Use of Carbohydrate, Protein and Fat to Characterise Wastewater in Terms of its Major Elemental Constituents and Energy

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Yao Yao

School of Chemical Engineering and Analytical Science

Contents

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Abstract

A method has been developed to determine the elemental composition, ThOD and heat of combustion of a simulated wastewater sample, based on the analysis of its carbohydrate, protein and fat components. According to the human dietary habits, the empirical contributions of carbohydrates, proteins and fats from selected types of food to the wastewater are considered. Their generic formulae are hereby calculated as $CH_{1.826}O_{0.913}$, $CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$ and $CH_{1.838}O_{0.118}$, respectively.

Carbohydrates are sub-grouped into mono-, di- and poly-saccharides and their heats of combustion are studied separately. Considering the constituents of the three groups in food, the overall heat of combustion of carbohydrates is calculated as 16.51 kJ g^{-1}. Furthermore, a relationship between functional groups in amino acids and the heats of combustion has been summarised and consequently a protein with its generic formula can be deconstructed as a combination of glycine and extra functional groups. The heat of combustion of protein is thus obtained as 18.20 kJ g^{-1}. Moreover, the linear relationship between heats of combustion and the number of carbons for both saturated and unsaturated fats are provided individually. The fat with its generic formula can be considered as a specific combination of saturated and unsaturated fats and the heat of combustion calculated as 38.93 kJ g^{-1}.

The generic formulae are applied to calculate the ThOD values of wastewater samples. The results are compared with the experimental measurements of COD and the results of calculated generic formulae previously reported[1]. It shows the generic formulae in this project are able to provide a closer estimation of the experimental results than the previous formulae, giving an average ratio of 0.96 compared with 0.84.

The heat of combustion of NESCAFÉ® Coffee-Mate Original has been measured experimentally and the energy value is also simulated by the overall heats of combustion of the three categories. A measurement after dissolving and drying samples is also conducted to mimic the experiment for wastewater sample. It is found the simulation only give 1.7% difference to the original measurement, while the dissolved-dried process significantly underestimates the energy value of the product because of the removal of volatile components and partial oxidation of the samples.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application or another degree or qualification of this or any other university or other institute of learning.

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About the Author

Yao Yao received bachelor degree of environmental engineering from Dalian polytechnic University (Liaoning, China) and was awarded outstanding graduates of Dalian in 2006.

She has got three-year work experience in China. After graduation, she worked as Laboratory Technician in the R&D department of Jinzhou New Century Quartz Glass Co., Ltd. After one year, she worked as Management Assistant in The Water-Diversion Project Office of Jinzhou Development Zone. After the project, she became a technological assistant in the Environmental Protection Office of Jinzhou development zone.

She travelled with her husband to the UK in 2009. After two years prepared of English study and culture adaption, she pursued an MPhil degree of Chemical Engineering at the School of Chemical Engineering and Analytical Sciences in the University of Manchester in 2012 with the supervision of Dr Alastair Martin.

During her MPhil study, she attended two academic conferences, which were ChemEngDay2013 in London and International Desalination Conference (IWA) 2013 in Qingdao(China), with research poster presentations. She also award the winner of 60 seconds of PhD in graduate school 2013.

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Chapter 1. Introduction

1.1 Wastewater treatment and its applications

Wastewater treatment is a technology which applies physical, chemical and biological methods to remove pollutants from water in order to reduce the pollution. Along with technological development, the goals of water treatment are not only to gain "fresh" water, but also recycle pollutants during the process.

As the source of fresh water is limited, it is desirable to reuse treated wastewater. Treated water has been widely applied in industries as coolant[2]. Municipal water discharged by wastewater treatment plants, which has achieved the reuse standard, can be used as landscape water or other non-drinking water applications[3]. Desalinated sea water is also another potential water source[4]. However, the expensive material cost and high power consumption are the key obstacles to expand its applications[5].

Sewage contains nitrogen and phosphate which are important components in fertilizers[6]. Baba *et al.* stated that the product yields of field using sludge as fertilizer has been increased by up to 20%[7]. In Sweden, more than 26% of sewage sludge was used as a fertilizer on farmland in 2008[8]. In Japan, it has been used as a fertilizer for a long time with no harm to human health[9]. Although some reports regarded the introduction of heavy metals with sludge to soil as a potential hazard, research has shown that small amount of metals based on N in agricultural system has no negative environmental effect on sugar cane in southern tropical soil[10].

1.2 Energy in wastewater

Historically, methane has been produced from human excreta in digestion tanks. This is still an important source of domestic fuel in some developing countries[11]. Modern technology enables energy generation for wastewater treatment. Sludge from biological wastewater treatment is also a good raw material for methane or hydrogen generation, which are bio-fuel and renewable energy.

McCarty *et al*. compared modern wastewater treatment technologies in domestic wastewater, such as microbial fuel cells (MFCs), chemical fuel cells (CFCs), aerobic and anaerobic conversions, etc. and concluded that the anaerobic treatment has the most promising prospect for capturing organic energy from wastewater. To improve the performance of the anaerobic treatment, raising the production efficacy and reducing the energy consumption are the two targets. Furthermore, increasing the methane yield, decreasing the hydrogen sulfide and reusing are the main research fields in the anaerobic method[12].

Frijns *et al*. offered the prospects of municipal wastewater as a potential chemical and thermal energy carrier[13]. Organic components are recovered to biogas and the chemical energy is retrieved by the optimisation of sludge digestion. Warm domestic wastewater is also applied as a thermal resource in which energy is recovered through heat exchange.

Logan and Elimelech reviewed the membrane processes applied for energy recovery from wastewater[14]. Through microbial fuel-cell technology, organic matters in wastewater are utilised as a source to generate electricity.

Shizas *et al*. used bomb calorimeter to test oven-dried raw municipal wastewater to analyse its energy content[15]. The raw wastewater contained energy from 3.2 kJ g^{-1} dry weight, while the primary, secondary and anaerobically digested sludges possess 15.9, 12.4 and 12.7 kJ g^{-1} dry weights, respectively. Similar energy content values were also reported in Zanoni and Mueller's[16] and Vesilind and Ramsey's reports[17]. Oven-drying inevitably causes the losses of volatile components, such as acetic acid and propanoic acid. Hence the resulting energy content from this method may be considerably lower than the actual value.

Heidrich *et al*.[18] used freeze-dried wastewater samples to obtain the energy of 16.8 kJ L^{-1}, which is nearly 20% higher than that from Shizas' measurements[15]. Heidrich's method maintains the complete organic matter in original wastewater so the results were believed to be closer to the actual value. However, common freeze-drying method takes weeks before collecting enough solid samples for further analysis, which does not fulfill the requirements of engineering application.

