TOF-MS plot of an organic bio-oil obtained from a HTL treated sewage sludge sample is presented. It can already be seen visually that in ¹D many compounds eluted at the same retention time, which would lead to co-elutions when using 1D GC. Co-elutions can lead to false annotation and/or erroneous (semi-)quantification because of the overestimation of peak areas. Compared to LC, the last aspect is especially important in GC, as it is highly common to characterise sample composition or to compare samples based on relative peak areas.^{1,3,5,6,35,45,46,61,62,64} Another benefit of using 2D GC is the organisation of compounds on the GC×GC plots based on their chemical families which is widely used as another criterion to tentatively identify or confirm a compound in different samples, including biowaste samples.^{1,5,6,35,39,45,59}

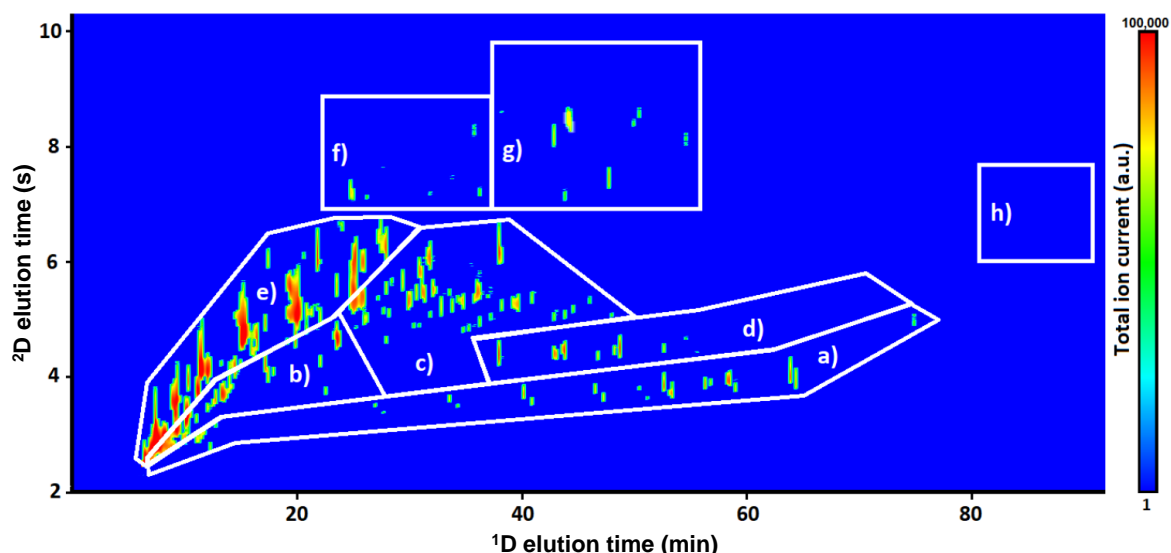


Figure 3. GC×GC-TOF chromatogram of the organic bio-oil fraction issued from hydrothermal liquefaction (HTL) of wet-waste sample, including sewage sludge, adapted from Cronin *et al.*⁵⁵ A non-polar × polar column set (60 m Rxi-1 × 1.0 m Rxi-17) was applied and the key regions are highlighted: (a) hydrocarbons; (b) aromatics, pyrroles, and phenols; (c) benzenamines, N-containing phenols, and other oxygenates; (d) long-chain alcohols and carboxylic acids; (e) pyrazines, pyridines, pyrimidines, imidazoles, and cyclic ketones; (f) pyrrolidines; (g) indoles; and (h) long-chain amides.

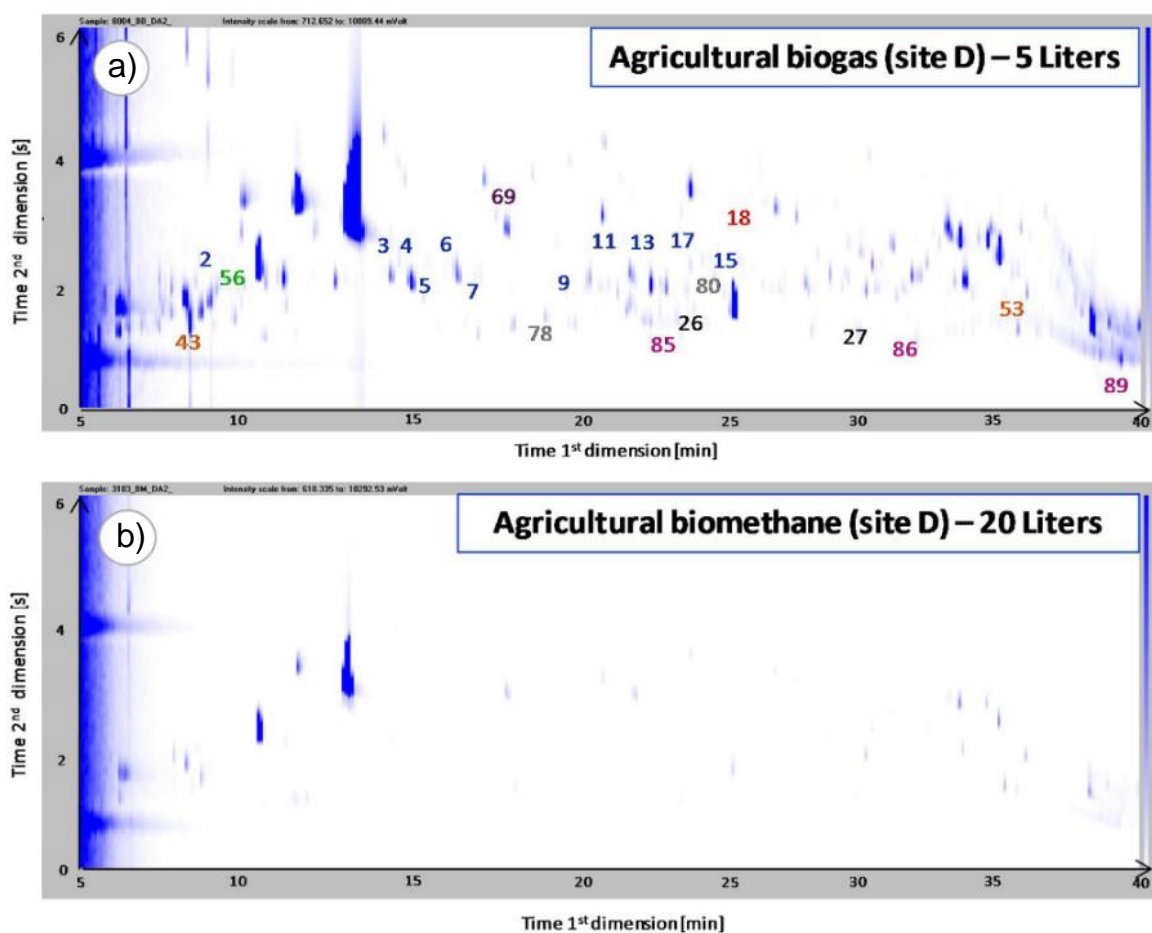
Figure 3 shows that the bio-oil obtained from the HTL treated sewage sludge contains numerous oxygenated and nitrogenated compounds. Subramaniam *et al.*⁵⁶ applied hydrotreatment on the bio-oil of HTL treated sewage sludge and food waste to remove heteroatom-containing compounds and yield non-polar hydrocarbons that would be suitable to be used in high-quality fuels. More specifically, they studied the stability of catalyst in this process in industrially relevant times. By applying GC×GC analysis, they could demonstrate that even when using the catalyst under strong conversion conditions (e.g., 1500 h), still the desired non-polar product was obtained from both converted feedstocks as it mainly consisted of linear and branched alkanes but also cycloalkanes, single ring alkyl aromatics, and double ring aromatics.

As can be seen from Table 1, generally, the organic phase of converted biowaste is analysed with GC×GC and only a few studies have focused on the aqueous wastewater phase (see section 2.6.1. for

265 more information on the direct GC×GC analysis of aqueous samples). Maddi *et al.*⁴¹ analysed the
266 aqueous wastewater obtained from the HTL conversion of different feedstocks and determined that the
267 converted sewage sludge consisted mainly of small polar compounds like organic acids, nitrogen
268 compounds, alcohols, aldehydes, and ketones. They also noted that compared to wastewater from food
269 industry, the converted municipal sludge contained higher amounts of nitrogenous compounds which
270 could be isolated for the production of agrochemicals, polymers, or other materials. However, quite low
271 quantities were detected implying that novel separation and concentration methods are needed to
272 valorise the wastewater of HTL treated sludge.

273
274 In addition to HTL, also the pyrolysis products of sewage sludge have been analysed with GC×GC to
275 perform a thorough chemical characterisation¹³ and assess the effect of different pyrolysis temperatures
276 on the obtained products.⁵¹ Although pyrolysis is sometimes considered the simplest thermochemical
277 conversion process, compared to HTL, the feedstock has to be dried and grinded into smaller pieces
278 before performing the pyrolysis. In terms of products, generally compounds with higher oxygen content
279 are obtained from pyrolysis compared to HTL produced bio-oils.^{40,46} Fan *et al.*⁵¹ applied GC×GC to
280 study the effect of pyrolysis temperature on the relative yields of some compound families in the
281 obtained sewage sludge bio-oil. For example, with increasing pyrolysis temperature also the proportion
282 of monoaromatics and polyaromatic ketones increased, which is valuable information when the aim is
283 to valorise those chemicals. The authors also noted that with 1D GC, usually hundreds of compounds
284 could be found in processed sewage sludge; however, their applied 2D GC allowed them to detect over
285 5000 compounds. Therefore, because converted sewage sludge is an especially complex mixture of
286 numerous compounds, fractionation can be used before the GC×GC analysis to improve the detection
287 and ease the data interpretation. Chorazy *et al.*¹³ used precipitation in n-pentane to separate the pentane-
288 soluble part (the choice of diluent was not elaborated) from poorly volatile asphaltene. The obtained
289 solution was fractionated into four parts by preparative column chromatography, which were then
290 analysed with GC×GC. Therefore, for the comprehensive analysis of processed sewage sludge or other
291 highly complex biowaste samples an additional pre-separation of the sample could be useful even before
292 the GC×GC analysis to ease the thorough chemical characterisation.