1.3 Major contents of domestic wastewater

Domestic wastewater comes from the human activities which may include diet, washing, excretion, etc. The pollutants in the water are complex due to the various sources and production processes, but researches showed the major components of domestic wastewater are proteins, fats and carbohydrates.

Liu and Liptak demonstrated and summarised the characteristics and sources of wastewater to indicate the complexity[19]. As shown in Table 1, most of typical organic compounds are found in domestic sewage, such as carbohydrates, fats, oils, greases, proteins, etc. It shows that domestic sewage is the most complex in comparison with industrial, commercial, agricultural and surface waters.

Other researchers[20-22] showed that proteins, fats and carbohydrates are the major organic contents of municipal wastewater. Heukelekian and Balmat determined the COD of municipal wastewater to be composed of 31% proteins, 16% carbohydrates and 45% lipids[20]. Raunkjaer *et al.* also demonstrated that the pollutants from their wastewater samples were composed from 28% proteins, 18% carbohydrates and 31% lipids[21]. Furthermore, Sophonsiri and Morgenroth quantitatively analysed COD values, proteins, carbohydrates and lipids contents in municipal, industral and agricultural wastewater and found after primary sedimentation, proteins, carbohydrates and lipids are the major three contributions to COD in municipal wastewater ranging from 92.6% to 99.9%[22].

Huang *et al.* applied neutral detergent method and GC-MS to identify the organic composition in domestic wastewater and found the major components in domestic wastewater are fibres, proteins and sugars, with TOC of 20.64%, 12.38% and 10.65% respectively[23]. In their research, the samples were collected at a sewage collection station. The raw sewage without screening or sedimentation could be the most possible samples in their study, which contains biomass and excrement with fibres. This explanation was supported that DNA and RNA are also detected with noticeable contents in their samples. If the biological active matters are omitted in Huang’s report, the major parts of wastewater are still proteins, carbohydrates and lipids, which are in accordance with the comments concluded from other studies.

Table 1 Chemical components and sources of wastewater[19].

	Domestic	Industrial	Commercial	Agricultural	Surface-water
Carbohydrates	√	√	√	—	—
Lipids	√	√	√	—	—
Pesticides	—	—	—	√	—
Phenols	—	√	—	—	—
Proteins	√	√	√	—	—
Priority pollutants	√	√	√	—	—
Surfactants	√	√	√	—	—
VOCs	√	√	√	—	—
Alkalinities	√	—	—	—	√
Chlorides	√	—	—	—	√
Heavy metals	—	√	—	—	—
Nitrogen	√	—	—	√	—
pH	√	√	√	—	—
Phosphorus	√	√	√	—	√
Priority pollutants	√	√	√	—	—
Sulphur	√	√	√	—	—
Hydrogen sulphide	√	—	—	—	—
Methane	√	—	—	—	—
Oxygen	√	—	—	—	√

1.4 Compositional analysis of wastewater and generic formulae of carbohydrates, fats and proteins

Before making fully use of wastewater, the preliminary job for engineers is to analyse its composition comprehensively. The selection of parameters, which reflects the information of raw wastewater and suggests the treatment process, is crucial for treatment unit design. Conventional parameters including COD, BOD, suspended solids (SS), TOC, etc. are the most important in this research area, evaluating pollutants in the water quantitatively. However, these parameters exhibit only a specific chemical property of raw wastewater, but not the actual components from the viewpoint of energy retrieval. Parameters like COD and TOC cannot unveil energies contained in them either. Another disadvantage of the conventional evaluation is its time consuming nature. Accomplishing a complete analysis of one wastewater sample from a plant requires a week or so, which invalidates the measurement as the components of wastewater normally change from day to day. Therefore, engineers opt to use generic formulae to reflect instant and reliable properties of raw wastewater, which enables them to evaluate the mass and energy balance more accurately. It is convenient to determine the contents of carbohydrates, proteins and fats dissolved and suspended in wastewater as well as in sediment. These nine measurements collectively can provide comprehensive information of the composition of wastewater, which is fundamental to the conduct of mass and energy balance calculations in wastewater. Moreover, fewer sample collections are required for the fast determination than the traditional method. Consequently the test cost can be significantly reduced.

Carbohydrate is the generic name of a classical chemicals which is polyhydric alcohol containing three elements, carbon, hydrogen and oxygen, and in food their ratio is approximately 1:2:1. Hence the generic formula of carbohydrate is written as $(CH_2O)_n$. It is classified into three groups, monosaccharides, disaccharides and polysaccharides. Two monosaccharide molecules connect each other to form disaccharides by inter-molecular loss of water. Solid sugar is always in open-chain form, while in solution it will form a ring structure by aldol condensation[24].

It is observed from Table 2, the generic formula of monosaccharides is $C_n(H_2O)_m$, which is slightly different from the original formula $(CH_2O)_n$. Henze *et al.* demonstrated the average formulae of carbohydrate is $C_{10}H_{18}O_9$, which is alternatively rewritten as $C_{10}(H_2O)_9$. The result agrees to $C_n(H_2O)_m$ formula with n = 10 and m = 9[1].

Fats are a group of organic chemicals which are made of fatty acids and glycerols. Fatty acid is a carboxylic acid containing long aliphatic chains. There are around 20 common fatty acids in food which consist of saturated and unsaturated acids, as shown in Table 3. The only criterion between them is whether the aliphatic tail contains an unsaturated bond. The generic formula of saturated fatty acids is $C_nH_{2n+1}COOH$. As the development of food science, the components of fats are conveniently found from references[24]. Henze *et al*. has summarised a generic formula for fats and oils as $C_8H_6O_2$[1].

Table 2. Formulae, H/C and O/C ratios of mono-, di- and trisaccharides[25].