293
294 Another way to valorise sewage sludge is to utilise the gas emitting from the anaerobic biodegradation
295 of the sludge. After purification and upgrading processes, the obtained biomethane can be used in gas
296 grids or as fuel instead of natural gas. GC×GC can be used to monitor the quality by studying the
297 elimination of the unwanted compounds and to make sure that the final gas meets the purity
298 requirements. The selection of the sampling device (e.g., Tedlar[®] bags, sorbent tubes) and conditions
299 (temperature, site location) have a strong impact on the quali-quantitative analysis of biogas.⁵⁷ Hilaire
300 *et al.*⁵⁷ used sorbent tubes (proven to be also suitable for the analysis of minor compounds⁷³) to collect
301 the compounds present in the gas sample, which were thereafter extracted with a mixture of
302 acetone/dichloromethane by pressurised liquid extraction. The use of sorbent tubes also helps to
303 eliminate the problem of storing and transporting gaseous samples; however, because of the solvents,
304 they had to add a solvent delay to the GC×GC analysis, which means that some especially volatile
305 compounds (such as short alkanes up to n-hexane) were not detected. Although the analysed gaseous
306 samples were much less complex (up to a few hundred compounds) than the previously discussed liquid
307 products (up to thousands of compounds), still the utilisation of GC×GC was beneficial. Compared to
308 1D GC, 80 % of co-elutions could be avoided with 2D GC and 89 standards were detected instead of
309 79. Nevertheless, there is room for improvement, as compounds with similar polarity like styrene and
310 o-xylene were not separated. The 2D plots helped to compare the samples and concluded that even when
311 two biogas samples were obtained from municipal solid waste landfills, their produced biogases were
312 chemically different. The GC×GC plots can also be used for the monitoring of purification processes,
313 like in Figure 4, where already visually it can be seen that the purification process was efficient for all
314 the analysed compounds, as their content had decreased significantly in the final biomethane.



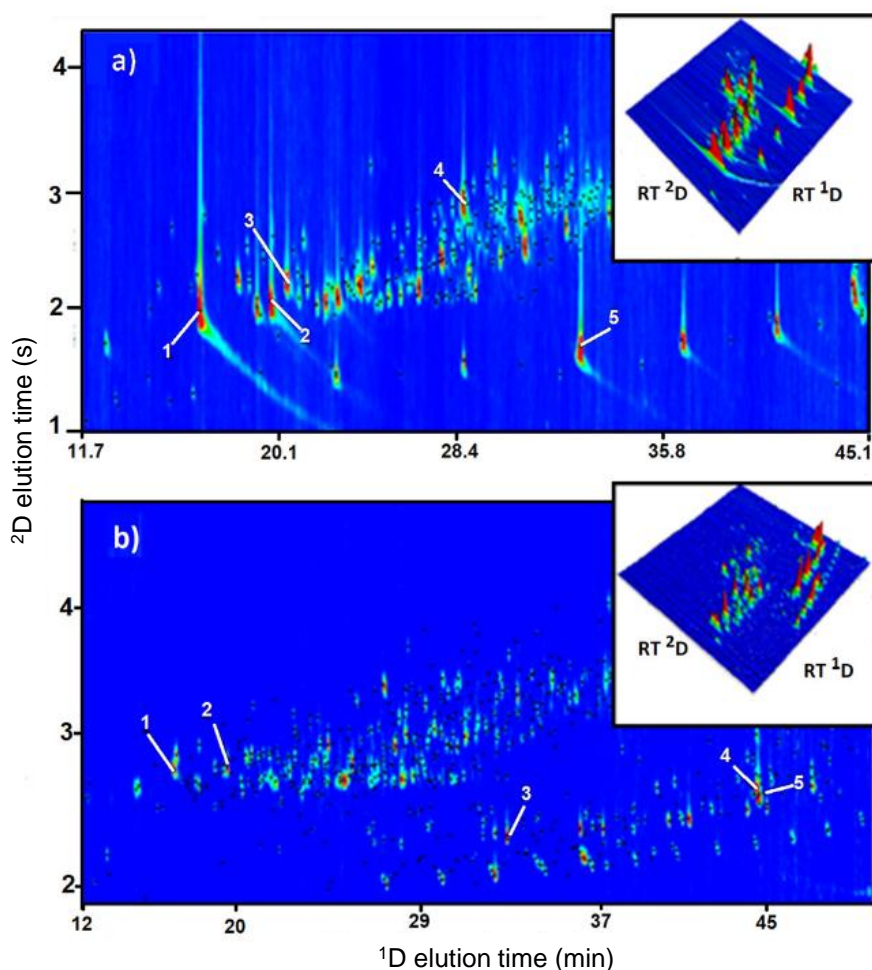
315
 316 *Figure 4.* GCxGC-MS of (a) raw biogas and (b) biomethane after purification process from anaerobic digester of
 317 agricultural residues. For comparison purposes, the same intensity scale was used. Numbers corresponding to
 318 standards: 1-17 mono-aromatic hydrocarbons; 18-22 poly-aromatic hydrocarbons; 23-32 alkanes; 33-38
 319 cycloalkanes; 39-41 alcohols; 42-44 ketones; 45-48 esters; 49-51 furans; 52-53 aldehydes; 54-59 sulphur organo-
 320 compounds; 60-74 halogen organo-compounds; 75-76 alkenes; 77-81 terpenes; and 82-89 siloxanes. Adapted from
 321 Hilaire *et al.*⁵⁷
 322

323 As the examples on the GCxGC analysis of processed sewage sludge showed, 2D chromatography has
 324 several advantages over 1D chromatography for the analysis of converted biowaste samples. The same
 325 conclusions have been made for the analysis of other feedstocks^{1,5,45} and also for 2D LC^{15,53} analyses
 326 reviewed here. However, as the aim of this review is not to demonstrate the benefit of using 2D instead
 327 of 1D chromatography, we will not specially focus on this aspect under the following paragraphs.
 328
 329

330 2.3. Biowaste containing fatty acids / triglycerides

331 Depending on the type of biowaste, triglycerides (TAGs) and fatty acids can be the major (e.g., animal
 332 fat, used cooking oil) but also the minor (e.g., residues of oily seeds, coconut) components. For example,
 333 the residual cake of crambe seed obtained after extracting the non-edible oil intended for biodiesel
 334 production can still contain some residual lipids. Onorevoli *et al.*¹ studied with GCxGC the pyrolysis
 335 bio-oils of the crambe seed cakes produced with three different oil extraction processes. They concluded
 336 that compressed propane extraction performed the best as lowest amounts of fatty acids were detected
 337 with GCxGC. However, one must be cautious with this conclusion as although the pyrolysis of TAGs
 338 yields fatty acids, also other compounds such as ketones or alkanes can be produced.⁷⁴ Another aspect
 339 is that crambe seed oil is known to contain high amounts of erucic (C22:1, around 60 %) and oleic acid
 340 (C18:1, around 20 %).⁷⁵ However, the longest detected fatty acid was palmitic acid (C16:0), which
 341 suggest that erucic and oleic acid were not enough volatile to be analysed with the applied GCxGC

342 method - 60 m ¹D column and final oven temperature 280 °C. At the same time, fatty acid methyl esters
 343 (FAMES) of erucic and oleic acid were detected. However, without another method suitable for the
 344 analysis of fatty acids (e.g., derivatisation that does not produce methyl esters), it is difficult to conclude
 345 if all fatty acids were detected or not. Nunes *et al.*¹² highlighted another issue with fatty acids when
 346 analysing the pyrolysis bio-oils of oil extraction residues of tropical fruits. They noted that fatty acids
 347 have a “tail” on the 2D plot and some of them (e.g., stearic and oleic acid) co-elute even in 2D GC,
 348 meaning that additional approach such as MS and spectral deconvolution may be needed for the
 349 identification. Other polar analytes like phenols, alcohols, or acids besides fatty acids,⁷⁶ might also
 350 exhibit tailing, which can interfere with the detection and identification of trace-level analytes. For
 351 example, in Figure 5a there are intense tailings for the main compounds but not as much in Figure 5b,
 352 where the main compounds are less polar. One option to avoid this issue is to apply derivatisation, which
 353 enables the analysis of poorly volatile compounds and improves the chromatographic separation of polar
 354 compounds.⁴⁷



355
 356 *Figure 5.* GC×GC-TOF-MS chromatograms of two liquid phases extracted from the pyrolysis product of coconut
 357 fibres. a) Acidic phase obtained by extracting the wastewater with organic solvent b) neutral phase obtained from
 358 bio-oil after the removal of the wastewater. Main components in acidic wastewater phase (a): (1) phenol; (2) 4-
 359 methyl-phenol; (3) 2-methoxy-phenol; (4) 2,6-dimethoxyphenol; (5) dodecanoic acid. Main components in neutral
 360 organic phase (b): (1) 5-methyl-2-furfural; (2) 2,3-dimethyl-2-cyclopenten-1-one; (3) dodecanoic acid methyl
 361 ester; (4) 9,12-octadecadienoic acid methyl ester; (5) 9-octadecenoic-(Z) acid methyl ester. Adapted from Schena
 362 *et al.*⁹

363
 364
 365 In terms of different derivatisation methods, the ones applying silylation or methylation reactions have
 366 been used before the GC×GC analysis of biowastes. Beccaria *et al.*⁵⁹ utilised both derivatisations for the
 367 analysis of animal fat waste, which is a viable alternative for the production of biodiesel. At this moment,
 368 mostly oleaginous crops are grown for this aim, where the TAGs are derivatised into FAMES or other

369 esters that can be used as biodiesel. However, animal fats also contain harmful compounds like non-
370 esterified fatty acids and other oxygenated compounds in low concentrations (1-5 %) that can decrease
371 the yield and quality of biodiesel. For example, high levels of acids can be severely corrosive to metals,
372 such as steel in engines.¹³ Therefore, it is necessary to characterise the minor compounds to develop a
373 suitable pretreatment method for their removal. Beccaria *et al.*⁵⁹ applied derivatisation with *N,O*-
374 bis(trimethylsilyl)trifluoroacetamide (BSTFA) reagent on animal fat that silanised only active
375 hydrogens in polar groups of minor compounds, thereby keeping the TAGs intact. By using this
376 approach, it was possible to analyse separately fatty acid derivatives that do not originate from TAGs.
377 Then they applied column chromatography (preparative LC) as a sample preparation step before the
378 GC×GC analysis to separate the minor compounds from the matrix consisting of alkanes and TAGs.
379 They determined that the sample contained over 150 minor components, including alcohols, aldehydes,
380 sterols, and other oxygenated compounds. The authors also determined the TAG composition with
381 GC×GC by applying derivatisation with MeOH and BF₃, a procedure suitable for TAG *trans*-
382 esterification that yields FAMES. Thanks to applying these derivatisations, they were able to analyse
383 poorly volatile compounds, such as TAGs (converted to FAMES, up to C24:0) and sterols.

384
385 In-situ gas phase derivatisation has also shown to be suitable for the detection of compounds that exhibit
386 derivatisation issues such as poor stability of derivatives or low derivatisation efficiency. Madsen *et al.*⁴⁷
387 applied silylation with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) reagent before
388 GC×GC to analyse and compare different bio-oils from HTL treated biomasses, including a residue of
389 bioethanol production. For the in-situ silylation, the dissolved HTL bio-oil was placed on a filter, which
390 was thereafter thermally desorbed in the presence of MSTFA. The compounds, including derivatised
391 ones, were trapped and finally desorbed into the ¹D column. This in-situ gas phase derivatisation was
392 highly efficient and enabled the detection of low-volatile compounds, from which C20, C22, C24, and
393 C26 fatty acids were reported for the first time for some samples. This improvement was significant
394 because it enabled the analysis of fatty acids that were the main components of the analysed HTL bio-
395 oils. In addition, the applied approach enabled the detection of monoglycerides that usually have lower
396 silylation efficiency in solution and therefore detection problems. However, because of the utilisation
397 of the derivatisation reagent, a longer solvent delay had to be used, which means that the most volatile
398 compounds were not detected.