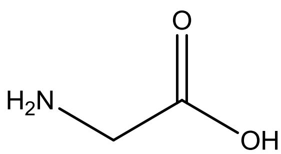
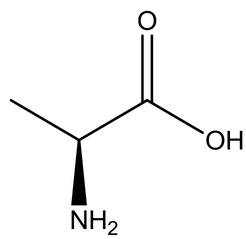
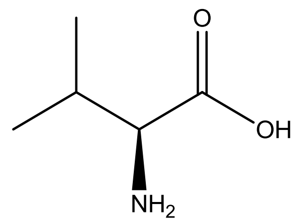
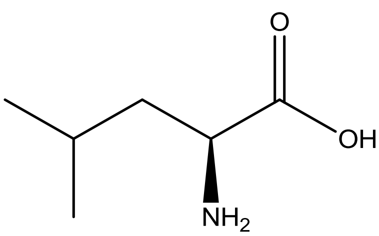
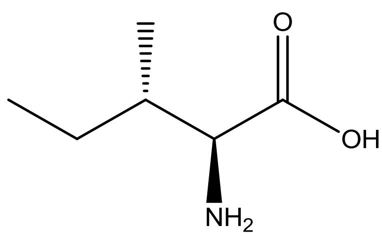
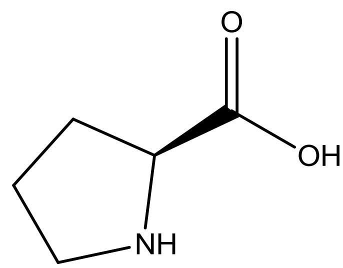
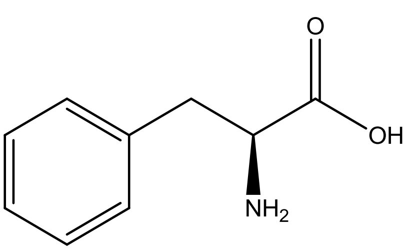
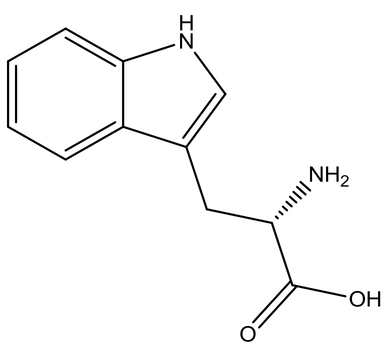
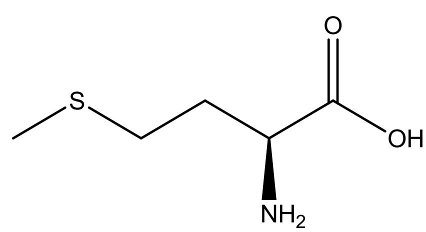
	Carbohydrate	Formula	H/C	O/C
Monosaccharide	Glyceraldehyde	$C_3H_6O_3$	2.000	1.000
	Threose	$C_4H_8O_4$	2.000	1.000
	Xylose	$C_5H_{10}O_5$	2.000	1.000
	Glucose	$C_6H_{12}O_6$	2.000	1.000
Disaccharide	Sucrose	$C_{12}H_{22}O_{11}$	1.833	0.917

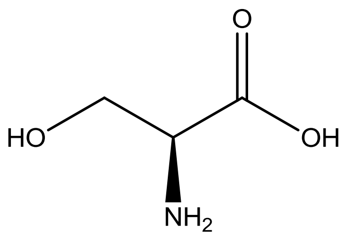
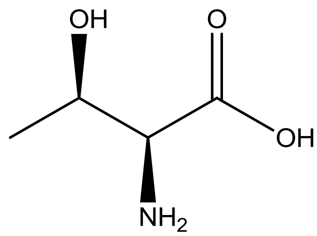
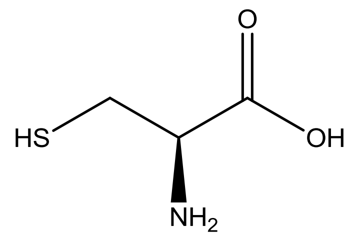
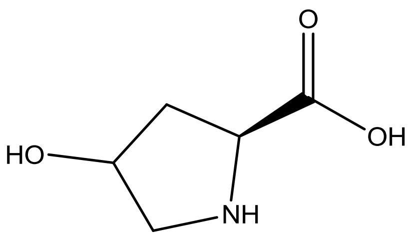
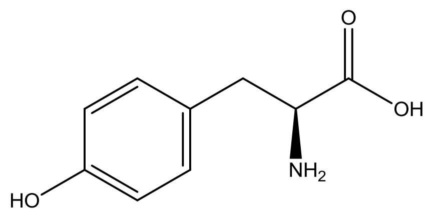
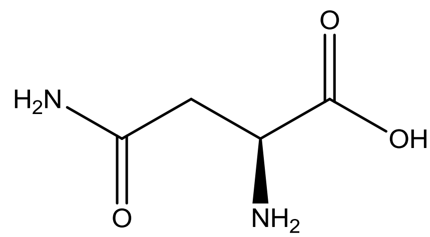
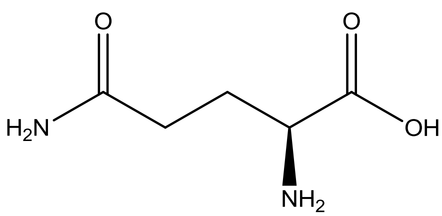
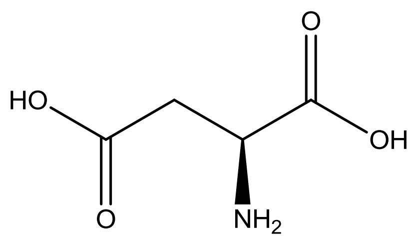
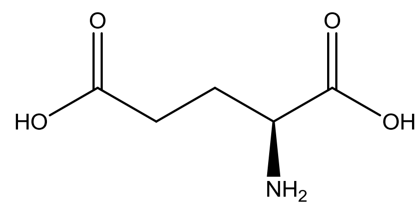
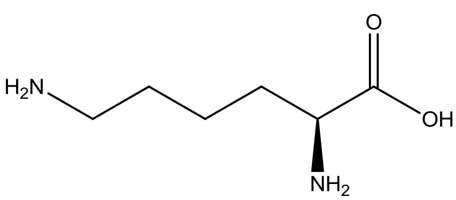
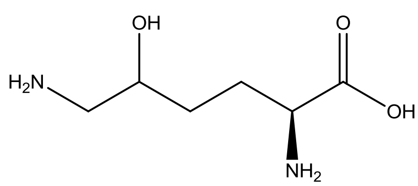
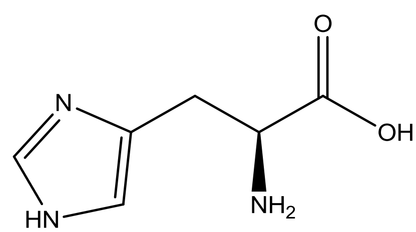
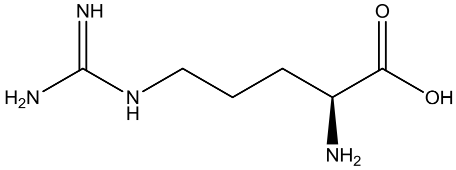
Table 3 Common fatty acids in food[24].