399
400 Fatty acids were also one of the most intense compounds detected in the bio-oil as well as in the aqueous
401 wastewater fraction of HTL processed food waste from canteens.⁴⁶ However, in this study, derivatisation
402 was not applied and the results are presented by compound classes, without information about the
403 identified fatty acids. Based on the before discussed publications and low final oven temperature of the
404 ¹D separation (280/300 °C) we presume that the long-chain fatty acids were not detected, thus implying
405 that the fatty acid content might have been even higher. Hence, one must consider the possible
406 composition of the analysable sample, as derivatisation might be needed for the GC×GC analysis of
407 other poorly volatile (e.g., carbohydrate oligomers¹²), polar (phenols, alcohols, or other acids⁷⁶), or
408 thermally unstable compounds. Derivatisation can also help to avoid contaminating the injector and/or
409 columns, which can lead to peak tailing, low signal-to-noise ratio, and shift in retention time.⁷¹ However,
410 at this moment, utilisation of derivatisation in the GC×GC analysis of biowastes remains unfortunately
411 scarce.

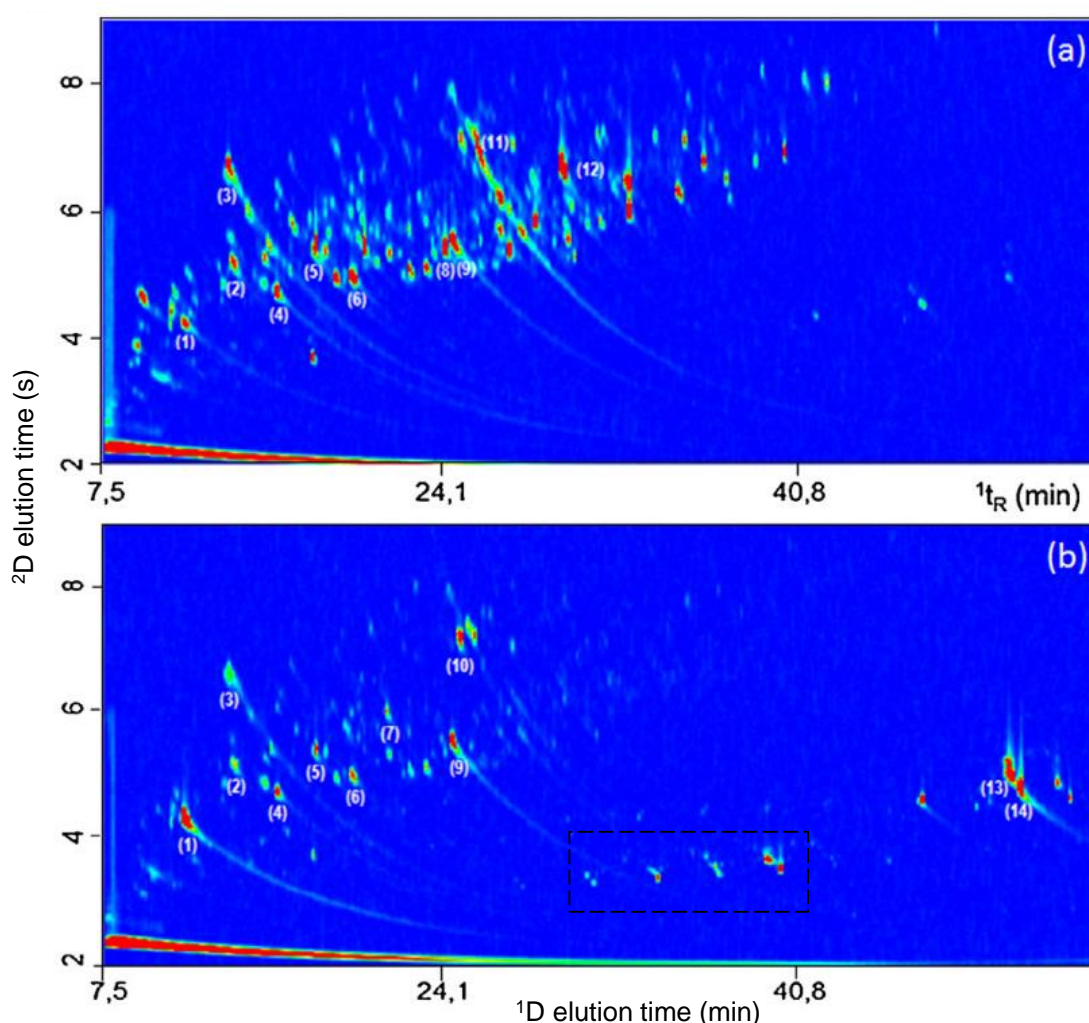
412 413 414 **2.4. Agricultural waste**

415
416 Agricultural waste is one of the biggest sources of residual biomass used for the production of biofuels
417 and biochemicals.^{3,17} This includes wastes consisting largely of lignocellulosic material (rice husk,
418 wheat straw, sugarcane bagasse), which can also contain some residual compounds like caffeine in spent
419 coffee or nicotine in tobacco residues. In the field of agricultural wastes, 2D chromatography has been
420 used for the analysis of both processed and unprocessed biowastes, as is explained under the following
421 sections.

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2.4.1. Analysis of processed agricultural waste - GC×GC and RPLC×RPLC

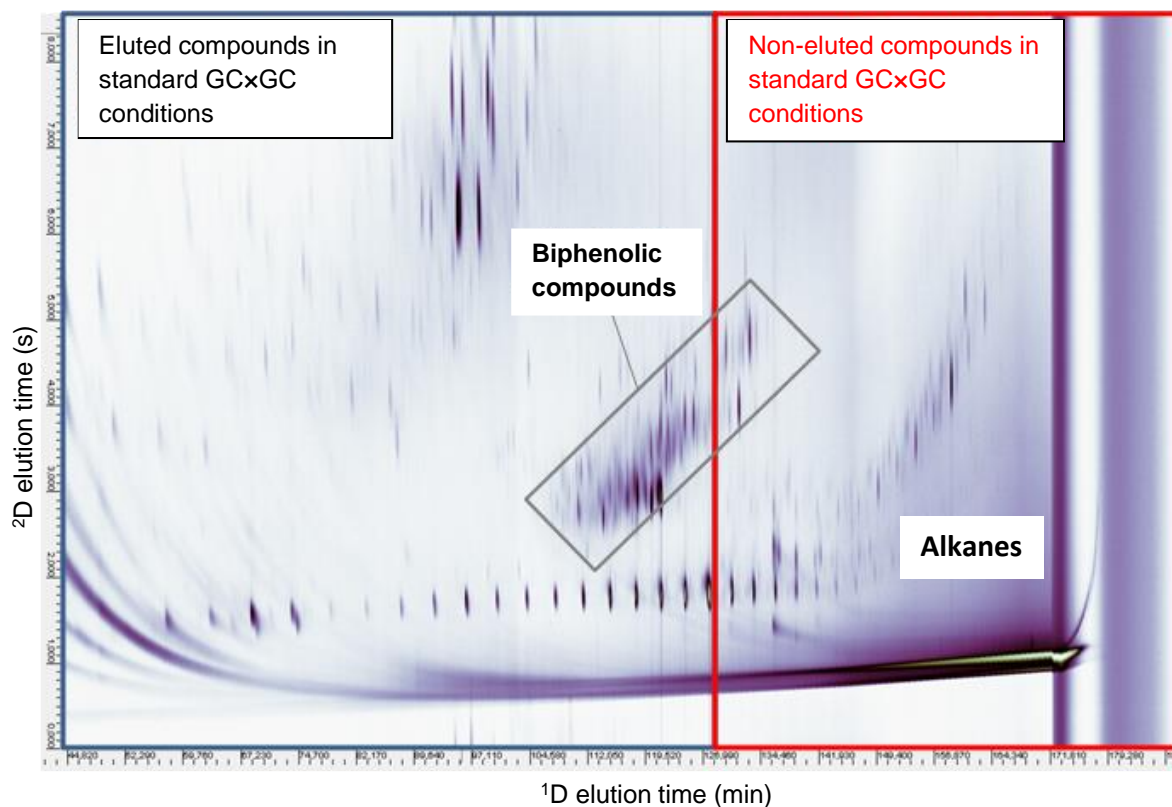
For the analysis of thermally processed agricultural wastes, GC×GC has again been applied to thoroughly characterise the converted waste^{2,6,8,35,36,61} or study how the reaction conditions affect the composition and distribution of the products.^{3,38-40,60} In addition, GC×GC has also been used to determine if different lignocellulosic biowastes can be processed together. For example, Barros *et al.*⁵ studied the fast pyrolysis organic bio-oil of both sugarcane straw and bagasse and noted that the bio-oils differed only by the relative amount of the same compounds and therefore could be valorised together. They detected various oxygenated compounds but also hydrocarbons, which were not detected with 1D GC. However, another study highlighted that bio-oils from different wastes of the same plant (here mango) could be chemically dissimilar.⁴⁵ They saw (Figure 6) that from mango seed waste the tegument aka seed coat could be preferred for producing phenolic chemicals (content of phenols > 30 % in the pyrolysis bio-oil) while the almond was of interest for making liquid fuels because of the higher amounts of long-chain fatty acids and hydrocarbons.



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Figure 6. GC×GC-TOF-MS plots of pyrolysis bio-oil from mango seed (a) tegument and (b) almond. Major compounds: (1) 2-furanomethanol; (2) cyclopentanedione; (3) 2(5H)-furanone; (4) phenol; (5) 1,2-cyclopentanedione, 3methyl-; (6) phenol, 3-methyl; (7) maltol; (8) phenol, 2-methoxy-4-methyl; (9) 1,2-benzenediol; (10) sugar; (11) 2-furancarboxaldehyde, 5-(hydroxymethyl)-; (12) phenol, 2,6-dimethoxy; (13) octadecenoic acid; (14) octadecanoic acid. The rectangle with dashed black lines indicates some of the main detected hydrocarbons in the mango seed almond. Adapted from Lazzari *et al.*⁴⁵

However, it must be kept in mind that depending on the harshness of the conversion process applied on

448 the polymeric lignocellulosic material, the yielding product can also contain oligomers. This was
449 observed by Güvenatam *et al.*⁶⁰ when analysing depolymerised wheat straw lignin, as oligomers were
450 detected when using MALDI-TOF-MS but not with GC×GC where the final oven temperature was
451 250 °C). Therefore, Joffres *et al.*³⁹ decided to apply also high temperature GC×GC in addition to the
452 standard GC×GC for the analysis of hydroconverted wheat straw lignin, where the oven final
453 temperatures were 360 °C and 300 °C, respectively. As can be seen in Figure 7, a significant number of
454 heavy alkanes and biphenolics would have remained undetected when using the standard GC×GC.
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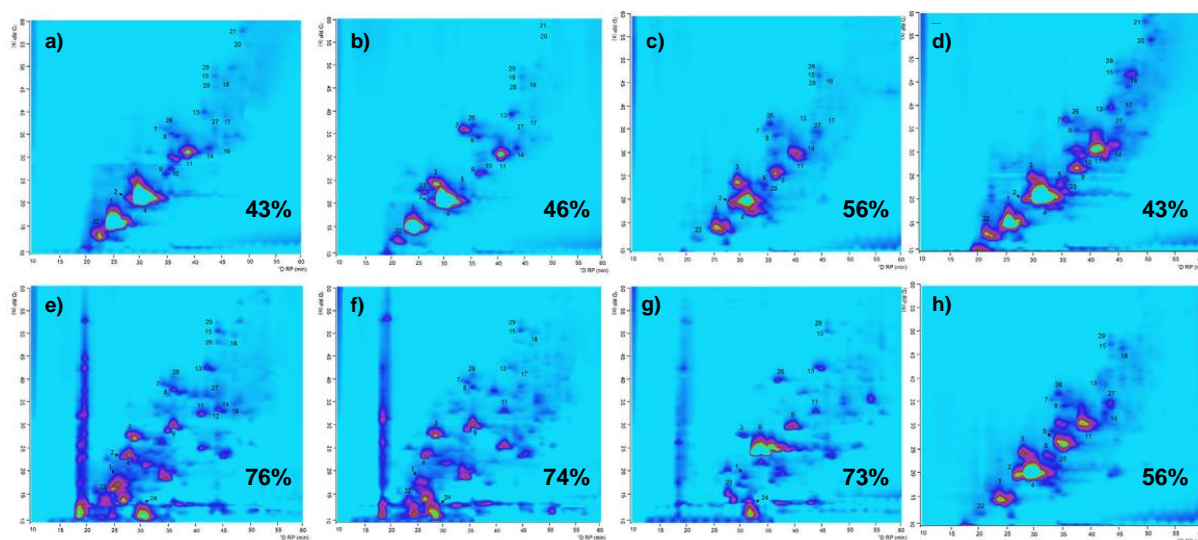
456
457 *Figure 7.* High temperature GC×GC-FID (conventional column setup: 30 m ZB column in ¹D and 2 m ZB50
458 column in ²D) plot of bio-oil obtained from the hydroconversion of wheat straw lignin. Adapted from the
459 Supplementary Information (SI) of Joffres *et al.*³⁹
460

461 In addition to the GC×GC analysis of the bio-oil fraction of a pyrolysed rice husk sample, Lazzari *et al.*³⁶
462 performed also the direct 1D reversed-phase LC (RPLC) analysis of the aqueous phase. They noted
463 that if LLE and GC×GC had been used to analyse the aqueous phase, presumably some highly polar
464 compounds would have not been detected, and a lower concentration would have been determined for
465 the detected polar analytes. The issues with the analysis of polar and low-volatile analytes suggests that
466 2D LC could be more suitable for the quali-quantitative analysis of polar compounds in biowastes as it
467 also enables the direct analysis of the wastewater. At least for agricultural biowastes, some research
468 involving 2D LC has already been conducted.
469

470 In 2D LC, there are no “conventional” column sets as various columns and techniques have been
471 combined while keeping the highest possible orthogonality and the feasibility of connecting the two
472 dimensions in mind. One of the most common on-line 2D LC techniques is RPLC×RPLC because of its
473 ease of implementation. Although similar chemical interactions take place in the two dimensions,
474 separation can be enhanced by using different stationary phases, organic solvent, temperature, or, in the
475 case of ionisable analytes, pH in the two dimensions.
476

477 Tomasini *et al.*⁷ and Lazzari *et al.*^{17,37} demonstrated by analysing with RPLC×RPLC method the
478 wastewater of pyrolysed agricultural residues that merely applying columns with different separation
479 selectivities (amide column in ¹D and C8⁷ or C18^{17,37} column in ²D) provided an improved separation of

480 the small oxygenated compounds, plus caffeine in coffee wastes. Additionally, it was experimentally
481 proven that with 2D LC it is possible to detect and separate some polar compounds (such as 5-
482 (hydroxymethyl)furfural) which require derivatisation to be detectable with the GC×GC analysis.³⁷ For
483 the orthogonality value, Tomasini *et al.*⁷ obtained 47 % (not clear if it was obtained for converted
484 coconut or sugarcane residues) and Lazzari *et al.*^{17,37} 33-74 %, depending on the processed sample
485 (Figure 8). The orthogonality values and the distribution of compounds on the 2D plot demonstrate that
486 the performance of RPLC×RPLC heavily depends on the chemical composition of the agricultural (and
487 non-agricultural crambe seed) feedstock. It can be seen in Figure 8 that the highest orthogonality values
488 were obtained for the processed samples of crambe seed, cottonseed, and coffee silverskin, suggesting
489 that these wastewaters contained chemically the most different analytes. In addition, it can be seen that
490 the analytes are upward diagonalised on the 2D plot, demonstrating the dependence of the two
491 dimensions.
492



493 *Figure 8.* RPLC×RPLC-DAD plots with orthogonality values for the aqueous wastewater phases from the
494 pyrolysis of (a) sugarcane bagasse; (b) coconut fibre; (c) almond of mango seed; (d) pineapple leaves; (e) crambe
495 seed; (f) cottonseed; (g) coffee silverskin; and (h) cassava peel. Adapted from Lazzari *et al.*¹⁷, where the
496 compound names corresponding to the peak numbers can be found.
497

498
499 Lignocellulosic biowaste such as sugarcane, rice, and wood residues can also be used for the production
500 of bioethanol by biochemical conversion. As this is a multi-step process, it is necessary to also study the
501 intermediate products to improve the valorisation process. To analyse the carbohydrates in the aqueous
502 fraction of chemically pretreated wheat straw, Reymond *et al.*¹⁶ developed a RPLC×RPLC method.
503 Compared to 2D GC, 2D LC has here advantages, as there is no need to derivatise the carbohydrates,
504 including oligomers. Additionally, the pretreatment yielded lignin-carbohydrate complexes, commonly
505 not produced with thermochemical processes.¹⁵ Using a highly retentive porous graphitic carbon column
506 and isopropanol eluent as first dimension, in combination with a more conventional C18 column and
507 acetonitrile eluent, a high orthogonality value of 75 % was observed for the compounds of interest.
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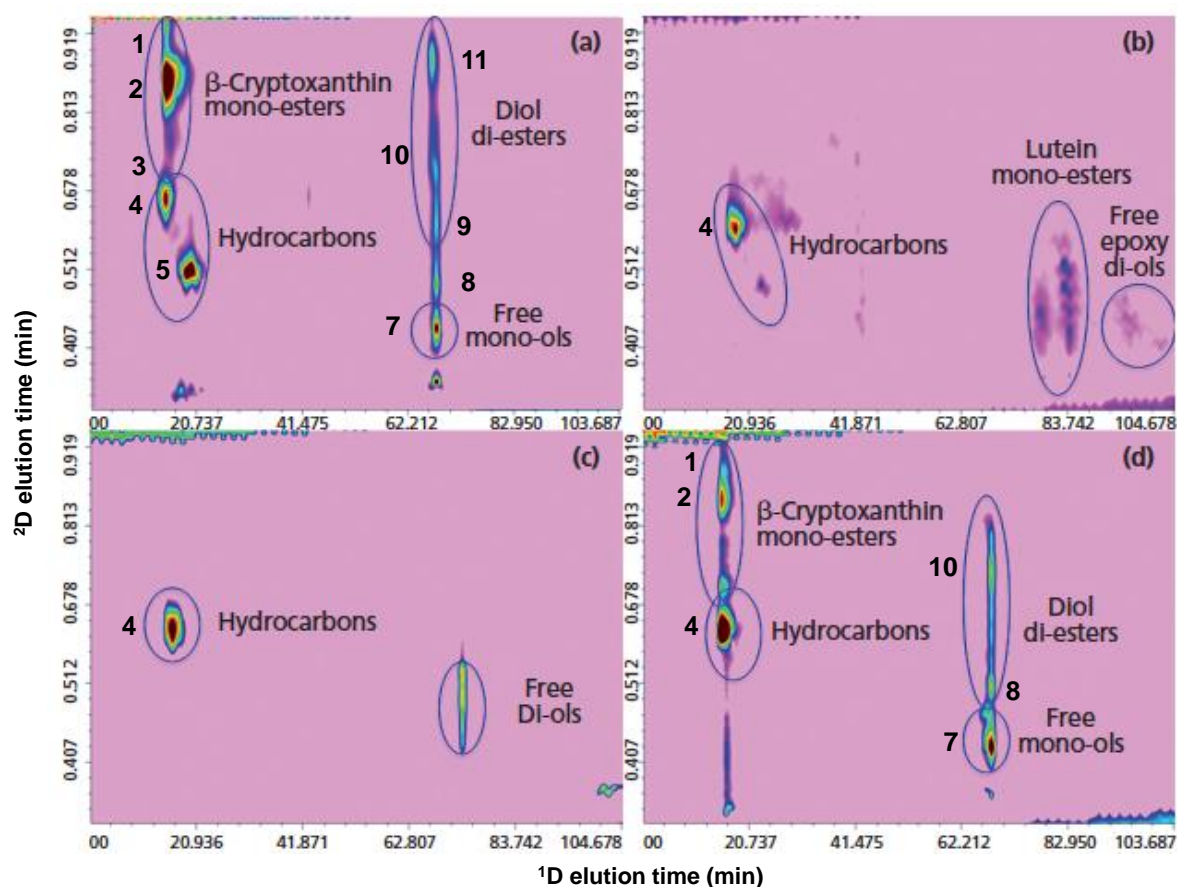
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510 **2.4.2 Analysis of extracts from unprocessed agricultural biowaste – NPLC×RPLC and** 511 **HILIC×RPLC**

512
513 Some valuable compounds can be isolated from biowaste without chemical conversion. These include
514 bioactive compounds in food waste, which are already in the suitable form and can be isolated from the
515 rest of the matrix using consecutive extractions.^{4,10,19} The extracts have to be thoroughly characterised
516 to identify the bioactive compounds and determine if the biowaste is suitable for the valorisation. For
517 these aims, on-line 2D methods have been used, where the RPLC is coupled to normal-phase (NPLC)
518 or hydrophilic interaction LC (HILIC). When applying these approaches, 2D LC separations with
519 different interactions are combined, thus higher orthogonality could be obtained.

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Donato *et al.*¹⁰ applied NPLC×RPLC for the analysis of carotenoids in overripe fruits (Figure 9). The NPLC allowed the separation of carotenoids into seven groups based on polarity, whereas the RPLC provided an additional separation within each group based on their increasing hydrophobicity and decreasing polarity. Based on the results, the authors concluded that the overripe fruits still contained valuable carotenoids that could be valorised. One important aspect to mention about the NPLC×RPLC technique (also applies to HILIC×RPLC, see next paragraph) is the incompatibility of the eluents commonly used in the two dimensions in terms of viscosity, miscibility, and solvent strength. For example, hexane is a weak solvent in NPLC but a strong solvent in RPLC. This can lead to adverse or even detrimental effects in the 2D separation, such as peak splitting, broadening, and breakthrough - analytes eluting with the dead volume.⁷⁷ As Donato *et al.*¹⁰ focused on the analysis of nonpolar carotenoids, they could use RPLC in almost completely non-aqueous mode (10 % H₂O in acetonitrile to 100 % isopropanol instead of the commonly used H₂O to acetonitrile elution) making the mobile phases more compatible. Although the orthogonality value was not reported, it is possible to see from Figure 9 that the developed 2D method yielded acceptable separation of the targeted compounds.