Saturated acids		Unsaturated acids	
Common names (lipid numbers)	Chemical formula	Common names (lipid numbers)	Chemical formula
Butyric acid (C4:0)	$C_4H_8O_2$	Palmitoleic acid (C16:1, cis-9)	$C_{16}H_{30}O_2$
Caproic acid (C6:0)	$C_6H_{12}O_2$	Oleic acid (C18:1, cis-9)	$C_{18}H_{34}O_2$
Caprylic acid (C8:0)	$C_8H_{16}O_2$	Linoleic acid (C18:2, cis,cis-9,12)	$C_{18}H_{32}O_2$
Capric acid (C10:0)	$C_{10}H_{20}O_2$	α-Linolenic acid (C18:3, cis,cis,cis-9,12,15)	$C_{18}H_{30}O_2$
Lauric acid (C12:0)	$C_{12}H_{24}O_2$	γ-Linolenic acid (C18:3, cis,cis,cis-6,9,12)	$C_{18}H_{30}O_2$
Myristic acid (C14:0)	$C_{14}H_{28}O_2$	Arachidonic acid (C20:4, cis,cis,cis,cis-5,8,11,14)	$C_{20}H_{32}O_2$
Palmitic acid (C16:0)	$C_{16}H_{32}O_2$	EPA (C20:5, cis,cis,cis,cis,cis-5,8,11,14,17)	$C_{20}H_{30}O_2$
Stearic acid (C18:0)	$C_{18}H_{36}O_2$	DPA (C22:5, cis,cis,cis,cis,cis-7,10,13,16,19)	$C_{22}H_{34}O_2$
Arachidic acid (C20:0)	$C_{20}H_{40}O_2$	DHA (C22:6, cis,cis,cis,cis,cis,cis-4,7,10,13,16,19)	$C_{22}H_{32}O_2$
Behenic acid (C22:0)	$C_{22}H_{44}O_2$		

Protein possesses large molecular weight and highly complex structures. Its functional group is made of amino acids which include a central carbon, a side-chain (R), an amino and a carboxyl groups (Table 4). The generic formula is written as $H_2NCHRCOOH$ or $(NH_3)^+$ $CHR(COO)^-$. Peptide bonds, which are formed by inter-molecular dehydration condensation between amino and carboxyl groups, build the elementary scaffold of a protein[24]. It is challenging to give a generic formula of all proteins due to their structural complexity and elemental variety. For some amino acids, the generic formula could be written in form of $C_{\alpha}H_{\beta}O_{\gamma}N_{\delta}S_{\varepsilon}$. An empirical generic formula of proteins given by Henze *et al*. is $C_{14}H_{12}O_{7}N_{2}$[1].

Table 4 Structures and chemical formulae of common amino acids.

Structure Name Chemical formula	Structure Name Chemical formula	Structure Name Chemical formula
 Glycine $C_2H_5O_2N$	 L-alanine $C_3H_7O_2N$	 L-valine $C_5H_{11}O_2N$
 L-leucine $C_6H_{13}O_2N$	 L-isoleucine $C_6H_{13}O_2N$	 L-proline $C_5H_9O_2N$
 L-phenylalanine $C_9H_{11}O_2N$	 L-tryptophan $C_{11}H_{12}O_2\ N_2$	 L-methionine $C_5H_{11}O_2NS$

Structure Name Chemical formula	Structure Name Chemical formula	Structure Name Chemical formula
 L-serine $C_3H_7O_3N$	 L-threonine $C_4H_9O_3N$	 L-cysteine $C_3H_7O_2NS$
 L-4-hydroxy-proline $C_5H_9O_3N$	 L-tyrosine $C_9H_{11}O_3N$	 L-asparagine $C_4H_8O_3\ N_2$
 L-glutamine $C_5H_{10}O_3\ N_2$	 L-aspartic acid $C_4H_7O_4N$	 L-glutamic acid $C_5H_9O_4N$
 L-lysine $C_6H_{14}O_2\ N_2$	 L-5-hydroxy-lysine $C_6H_{14}O_3\ N_2$	 L-histidine $C_6H_9O_2\ N_3$
 L-arginine $C_6H_{14}O_2\ N_4$		

1.5 Objectives

The study will attempt to summarise the chemical formulae of typical chemicals in carbohydrates, proteins and fats in food individually. When combined with their published proportion in food [26], the generic formulae of the three categories are derived. Using the heats of combustion of typical compounds and their proportions, the general heat of combustion of the three categories will be calculated.

The generic formulae and the heat of combustion based on food can be considered as the properties of organic components in wastewater on the assumption that food is the dominant source of the components in wastewater.

To verify these results, ThOD values of selected domestic wastewater samples will be calculated based on the generic formulae and compared with reported experimental measurements[21] and the formulae model proposed by Henze *et al*[1]. Moreover, the heat of combustion of NESCAFÉ® Coffee-Mate Original will be measured experimentally. It also will be compared with the predicted value based on the generic formulae and the overall heat of combustion of carbohydrates, proteins and fats and their proportions in the product.

Chapter 2. Induction of generic formulae

The generic formulae of carbohydrates, proteins and fats are worked out using different approaches, depending on the structural similarity within each category. Based on the degree of polymerisation (DP), carbohydrates are divided into three groups, of which the formulae are analysed separately. The generic formula of carbohydrates is then calculated by multiplying the individual formulae of three groups by their contents in major foods.

The contents of 18 essential amino acids in 22 foods are extensively studied. The molar fractions of amino acids in each food is calculated based on experimental measurements listed in ref [26]. According to the element ratios of amino acids, the ratios of each food from amino acids are hence calculated. The generic formulae of proteins is obtained by taking the average.

The formulae of fatty acids are complemented by a small correction, which is one-third of glycerol piece in a glyceride molecule. After calculating the molar fractions of fatty acids in selected food, the element ratios of each food are studied and their contributions of fat to wastewater are estimated. As a consequent, the generic formula of fat is calculated by multiplying the element ratios of foods by their contribution percentages.

2.1 Carbohydrates

Carbohydrates are undoubtedly one of the most important nutrients and the major energy carriers for human activities. The term is derived from their empirical formula $C_m(H_2O)_n$ (both m and n are integers and are either equal or different values) while depending on DP, they are structurally classified as three major chemical groups: monosaccharides, disaccharides and polysaccharides. The latter two groups are products of various condensations or polymerizations of the former one. The most usual sugars, common examples of monosaccarides and disaccharides, are glucose and sucrose, with molecular formula of $C_6H_{12}O_6$ and $C_{12}H_{22}O_{11}$, respectively. Glucose is also the monomer of typical polysaccharides $(C_6H_{10}O_5)_n$, such as starch and cellulose.

It is observed from Table 1 (Page 13) The H/C and O/C ratios of monosaccharides are constant but decline as the DP develops due to the dehydration condensations between glucose units.

Therefore, a linear relationship between the numbers of H (n_H) and C (n_C) in a polysaccharide molecule is built up as

$$\mathbf{n_H = \frac{5}{3} n_C + 2} \quad (1)$$

Consequently, the relationship between the H/C ratio ($r_{H/C}$) and the number of carbon is obtained by dividing n_C on both hands of eq. 1.

$$\mathbf{r_{H/C} = \frac{n_H}{n_C} = \frac{5}{3} + \frac{2}{n_C}} \quad (2)$$

Considering DP of polysaccharides ranging from 40 to 3000[27], the average H/C ratio ($\bar{r}_{H/C}$) is calculated from a definite integral of eq. 2 divided by a definite integral of unity to n_C. Both of the definite integrals have the identical interval [40, 3000].

$$\mathbf{\bar{r}_{H/C} = \frac{\int_{40}^{3000} \frac{5}{3}+\frac{2}{n_C}\, dn_C}{\int_{40}^{3000} 1\, dn_C} = \frac{(\frac{5}{3}n_C+2\ln n_C)|_{40}^{3000}}{n_C|_{40}^{3000}} = \frac{5}{3} + \frac{2(\ln 3000-\ln 40)}{3000-40} = 1.670} \quad (3)$$

The average O/C ratio of polysaccharides is calculated through the same approach, which is 0.835. Alternatively, it is also able to obtain this ratio by simply halving the $\bar{r}_{H/C}$ as the number of H is always twice as many as the number of O in carbohydrate formulae.

According to McCance and Widdowson's analysis on the composition of food[26], ten categories of food contribute carbohydrates predominantly to the human diet. The partially digested residues are transferred to municipal wastewater due to partial digest. They are grouped as grain, milk, alcohol, vegetable, fruit, nut, sugar and confectionery, sauce, soup and others. The former five are considered as the major contributors (Class A) of carbohydrates due to the dietary habits of human beings while the remaining five are regarded as minor contributors (Class B). The sugar (mono- and disaccharides) and polysaccharides (starch and dietary fibers) contents of all the categories of food are listed in Table 5 and illustrated in Figure 1. Empirical percentages of carbohydrate contributions from both classes are allocated, which are 80.0% and 20.0%, respectively. For simplicity, individual category of food in a class has the equivalent contribution to municipal wastewater ($c_{m,a}$ and $c_{n,b}$)and mono- and disaccharides have the same proportion in sugar content. As shown in Table 5, the total percentages of sugars and polysaccharides in wastewater are calculated based on the equations below:

$$\mathbf{c_{t,sugar}}\% = \sum_{\mathbf{A}} \mathbf{c_{m,A}} \times \mathbf{c_{m,suger}} + \sum_{\mathbf{B}} \mathbf{c_{n,B}} \times \mathbf{c_{n,suger}} \tag{4}$$

$$\mathbf{c_{t,polysaccharide}}\% = \sum_{\mathbf{A}} \mathbf{c_{m,A}} \times \mathbf{c_{m,polysaccharide}} + \sum_{\mathbf{B}} \mathbf{c_{n,B}} \times \mathbf{c_{n,polysaccharide}} \tag{5}$$

Table 5. Sugar and polysaccharide proportions in various types of food and the calculation of their contents in wastewater. (approximate percentage data retrieved and summarised from reference [26])

		Empirical percentages of carbohydrate contribution	Sugar contents (mono- and disaccharides)	polysaccharide contents (starch and dietary fibers)	Relative sugar contents	Relative polysaccharide contents
Class A	grain	16.0%	10.0%	90.0%	1.6%	14.4%
Total content: 80.0%	milk	16.0%	100.0%	0.0%	16.0%	0.0%
	alcohol	16.0%	100.0%	0.0%	16.0%	0.0%
	vegetable	16.0%	50.0%	50.0%	8.0%	8.0%
	fruit	16.0%	75.0%	25.0%	12.0%	4.0%
Class B	nut	4.0%	30.0%	70.0%	1.2%	2.8%
Total content: 20.0%	sugar and confectionery	4.0%	95.0%	5.0%	3.8%	0.2%
	sauce	4.0%	80.0%	20.0%	3.2%	0.8%
	soup	4.0%	40.0%	60.0%	1.6%	2.4%
	others	4.0%	0.0%	100.0%	0.0%	4.0%
				Total percentage:	63.4%	36.6%