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Figure 9. NPLC×RPLC-PDA plots of (a) hybrid persimmon-apple; (b) banana pulp; (c) banana peel; and (d) nectarine. Some of the identified peaks: β -Cryptoxanthin-C16:0 (1); β -Cryptoxanthin-C14:0 (2); β -Cryptoxanthin-C16:1 (3); β -Carotene (4); Lycopene (5); β -Cryptoxanthin (7); Anteraxanthin-C14:0 (8); Anteraxanthin-C14:0-C14:0 (9); Lutein-C14:0-C14:0 (10); and Zeaxanthin-C14:0-C14:0 (11). Adapted from Donato *et al.*¹⁰, where also the other peak annotations can be found.

Another option to reduce the negative effect of solvent incompatibility is to use loops with a slightly bigger volume than is technically necessary to transfer the solution from 1D to 2D.⁷⁸ This way, the effluent is diluted with the mobile phase of 2D. This approach was used by the Herrero group^{4,19} in the HILIC×RPLC analysis of possibly bioactive (poly)phenolic compounds, such as anthocyanins, in residual black chokeberry pomace⁴ and grapevine canes.¹⁹ HILIC is a technique that uses a polar stationary phase like NPLC, but in combination with a hydro-organic mobile phase. For the black chokeberry pomace and grapevine cane extracts orthogonality values of 76 % and 78 % were calculated,

550 respectively. In addition, the peaks were not diagonalised on the 2D plots, which confirms that the
551 correlation between the selected dimensions was low. With the grapevine cane extract also a
552 RPLC×RPLC setup was tested; however, much poorer orthogonality of 45 % was obtained.¹⁹ Both
553 studies concluded that the utilisation of the developed HILIC×RPLC methods allowed them to identify
554 compounds never before described in the corresponding biowastes. In addition, polyphenol oligomers
555 up to heptamers were identified, which would be impossible with GC×GC because of the low volatility
556 of these oligomers. The authors also concluded that these biowastes have potential for the valorisation
557 of (poly)phenolic compounds.

558 In terms of modulation, researchers have developed other techniques to reduce the solvent
559 incompatibility issue between two complementary LC dimensions in on-line setups. Although, as far as
560 we know, these have not yet been applied in-between HILIC/NPLC and RPLC dimensions for the 2D
561 analysis of valorisable biowastes, they still are worth mentioning in the name of future studies. For
562 example, by using additional ports in the valve of the modulation, active-solvent modulation (ASM) can
563 be performed where the ¹D effluent is diluted by the ²D eluent. Another quite popular approach is the
564 stationary-phase-assisted modulation (SPAM) where instead of empty loops trapping columns are used
565 to trap the analytes and “replace” the eluent of the ¹D effluent with ²D eluent (see section 2.5.4. for an
566 application in LC×SFC). Although these approaches require modifying the valve of the modulator, they
567 have demonstrated decrease of undesired solvent mismatch effects but also improved detection
568 sensitivity because of the concentration of the analytes. Further reading on various modulation types
569 and applications in 2D LC can be found in previously published reviews.^{24,79}
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571 **2.5. Waste from wood industry or energy crops**

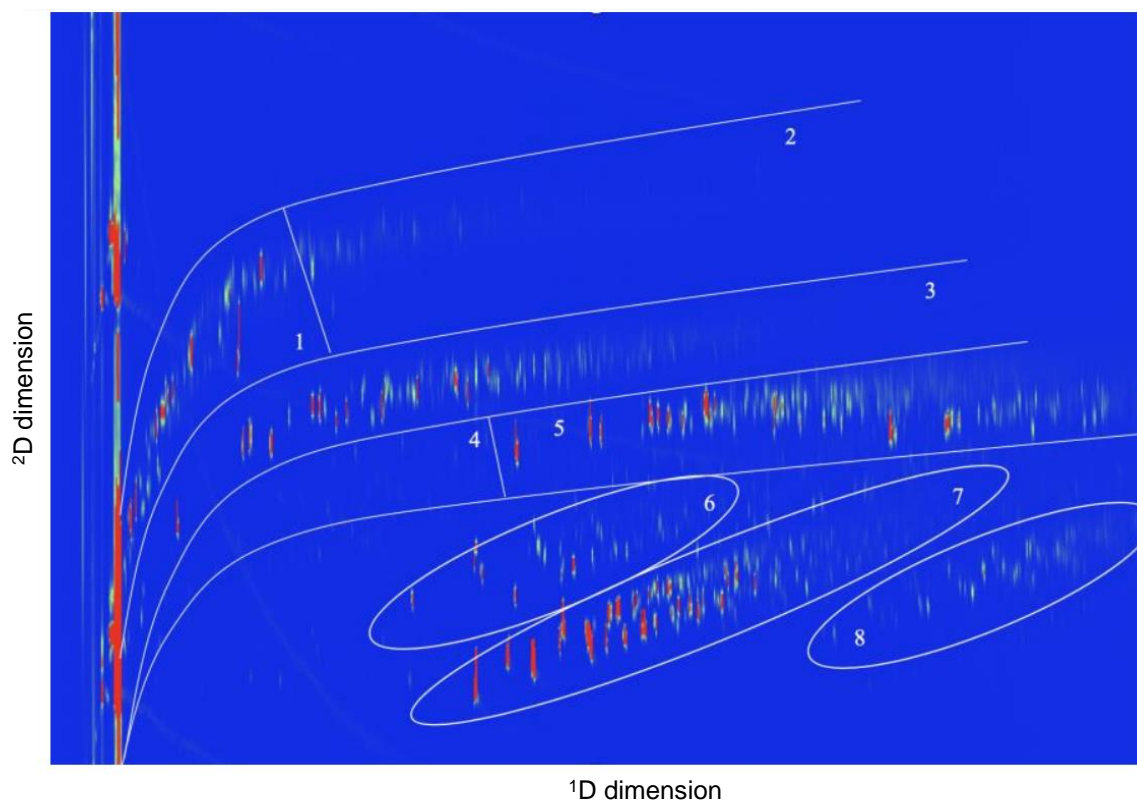
572 Wood and energy crops are lignocellulosic biomasses, from which mainly three different types of wastes
573 have been analysed with 2D chromatographic methods. One is lignin, which is a by-product obtained in
574 large quantities from the paper industry when it is separated from (hemi)cellulose. However, lignin is
575 the largest renewable resource for aromatics and could be valorised by the isolation of aromatic/phenolic
576 chemicals (e.g., vanillin for food flavouring) or used for the synthesis of novel materials such as lignin-
577 based hydrogels for wound healing and food packaging.⁸⁰ Another waste is the residual stillage obtained
578 from the production of bioethanol, which can be used for the same purposes, as it mainly consists of
579 lignin but also residual (hemi)cellulose and proteins.³⁴ Finally, the mechanical processing of wood
580 produces wastes, such as woodchips or sawdust. Sometimes it is not specified if the wood sample is a
581 residue or not, but because the material is chemically identical, the same 2D method can be applied.
582

583 **2.5.1. GC×GC and GC-GC**

584
585 As far as we know, pyrolysed wood lignin is the only biowaste that has been analysed with GC-GC
586 since 2010. This was done Olcese *et al.*⁶⁷ to additionally separate four compounds (1-undecene,
587 guaiacol, p- and m-cresol) in one heart-cutting zone for quantitative analysis purposes. However, it is
588 unclear if the other chromatographic areas were without co-elution, as GC×GC analysis was not
589 presented for the same sample. Lago *et al.*⁵⁰ used GC×GC to compare the pyrolysis products of Kraft
590 lignin to the hydrolysis lignins of different origins - aspen wood, sugarcane bagasse, Napier grass, and
591 wheat straw. The study of the relative abundances of the main phenolic compounds demonstrated that
592 the processing technique (Kraft pulping vs. hydrolysis) had a bigger effect on the final composition than
593 the origin of lignin, as the processed hydrolysis lignins were similar to each other but not so much to the
594 Kraft lignin sample. The fact that converted Kraft lignin differed the most could already be made when
595 looking at the 2D plots – Kraft lignin had higher proportions of phenolic compounds compared to
596 hydrolysis lignin, which is expected as the second contains also residual cellulose and hemicellulose.⁵⁰
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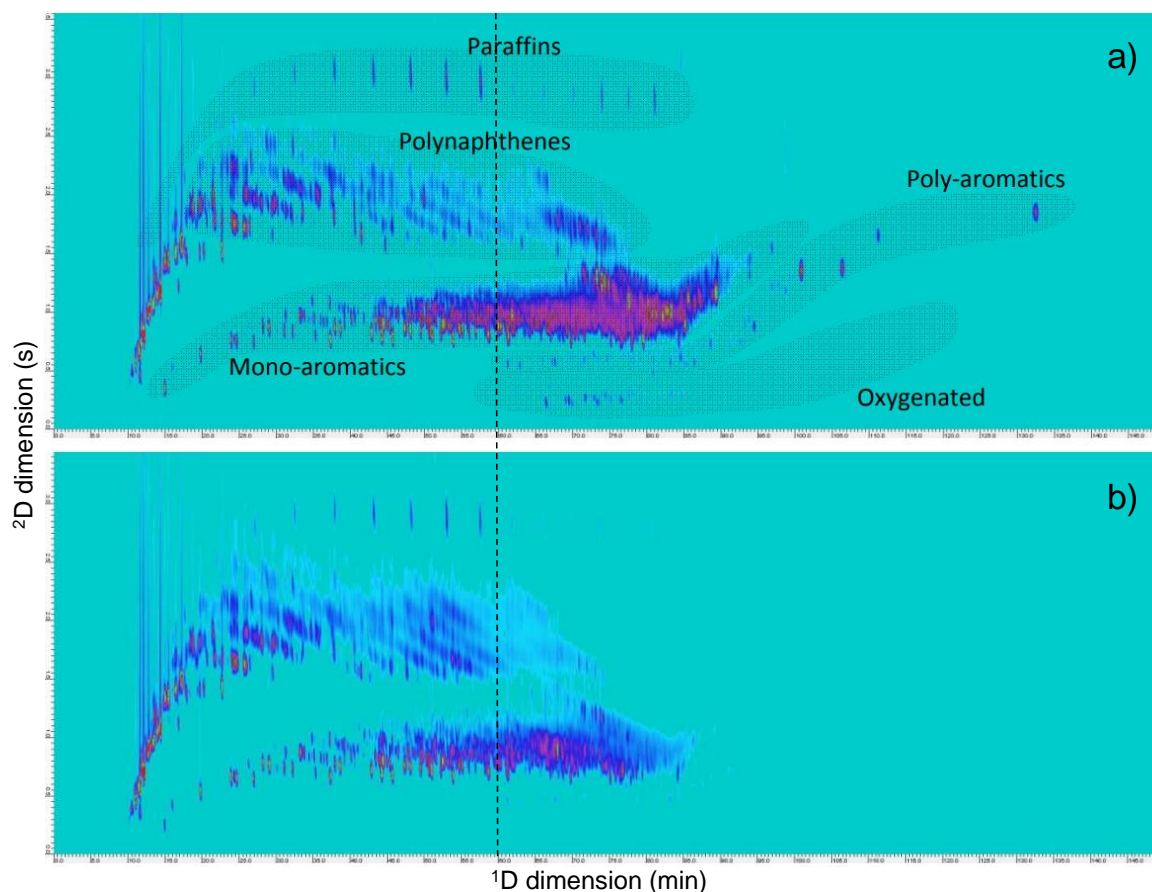
598 The systematic distribution of chemical families on the 2D plots can be used to monitor treatment
599 processes without identifying the exact chemical formulas of the compounds. For this, the reversed
600 (polar x non-polar) column setup can be useful, where in ¹D, the separation is based on both the boiling
601 point and polarity, while in ²D only on polarity. This can improve the resolution of some specific
602 compound groups, such as speciation of oxygenated compounds into chemical families, separately from