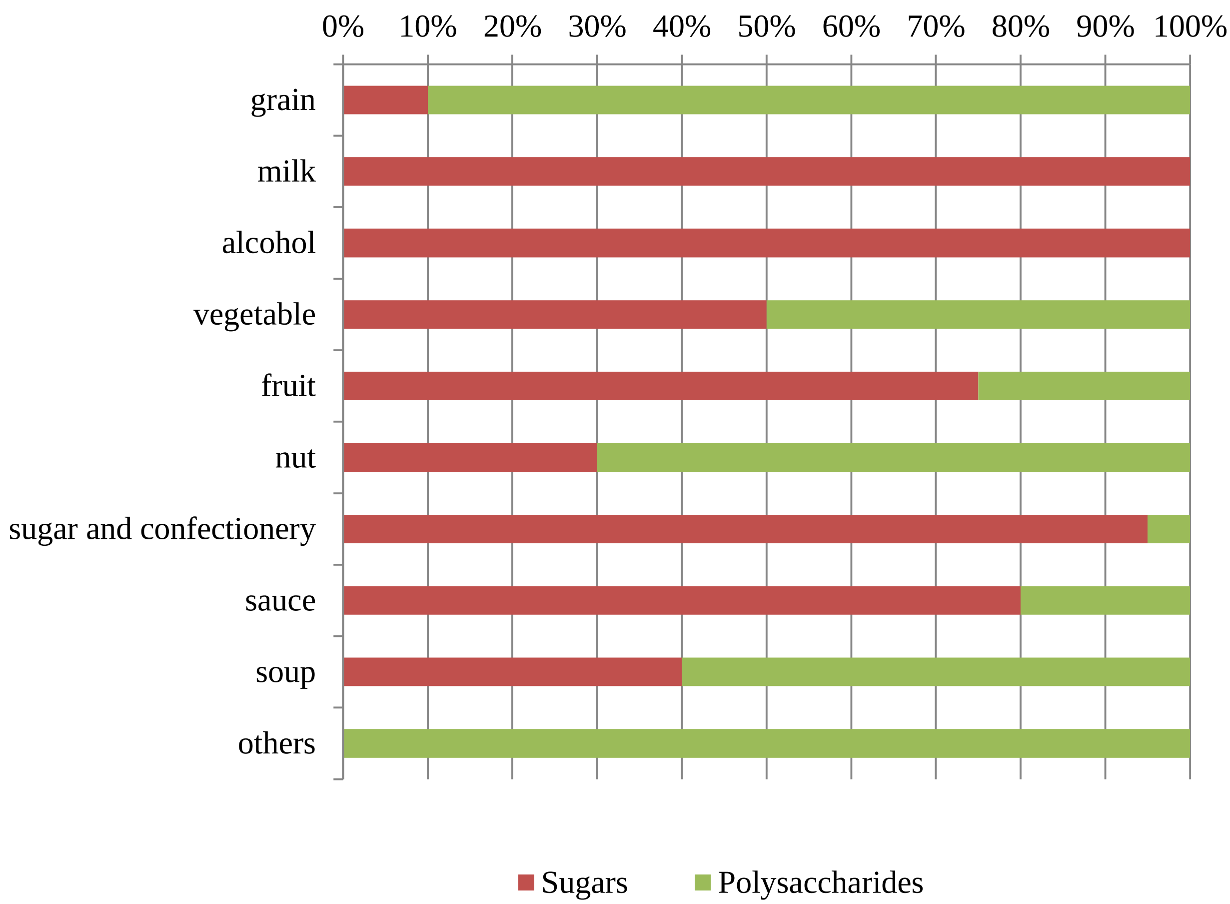


Figure 1 Sugars and polysaccharides proportions of various categories of food.

Combining with the H/C and O/C ratios of mono-, di- and polysaccharides, the overall ratio of carbohydrates in municipal wastewater are calculated, as shown in Table 6. As a consequence, the generic formula of carbohydrate is $CH_{1.826}O_{0.913}$.

Table 6. Relative H/C and O/C ratios of mono-, di- and polysaccharides.

	H/C	O/C	Content in wastewater	relative H/C	relative O/C
Monosaccharide	2.000	1.000	31.7%	0.634	0.317
Disaccharide	1.833	0.917	31.7%	0.581	0.291
Polysaccharide	1.670	0.835	36.6%	0.611	0.306
			Total ratio:	1.826	0.913

2.2 Proteins

Due to the structural varieties of proteins, the classification method applied to carbohydrates is not valid. Alternatively, the generic formulae of proteins are derived from their building-blocks, amino acids. In this project, 18 common amino acids, including 9 essential amino acids, are examined. Their H/C, O/C, N/C and S/C are listed in Table 7.

Table 7 Formulae, abbreviations and element ratios of common amino acids.

Name	Formula	Abbr.	H/C	O/C	N/C	S/C
L-Alanine	$C_3H_7O_2N$	Ala	2.333	0.667	0.333	0.000
L-Arginine	$C_6H_{14}O_2N_4$	Arg	2.333	0.333	0.667	0.000
L-Aspartic acid	$C_4H_7O_4N$	Asp	1.750	1.000	0.250	0.000
L-Cysteine	$C_3H_{12}O_2NS$	Cys	4.000	0.667	0.333	0.333
L-Glutamic acid	$C_5H_9O_4N$	Glu	1.800	0.800	0.200	0.000
Glycine	$C_2H_5O_2N$	Gly	2.500	1.000	0.500	0.000
L-Histidine	$C_6H_9O_2N_3$	His	1.500	0.333	0.500	0.000
L-Isoleucine	$C_6H_{13}O_2N$	Ile	2.167	0.333	0.167	0.000
L-Leucine	$C_6H_{13}O_2N$	Leu	2.167	0.333	0.167	0.000
L-Lysine	$C_6H_{14}O_2N_2$	Lys	2.333	0.333	0.333	0.000
L-Methionine	$C_5H_{11}O_2NS$	Met	2.200	0.400	0.200	0.200
L-Phenylalanine	$C_9H_{11}O_2N$	Phe	1.222	0.222	0.111	0.000
L-proline	$C_5H_9O_2N$	Pro	1.800	0.400	0.200	0.000
L-Serine	$C_3H_7O_3N$	Ser	2.333	1.000	0.333	0.000
L-Threonine	$C_4H_9O_3N$	Thr	2.250	0.750	0.250	0.000
L-Tryptophan	$C_{11}H_{12}O_2N_2$	Trp	1.091	0.182	0.182	0.000
L-Tyrosine	$C_9H_{11}O_3N$	Tyr	1.222	0.333	0.111	0.000
L-Valine	$C_5H_{11}O_2N$	Val	2.200	0.400	0.200	0.000

According to the component analysis of food, cereals, eggs, meat, seafood and vegetables are the major contributors of protein to human diet and the domestic wastewater. Typical foods from these categories are extensively selected. The mass contents of examined amino acids in these foods are retrieved from the McCance and Widdowson's handbook[26], which are further converted into molar fractions by dividing the molecular weights of amino acids, as shown in Table 9. Combining the individual H/C ratio of all amino acids, the H/C ratio of each food is calculated, following the equation below:

$$\mathbf{H/C\,(food)} = \sum_{\mathbf{i}} \mathbf{n(i)} * \mathbf{H/C(i)} \tag{6}$$

where n(i) is the molar content of the i^{th} amino acid in the food and H/C(i) is the H/C ratio of this amino acid. Similarly, the O/C, N/C and S/C ratios of food are calculated by replacing the corresponding elementary ratios of amino acids. (Table 10)

Consequently, the generic element ratios of proteins in food are worked out by averaging those of various types of food, as shown in Table 8. Statistical analysis indicates that all the average ratios but the S/C come with a small standard deviations. However, the small confidence interval of the S/C ratio still supports the average is accurate and reliable. Therefore, the generic formula of proteins is written as $CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$.

Table 8 Calculation of the generic formula of proteins and the error analysis.

	H/C	O/C	N/C	S/C
Number of Samples	22	22	22	22
Average	2.063	0.626	0.282	0.008
Standard Deviation	0.029	0.018	0.016	0.002
Coefficient of Variance	0.014	0.029	0.057	0.250
Confidence Interval (95.0%)	0.0137	0.0083	0.0073	0.0010

Table 9 Molar fractions of common amino acids in selected foods (calculation based on data in ref [26]).

Name	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
barley pearl	0.065	0.038	0.058	0.026	0.222	0.071	0.019	0.037	0.071	0.024	0.010	0.043	0.131	0.053	0.039	0.012	0.023	0.059
bran wheat	0.082	0.058	0.080	0.029	0.181	0.111	0.025	0.033	0.065	0.038	0.009	0.033	0.070	0.059	0.038	0.009	0.023	0.055
cornflour maize	0.110	0.031	0.061	0.017	0.168	0.064	0.023	0.037	0.124	0.024	0.011	0.039	0.102	0.062	0.040	0.004	0.028	0.054
wholemeal	0.056	0.036	0.051	0.029	0.254	0.073	0.018	0.035	0.070	0.022	0.010	0.037	0.125	0.069	0.031	0.008	0.023	0.052
brown flour	0.045	0.032	0.043	0.028	0.290	0.056	0.018	0.034	0.068	0.020	0.010	0.036	0.141	0.070	0.030	0.008	0.022	0.049
white flour	0.045	0.026	0.043	0.028	0.294	0.056	0.018	0.038	0.070	0.017	0.009	0.038	0.144	0.070	0.030	0.008	0.019	0.048
beef	0.089	0.048	0.089	0.013	0.145	0.092	0.029	0.048	0.075	0.077	0.015	0.033	0.055	0.053	0.048	0.008	0.026	0.056
lamb	0.085	0.046	0.090	0.014	0.150	0.087	0.027	0.047	0.072	0.088	0.015	0.031	0.053	0.054	0.051	0.009	0.026	0.054
pork	0.081	0.045	0.089	0.014	0.144	0.093	0.037	0.045	0.071	0.087	0.016	0.031	0.055	0.053	0.048	0.008	0.027	0.054
chichen	0.087	0.048	0.092	0.014	0.150	0.089	0.026	0.048	0.077	0.082	0.015	0.036	0.049	0.051	0.047	0.008	0.026	0.055
white and fatty fish	0.097	0.046	0.099	0.012	0.130	0.078	0.023	0.051	0.082	0.084	0.016	0.032	0.046	0.060	0.051	0.007	0.024	0.062
crustacea	0.094	0.059	0.101	0.013	0.132	0.108	0.015	0.044	0.082	0.067	0.016	0.030	0.047	0.060	0.048	0.007	0.025	0.051
molluscs	0.081	0.056	0.108	0.017	0.123	0.088	0.020	0.047	0.075	0.070	0.016	0.032	0.047	0.063	0.050	0.009	0.030	0.069
potatoes	0.060	0.042	0.202	0.015	0.127	0.065	0.018	0.046	0.068	0.054	0.011	0.038	0.049	0.058	0.047	0.011	0.025	0.064
tomatoes	0.085	0.038	0.272	0.017	0.000	0.074	0.029	0.046	0.065	0.062	0.009	0.033	0.044	0.077	0.059	0.013	0.022	0.056
peas	0.066	0.077	0.117	0.013	0.155	0.075	0.020	0.047	0.074	0.073	0.006	0.040	0.047	0.058	0.047	0.007	0.021	0.056
mushrooms	0.118	0.077	0.076	0.015	0.108	0.077	0.019	0.039	0.063	0.069	0.015	0.028	0.101	0.059	0.052	0.011	0.024	0.049
cabbage	0.102	0.085	0.087	0.016	0.104	0.113	0.029	0.041	0.071	0.037	0.008	0.033	0.057	0.070	0.055	0.009	0.019	0.063
lettuce	0.079	0.042	0.140	0.000	0.113	0.090	0.017	0.047	0.077	0.043	0.013	0.050	0.074	0.052	0.057	0.007	0.024	0.075
almonds	0.062	0.080	0.101	0.017	0.213	0.101	0.021	0.038	0.068	0.022	0.008	0.042	0.060	0.048	0.029	0.006	0.023	0.062
peanuts	0.060	0.090	0.119	0.015	0.173	0.104	0.022	0.036	0.068	0.034	0.007	0.042	0.052	0.064	0.030	0.008	0.030	0.049
eggs	0.079	0.045	0.105	0.019	0.106	0.053	0.020	0.055	0.082	0.055	0.019	0.040	0.043	0.097	0.056	0.012	0.029	0.083

Table 10 Element ratios contributed from common amino acids in selected food (calculation based on data in ref [26]).

Name	H/C	O/C	N/C	S/C
barley pearl	2.020	0.609	0.261	0.009
bran wheat	2.086	0.639	0.292	0.011
cornflour maize	2.042	0.599	0.262	0.007
wholemeal	2.029	0.626	0.262	0.011
brown flour	2.006	0.625	0.253	0.010
white flour	2.004	0.625	0.249	0.010
beef	2.058	0.611	0.285	0.006
lamb	2.062	0.612	0.284	0.006
pork	2.056	0.609	0.287	0.006
chichen	2.059	0.611	0.284	0.006
white and fatty fish	2.067	0.609	0.282	0.005
crustacea	2.082	0.630	0.292	0.006
molluscs	2.071	0.617	0.285	0.007
potatoes	2.014	0.654	0.271	0.006
tomatoes	2.046	0.673	0.287	0.006
peas	2.058	0.619	0.290	0.005
mushrooms	2.080	0.594	0.296	0.006
cabbage	2.108	0.627	0.310	0.006
lettuce	2.008	0.625	0.272	0.001
almonds	2.038	0.639	0.290	0.006
peanuts	2.043	0.642	0.299	0.006
eggs	2.061	0.608	0.271	0.008

2.3 Fats

The generic formula of fats is derived using a combination of the methods applied to carbohydrates and proteins. The structural varieties of fatty acids result that some fat contributors are selected as samples. On the other hand, according to human dietary habits, the contributions from various types of foods are noticeably distinct. Therefore, the generic formula of fat in food is a weighted average of the generic formulae in each food.

Figure 2 shows an introductory formation of fat from fatty acids and glycerol. The generic formula of fats is simply divided into three pieces, each of which consists of the formulae of fatty acid and the one-third of glycerol remains after the “formation”, $CH_{0.667}$.(Table 11) The corrected $(H/C)_c$ and $(O/C)_c$ of fatty acids are able to represent the elementary ratio in fat.

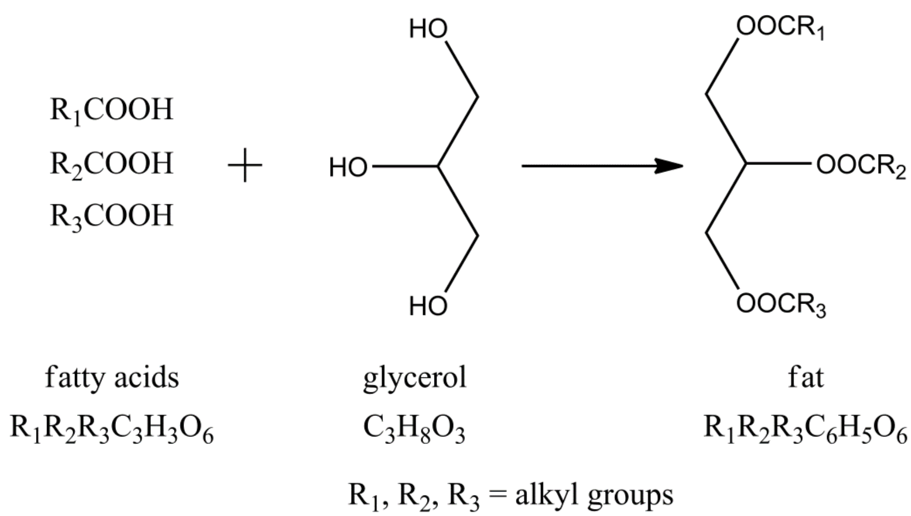


Figure 2 Generic triglyceridisation of carboxylic acids with glycerol.