603 non-oxygenated compounds.^{34,39,60,66,81} For example, Hita *et al.*³⁴ applied the reversed setup to compare
604 separately and jointly the HTL and hydrodeoxygenation processes for the valorisation of low-weight
605 chemicals (mainly alkylphenols and aromatics) from bioethanol stillage residue, obtained from the
606 enzymatic hydrolysis of eucalyptus. They compared the processes by quantifying together the
607 compounds in the different chemical classes formed on the 2D plots (Figure 10). The results
608 demonstrated that the direct hydrodeoxygenation and the two-step HTL-hydrodeoxygenation process
609 yielded the highest amounts of the desired monomers, but the joint process had also technical
610 advantages, such as low char formation.
611



612
613 *Figure 10.* Exemplary GCxGC-FID (reversed column setup) chromatogram of bio-oil obtained from
614 hydrotreating wood lignin. Classified regions based on chemical nature: (1) cyclic alkanes; (2) linear alkanes; (3)
615 aromatics; (4) ketones; (5) naphthalenes; (6) guaiacols; (7) alkylphenolics; (8) catechols. Adapted from the SI of
616 Hita *et al.*³⁴
617

618 Mathieu *et al.*⁶⁴ applied also a reversed column setup to evaluate the efficiency of hydrodeoxygenation
619 on bio-oil obtained from HTL treated woodchips. In Figure 11, the GCxGC plots of bio-oil hydrotreated
620 under milder (70 bar, Figure 11a) and harsher (120 bar, Figure 11b) conditions are presented, where the
621 shaded background is used to visualise the compound classes. It can be seen that although both processes
622 yielded a low level of oxygenated compounds, the harsher conversion process was more successful as
623 the area of oxygenated compounds is clear in Figure 11b (only trace amounts were detected on closer
624 inspection). In addition, overall a lower signal was seen in ¹D after 60 min (dashed black line), which
625 indicates that the harsher hydrotreatment also decreased the amount of heavier components. Based on
626 their observations, the authors stated that the product obtained under harsher conditions (higher pressure
627 and temperature) could be suitable to be directly blended with diesel and used as transportation fuel.
628 Therefore, in their second study by Sauvanaud *et al.*⁶⁵ they applied the same GCxGC method and
629 determined that up to 20 wt % of bio-oil could be co-hydrotreated with diesel, yielding a product with
630 very low oxygen content that fits the road diesel specifications.
631



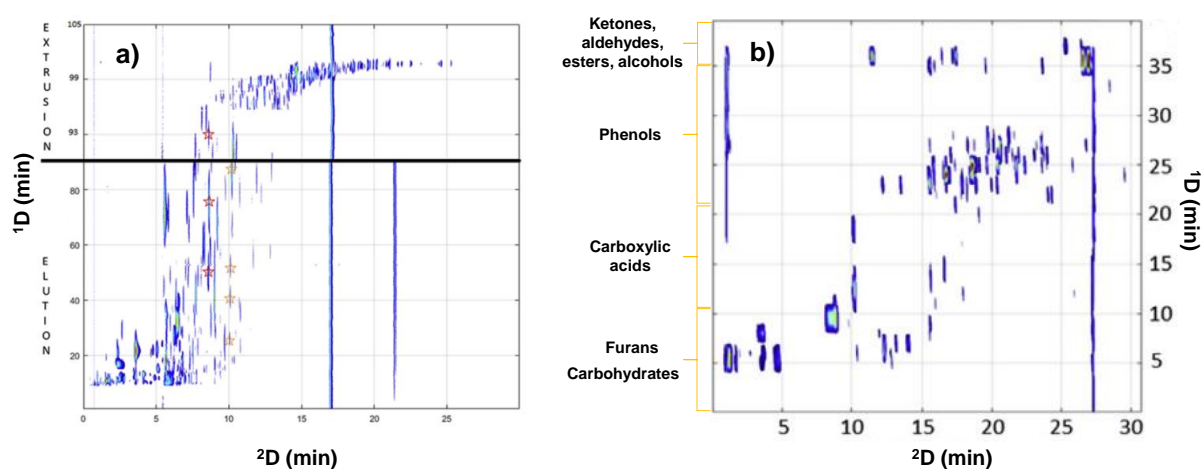
632
 633 *Figure 11. GC×GC-MS plots of the bio-oil (HTL of woodchips) hydrotreated under milder (a, 70 bar) and*
 634 *harsher (b, 120 bar) conditions. The shaded background visualises the compound classes and the dashed black*
 635 *line the 60 min in ¹D for the comparison. Adapted from the SI of Mathieu *et al.*⁶⁴*
 636

637
 638 As it was mentioned before, GC×GC has its limitations for the analysis of poorly volatile compounds.
 639 Besides derivatisation and high temperature GC×GC, another option to overcome this obstacle is to use
 640 pyrolysis-GC×GC, where the pyrolysis takes place in a pyrolyser directly connected to the GC×GC. For
 641 example, this has been done by researchers in Denmark for the analysis of HTL treated aspen wood,^{62,63}
 642 which has led to one of the most exhaustive biocrude characterisations. First, Pedersen *et al.*⁶³ performed
 643 fractional distillation of a biocrude from the HTL process, derivatised the higher boiling fractions with
 644 BSTFA, and analysed all the fractions with 1D GC. The residual solid of the distillation was analysed
 645 with pyrolysis-GC×GC. Thanks to this combined approach, they were able to determine over 85 % of
 646 the total composition and demonstrate that only 48.2 % is volatile below 350 °C - it must be kept in
 647 mind that generally even lower final oven temperatures are used with the standard GC×GC. The authors
 648 concluded that because the solid residue consisted mainly of aromatic compounds also suitable for fuel
 649 production, cracking of the residue could be used to valorise even more of the biocrude. Concerning
 650 pyrolysis-GC×GC, it should be noted that during the pyrolysis process, the compounds are fragmented
 651 and, to some extent, deoxygenated by the formation of pyrolysis products, mainly CO₂ and H₂O.
 652 Therefore, compared to the standard GC×GC, it is more difficult to identify the original compounds.
 653

654
 655 **2.5.2. CPC×RPLC**
 656

657 One technique used for the analysis of organic bio-oil⁵³ or wastewater phase⁴⁴ from the fast pyrolysis of
 658 softwood sawdust is centrifugal partition chromatography (CPC) combined with RPLC. CPC is a liquid-
 659 liquid chromatography method that enables the separation of compounds based on their partition
 660 coefficients, similarly to LLE but with higher efficiency. Therefore, compounds that have the same t_R

661 in RPLC but different K_D can be separated using CPC×RPLC. Because of the solvent incompatibility
 662 and flow rate discrepancy, only off-line setup is possible. Le Masle *et al.*⁵³ used CPC to separate the
 663 bio-oil into 280 fractions, from which the last 100 (obtained in heptane/ethyl acetate) were evaporated
 664 to dryness and dissolved in methanol, a solvent more compatible with the mobile phase of ²D. After
 665 analysing the fractions one-by-one with RPLC, a CPC×RPLC plot was obtained (Figure 12a). A good
 666 orthogonality with a value of 73 % was calculated in the CPC elution zone for the CPC×RPLC setup,
 667 which demonstrates that the two separations are sufficiently different. However, it can be seen on the
 668 2D plot (Figure 12a) that the peaks are very wide (a few to 10 min) in the ¹D dimension, highlighting
 669 the low peak capacity of the CPC method. In their second study,⁴⁴ the authors modified the CPC×RPLC
 670 method and applied it to the wastewater phase that generally contains a higher number of ionisable
 671 compounds than the organic bio-oil. They applied pH zone refining, which allowed them to improve the
 672 chemical organisation on the CPC dimension (see Figure 12b for the proposed compound ranges) that
 673 can help with compound annotation. In addition, the peaks are narrower in the ¹D dimension suggesting
 674 that the peak capacity of the CPC dimension was also improved.
 675



676
 677 *Figure 12.* CPC×RPLC-UV plots of liquid phases obtained from the fast pyrolysis of softwood sawdust. (a)
 678 Organic bio-oil, adapted from Le Masle *et al.*⁵³ where the star signs represent examples of avoided co-elutions,
 679 and (b) aqueous wastewater, adapted from Dubuis *et al.*⁴⁴.

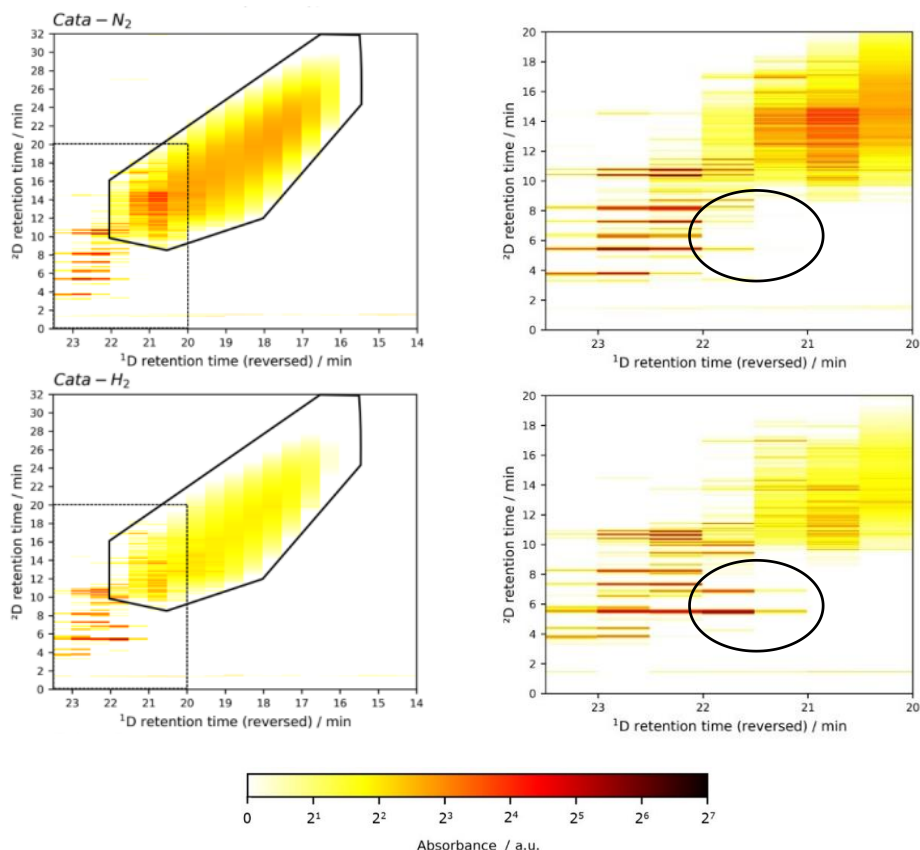
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682 2.5.3. SEC×RPLC and mGPC-RPLC

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684 Another option is to use size exclusion chromatography (SEC), where the separation is performed based
 685 on the size of the compound in the solution. Dubuis *et al.*¹⁵ developed a SEC×RPLC method for the
 686 analysis of aqueous phases from the fast pyrolysis of sawdust and biochemically pre-treated wheat straw.
 687 In addition to the separation based on size, also chemical organisation was obtained with the SEC
 688 dimension to some extent. The biochemical conversion produced lignin-carbohydrate complexes (as
 689 was also seen in the work by Reymond *et al.*¹⁶, see section 2.4.1.), which eluted after carbohydrates but
 690 before oxygenated aromatics in SEC. Gel permeation chromatography (GPC), a type of SEC but in
 691 organic media, was combined with RPLC by De Saegher *et al.*⁵⁴ to study the products of differently
 692 depolymerised Miscanthus lignin. As the authors aimed to have a fast and cost effective method to assess
 693 the conversion process, they applied the technique in off-line multiple heart-cutting mode on one
 694 selected part of ¹D and analysed only 19 GPC fractions with RPLC. Figure 13 shows 2D plots of two
 695 out of the four samples analysed by De Saegher *et al.*⁵⁴ The diagonal on the 2D plot demonstrates the
 696 correlation between the two dimensions, which is expected as the hydrophobicity (t_R in RPLC) of a
 697 lignin monomer/oligomer is correlated to its size (t_R in GPC). In addition, as only 19 fractions were
 698 collected from GPC, the peaks in ¹D axis are very wide. Nevertheless, the 2D plots allowed them to
 699 perform a rapid but also detailed screening of the differently processed samples in a graphical manner.
 700 For example, as the area surrounded by the polygon contains mainly oligomers, it was possible to
 701 conclude that the sample processed under H₂ had a higher degree of depolymerisation (lower signal). In
 702 addition, based on the analysis of standards, they could deduce that the same sample had a higher

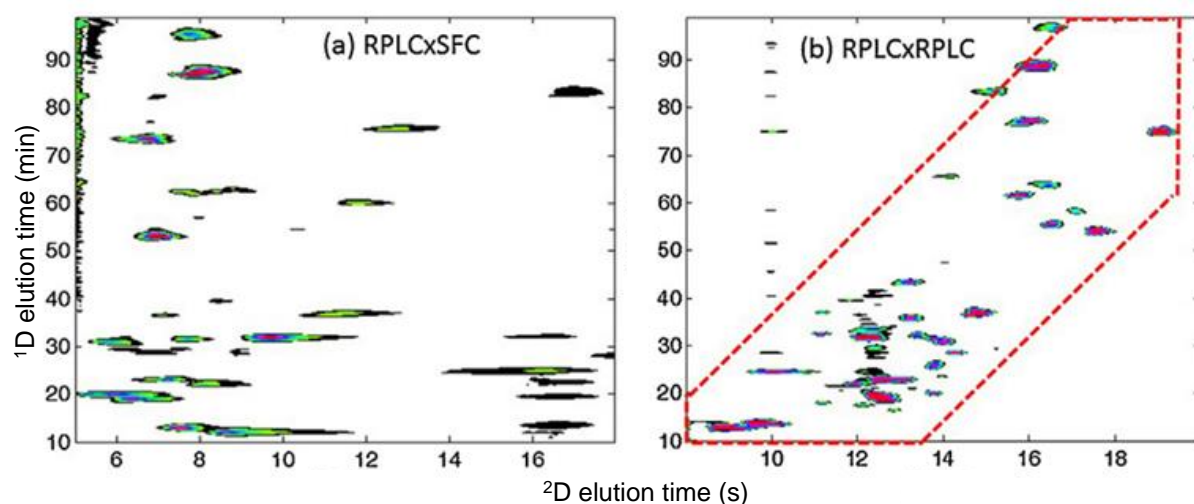
703 concentration of OH-groups because the signals were more intense, mainly in the area highlighted by
 704 the oval. Therefore, even when 2D techniques exhibit low orthogonality and peak capacity, they can
 705 still be beneficial for analysing or monitoring valorisation processes.
 706



707
 708 *Figure 13.* mGPC-RPLC-UV/VIS plots of converted Miscanthus lignin, the catalytic depolymerisation
 709 performed under N₂ (row 1) or H₂ (row 2). On the left side – the complete 2D plot, on the right side – subplot of
 710 the complete 2D plot. The area containing mainly oligomers is highlighted with a polygon. The area highlighting
 711 the difference in OH-groups is indicated with an oval. Adapted from the SI of De Saegher *et al.*⁵⁴
 712
 713

714 2.5.4. RPLC×SFC and RPLC×RPLC

715
 716 On-line 2D LC techniques have been used for the analysis of wood-derived waste. For example, RPLC
 717 has been combined with supercritical fluid chromatography (SFC), where pressurised gas (often CO₂)
 718 and an organic co-solvent such as methanol or acetonitrile are used as eluents. When a polar stationary
 719 phase is used in SFC, then similar separation mechanism to NPLC is achieved which can lead to higher
 720 orthogonality in combination with RPLC than can be achieved with RPLC×RPLC. Beside utilising less
 721 toxic eluents than NPLC, SFC also needs less time for re-equilibration, which is beneficial for very fast
 722 2D separations.⁸² Sarrut *et al.*⁶⁹ were the first to develop an on-line RPLC×SFC method and compare it
 723 to RPLC×RPLC based on the analysis of neutral compounds in bio-oil wastewater (Figure 14). Thanks
 724 to the uncorrelated RPLC×SFC dimensions, an orthogonality value close to 100 % was found,
 725 demonstrating the high separation power. For RPLC×RPLC, this value was close to 60 %, which was
 726 also seen by Le Masle *et al.*¹⁸ in a previous study. However, even after a through optimisation performed
 727 by Sarrut *et al.*⁶⁹, broader peaks were seen for 2D-SFC compared to 2D-RPLC (Figure 14a), due to
 728 solvent incompatibility during modulation, which leads to lower practical peak capacity and poorer
 729 sensitivity. Therefore, even though this work showed the great potential of combining RPLC with SFC,
 730 some technical issues were still observed with the on-line system that need solving to improve the peak
 731 capacity and sensitivity.
 732



733
734 *Figure 14.* (a) RPLC×SFC-UV and (b) RPLC×RPLC-UV analysis of the aqueous wastewater of a bio-oil. The
735 area surrounded by red dotted lines shows the separation space for RPLC×RPLC-UV. Adapted from Sarrut *et*
736 *al.*⁶⁹

737
738 To improve the sensitivity aspect, Sun *et al.*⁶⁸ applied and compared three trapping columns with
739 different chemistry instead of the more traditional collection loops. The applied method is called
740 trapping column-assisted modulation or SPAM (see section 2.4.2.), where after the ¹D separation, the
741 analytes are retained in the trapping column and subsequently transferred by flushing with ²D eluent to
742 ²D. In this manner, less RPLC mobile phase is transferred to the SFC, which lowers the issues caused
743 by mobile phase incompatibility. Also higher flow rates can be used in ¹D leading to shorter overall
744 analysis time (in this study it was cut by half). For the analysis of small phenolic compounds, an Agilent
745 Eclipse Plus Phenyl-Hexyl column performed the best as the trapping column and was selected for the
746 RPLC×SFC analysis of a depolymerised lignin sample. Due to the increased sensitivity that the trapping
747 columns can provide because of analyte concentrating effect, it was possible to identify compounds left
748 unnoticed when collection loops were used. Using trapping columns, an orthogonality value of 79 %
749 was achieved, which was a bit higher compared to 77 % obtained with classical collection loops. We
750 assume that the higher value was caused by the fact that more compounds could be detected when
751 trapping columns were used, some outside of the separation space observed when collection loops were
752 applied. However, as higher flow rates were used in ¹D, the main disadvantage for applying trapping
753 columns is the more severe undersampling for ¹D, which leads to lower practical peak capacity for the
754 RPLC×SFC method.

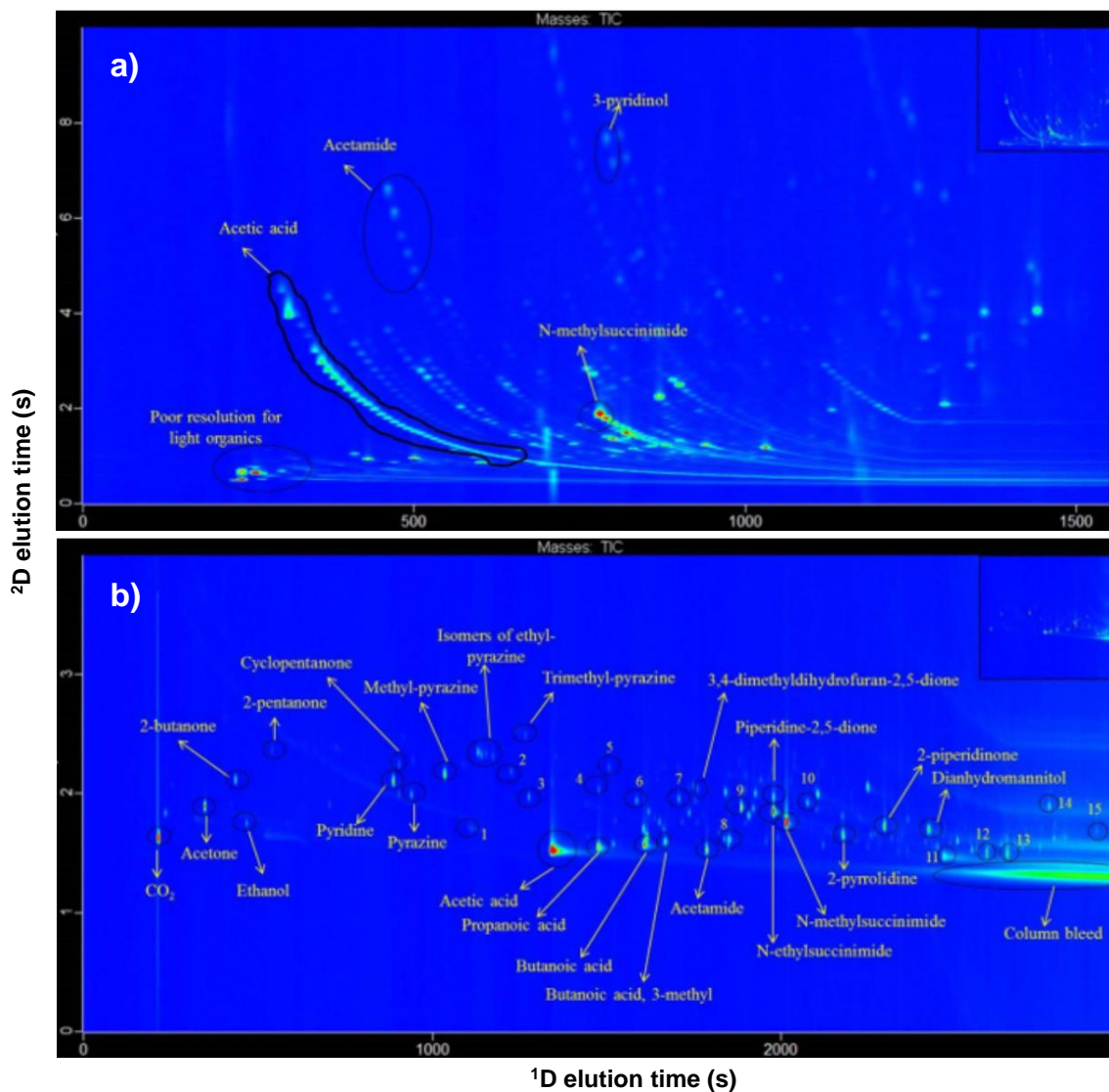
755 756 757 **2.6. Residues from algae processing**

758 Microalgae is aquatic microorganism that can be cultivated for the production of food supplements or
759 alternative liquid transportation fuels. After isolating the valuable components, the first process yields a
760 residue, which can be also converted into fuel or other materials such as bio-bitumen for roads^{48,49}.
761 Because it avoids the need of sample drying, HTL conversion is commonly used, which also yields a
762 valuable aqueous wastewater fraction containing around 20-35 % of the total carbon of the algal
763 feedstock. Besides the possibility of converting the organic compounds into useful chemicals or fuel,
764 the wastewater itself could be used for cultivating new algae.⁷²

765 766 **2.6.1. GC×GC**

767
768 Maddi *et al.*⁷¹ applied a conventional setup for the GC×GC analysis of wastewater from HTL
769 conversion. They were the only researchers to analyse the aqueous phase directly with GC×GC, thanks
770 to the low injected volume (1 μL, required due to large expansion volume of water), water tolerant
771 columns, and high split ratio (1:250), which led to low injected water volumes. However, one must be

772 careful when injecting water even in low amounts, as it can harm the stationary phase of polar columns
 773 or expand outside the injection liner.⁸³ Besides, as the sample mainly consisted of polar small
 774 compounds, deriving from the degradation of larger molecules – oxygenates and organic acids (e.g.,
 775 cyclopentanone, propanoic acid) from carbohydrates and lipids, and nitrogen heterocyclics (e.g.,
 776 pyridine, acetamide) from proteins,^{70,71} other problems emerged. Only a low number of compounds
 777 could be detected and some organic acids and nitrogen compounds showed drastic peak tailing (Figure
 778 15a). Instead of applying derivatisation, like it was done in the publications reviewed under section 2.3.,
 779 the authors tested a reversed column setup for the same sample, which demonstrated improved peak
 780 shape, peak capacity, and resolution (Figure 15b). This method allowed even the detection of CO₂ and/or
 781 CO₂-NH₃ salts in both sludge and algae HTL wastewaters, as no solvent delay had to be used because
 782 the *m/z* range started at 35. However, applying a polar column instead of a semi-polar column has also
 783 its disadvantages. The maximum allowed temperatures for polar GC columns are generally around
 784 260 °C, therefore, compounds with higher molecular weight are left undetected. Additionally, polar
 785 columns may nevertheless bleed at higher temperatures and the resulting signal can interfere with the
 786 detection of analytes.^{41,71} The reversed setup has shown to be suitable also for the analysis of bio-oil
 787 obtained from the HTL treatment of *Scenedesmus* microalgae residues after the extraction of valuable
 788 proteins. This bio-oil could be used as bio-bitumen as its rheological properties resemble a standard
 789 bitumen. By applying GC×GC, Geantet *et al.*⁴⁹ demonstrated that the vaporisable fraction of the bio-oil
 790 consisted mainly of linear and branched long-chain alkanes and alkenes; free fatty acids and their amide
 791 derivatives; and nitrogen-containing heterocyclic compounds.
 792



793
 794 *Figure 15.* GC×GC-TOF-MS plots of the wastewater obtained from the HTL conversion of algae with (a) the

795 conventional non-polar \times polar column combination, and (b) the reversed polar \times non-polar combination. Adapted
796 from Maddi *et al.*⁷¹, where also the names of the other identified compounds are presented.

797
798

799 **2.6.2. RPLC \times SFC**

800

801 RPLC \times SFC has also been applied for the analysis of wastewater from HTL treated algae by Teboul *et*
802 *al.*⁷² Again a high orthogonality value was obtained (75 %, only 45 % was seen for RPLC \times RPLC),
803 demonstrating the low correlation of the RPLC and SFC dimensions also for the analysis of algal HTL
804 wastewater. Because of the solvent incompatibility issue, off-line mode was used and it was proven that
805 water-rich fractions could be injected into the SFC dimension without detrimental effects on the overall
806 performance if the injection volume is suitably low (for the used setup 10 μ L was found as the limit).
807 Here emerges another advantage of the off-line LC \times SFC setup – the splitting of the ¹D effluent can be
808 easily done by injecting only a part of the ¹D fractions into ²D. This has not yet been possible with on-
809 line loop interfaces, where so far the full content of the loop is injected into the ²D causing the need for
810 low flow rates in ¹D to reduce the injected volume.⁸²

811

812 **3. Future analytical prospects**

813

814 Sample preparation remains a crucial step before efficient GC \times GC analysis. One technique that has
815 potential to be used more for the analysis of polar compounds is derivatisation, which is a very common
816 approach in 1D GC but has been applied only a few times for the GC \times GC analysis of (converted)
817 biowastes.²⁹ Another sample pre-treatment approach is column chromatography, which has proven to
818 be useful to simplify the data interpretation or to enable the GC \times GC analysis of a specific part of the
819 sample.^{13,59} In the future, this could be performed using automated HPLC systems and connected in off-
820 line or on-line mode to the 2D separation, which could lead to the utilisation of 3D chromatography for
821 the analysis of complex biowastes.

822

823 As can be seen from Table 1, the main detectors used for the 2D chromatographic analysis of biowastes
824 are (HR)MS, UV-Vis, and FID. However, more specific detectors (SCD/FPD, NCD/NPD, ECD, etc.)
825 could also be beneficial for the characterisation of sulphur, nitrogen, or halogenated compounds in
826 converted biowastes. To our knowledge, since 2010, only the nitrogen chemiluminescence detector
827 (NCD) has been applied in the field of biowastes by analysing the wastewater of processed microalgae.⁸⁴
828 Another promising detector for the analysis of compounds with characteristic spectroscopic responses
829 is the vacuum ultraviolet absorbance detector (VUV) because the VUV spectral data is presumably
830 orthogonal to the typical GC separation (e.g., more than in the case of MS, where the molecular mass is
831 largely correlated to the elution order).⁸⁵

832

833 In our knowledge, another aspect that has not yet been studied is the combined 2D GC and 2D LC
834 analysis of one biowaste sample. Generally, the conversion of biowaste yields both an organic phase
835 and an aqueous wastewater phase, for which 2D GC and 2D LC could be respectively suitable. One
836 reason why this kind of complementary study is difficult to perform is the lack of easily implementable
837 orthogonal 2D LC systems. As it could be seen from the previous paragraphs, orthogonal 2D LC
838 instruments still require further developments, for example, the on-line setups in terms of interfaces to
839 overcome the issues of incompatible solvents, pressurised gas in SFC, etc. However, 2D LC has great
840 potential to be complementary to 2D GC for the analysis of low-volatility compounds, as these are still
841 largely unknown in (processed) biowaste samples.^{15,63}

842

843 **4. Conclusions**

844

845 In this review, the aspects of the 2D chromatographic analysis of valorisable biowastes of different
846 origins ranging from sewage sludge to algae residues and published since 2010 were discussed. Both

847 2D GC and 2D LC methods proved to be powerful separation and analysis techniques for the thorough
848 characterisation of (processed) biowaste samples, which can aid with the selection of valorisation routes
849 (production of biochemicals or biofuels) suitable for a specific material. Furthermore, the 2D plots
850 obtained from the analysis demonstrated to be useful for the monitoring of conversion and additional
851 treatment processes, such as purification, deoxygenation, and depolymerisation.

852
853 From the different 2D techniques, GC×GC is the most widely used as it enables quite easily to have
854 orthogonal separation and classification of chemical families. Although the conventional column setup
855 has shown a good overall separation, the reversed setup has shown advantages for the analysis of polar
856 compounds and the speciation of oxygenates separately from non-oxygenated compounds, which is
857 especially beneficial for biofuel analysis. Therefore, considering that the analytes in converted biowastes
858 have a wide range of polarity, the reversed setup is nowadays more and more preferred. Depending on
859 the state of the (converted) biowaste, it can also contain poorly volatile compounds, for which
860 derivatisation, high temperature GC×GC, or pyrolysis-GC×GC can be used to some extent. However,
861 for the analysis of polar, low volatile, and thermally unstable compounds, especially in aqueous phase,
862 the utilisation of 2D LC may be more suitable. The on-line RPLC×RPLC is the most straightforward to
863 use in terms of on-line configuration; however, because of the correlated dimensions, generally low
864 orthogonalities were obtained. The off-line 2D LC methods involving CPC, SEC, GPC, and SFC
865 demonstrated a good orthogonality with RPLC, and because of their easier implementation and (for
866 some setups) also organisation of chemical families, they can be used for the monitoring of complex
867 samples without the need for sophisticated instruments. In addition, the solvent incompatibility issue
868 can be avoided, which is an obstacle in orthogonal on-line 2D LC instruments. However, some possible
869 solutions for on-line configurations were demonstrated, which led to promising on-line NPLC×RPLC,
870 RPLC×SFC, and HILIC×RPLC setups with higher orthogonalities than RPLC×RPLC.

871
872 Overall, the reviewed studies demonstrated that two-dimensional chromatography is a powerful
873 technique for the analysis of valorisable biowaste and has the potential to be applied more for this aim
874 in the future.

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878 **Author contribution**

879 **Eliise Tammekivi:** Conceptualisation, Writing – original draft, Writing – review & editing. **Christophe**
880 **Geantet:** Conceptualisation, Writing – review & editing. **Chantal Lorentz:** Conceptualisation, Writing –
881 review & editing. **Karine Faure:** Conceptualisation, Funding acquisition, Writing – review & editing.

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883 **Declaration of competing interest**

884 The authors declare that they have no known competing financial interests or personal relationships that
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