According to ref [26], dairy products, eggs, meats, seafood and cooking oils are the major contributors of fat to food, as well as wastewater. The molar fractions of various fatty acids in selected food types are calculated based on the data in ref [26].(Table 12) Multiplying them by the corrected $(H/C)_c$ and $(O/C)_c$ of each fatty acid and summing the products, the H/C and O/C ratios are worked out, as listed in Table 13. Assigning the empirical contents of each food, the fractional H/C and O/C ratios are calculated and the summations lead to the generic H/C and O/C ratios in food. So it’s concluded that the generic formula of fat is $CH_{1.838}O_{0.118}$.

Table 11 Corrected formulae and element ratios of selected fatty acids.

Name	Abbr.	Fomula	M.W. /g mol^{-1}	C	H	O	C_c	H_c	O_c	$(H/C)_c$	$(O/C)_c$
Butyric acid	C4:0	$C_4H_8O_2$	88.11	4	8	2	5	8.667	2	1.733	0.400
Caproic acid	C6:0	$C_6H_{12}O_2$	116.16	6	12	2	7	12.667	2	1.810	0.286
Caprylic acid	C8:0	$C_8H_{16}O_2$	144.21	8	16	2	9	16.667	2	1.852	0.222
Capric acid	C10:0	$C_{10}H_{20}O_2$	172.26	10	20	2	11	20.667	2	1.879	0.182
Lauric acid	C12:0	$C_{12}H_{24}O_2$	200.32	12	24	2	13	24.667	2	1.897	0.154
Myristic acid	C14:0	$C_{14}H_{28}O_2$	228.37	14	28	2	15	28.667	2	1.911	0.133
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.42	16	32	2	17	32.667	2	1.922	0.118
Stearic acid	C18:0	$C_{18}H_{36}O_2$	284.48	18	36	2	19	36.667	2	1.930	0.105
Arachidic acid	C20:0	$C_{20}H_{40}O_2$	312.53	20	40	2	21	40.667	2	1.937	0.095
Behenic acid	C22:0	$C_{22}H_{44}O_2$	340.58	22	44	2	23	44.667	2	1.942	0.087
Palmitoleic acid	C16:1	$C_{16}H_{30}O_2$	254.41	16	30	2	17	30.667	2	1.804	0.118
Oleic acid	C18:1	$C_{18}H_{34}O_2$	282.46	18	34	2	19	34.667	2	1.825	0.105
Linoleic acid	C18:2	$C_{18}H_{32}O_2$	280.45	18	32	2	19	32.667	2	1.719	0.105
α-Linolenic acid	C18:3	$C_{18}H_{30}O_2$	278.43	18	30	2	19	30.667	2	1.614	0.105
Arachidonic acid	C20:4	$C_{20}H_{32}O_2$	304.47	20	32	2	21	32.667	2	1.556	0.095
EPA	C20:5	$C_{20}H_{30}O_2$	302.45	20	30	2	21	30.667	2	1.460	0.095
DPA	C22:5	$C_{22}H_{34}O_2$	330.50	22	34	2	23	34.667	2	1.507	0.087
DHA	C22:6	$C_{22}H_{32}O_2$	328.49	22	32	2	23	32.667	2	1.420	0.087

Table 12 Molar fractions of common fatty acids in selected foods (calculation based on data in ref [26]).

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C20:0	C22:0	C16:1	C18:1	C18:2	C18:3	C20:4	C20:5	C22:5	C22:6
Ice cream	0.0329	0.0172	0.0076	0.0163	0.0185	0.0469	0.1240	0.0436	—	—	0.0071	0.0839	0.0057	0.0036	—	—	—	—
Cow's milk	0.0363	0.0172	0.0083	0.0163	0.0175	0.0490	0.1014	0.0394	—	—	0.0106	0.0984	0.0050	0.0054	—	—	—	—
Egg	—	—	—	—	—	—	0.1115	0.0327	—	—	0.0165	0.1519	0.0396	—	0.0026	—	—	0.0037
Olive oil	—	—	—	—	—	—	0.0468	0.0081	0.0013	—	0.0039	0.2549	0.0392	0.0025	—	—	—	—
Rapeseed oil	—	—	—	—	—	—	0.0175	0.0042	0.0026	0.0009	0.0094	0.1912	0.0820	0.0359	—	—	—	—
Sunflower oil	—	—	—	—	—	0.0004	0.0226	0.0221	0.0019	0.0021	0.0004	0.1168	0.1854	0.0011	—	—	—	—
Bacon	—	—	—	—	—	0.0070	0.1069	0.0503	—	—	0.0138	0.1551	0.0257	0.0022	—	—	—	—
Beef	—	—	—	—	—	0.0140	0.1049	0.0457	—	—	0.0248	0.1487	0.0071	0.0047	—	—	—	—
Lamb	—	—	—	—	—	0.0236	0.0944	0.0735	—	—	0.0051	0.1352	0.0089	0.0090	—	—	—	—
Pork	—	—	—	—	—	0.0070	0.1057	0.0485	—	—	0.0134	0.1551	0.0264	0.0032	—	—	—	—
Chinken	—	—	—	—	—	0.0057	0.1041	0.0250	—	—	0.0283	0.1409	0.0481	0.0025	—	—	—	—
Turkey	—	—	—	—	—	0.0044	0.0975	0.0352	—	—	0.0197	0.0761	0.0713	0.0036	—	—	—	—
Ham	—	—	—	—	—	0.0066	0.1018	0.0408	—	—	0.0149	0.1572	0.0339	—	—	—	—	—
Pork sausage	—	—	—	—	—	0.0083	0.1006	0.0478	—	—	0.0142	0.1607	0.0275	0.0022	—	—	—	—
Haddock	—	—	—	—	—	0.0066	0.0780	0.0214	—	—	0.0157	0.0503	0.0078	0.0014	0.0108	0.0397	0.0073	0.0746
Salmon	—	—	—	—	—	0.0210	0.0733	0.0137	—	—	0.0236	0.0807	0.0050	0.0029	0.0016	0.0271	0.0082	0.0335
Peanut	—	—	—	—	0.0005	0.0022	0.0417	0.0095	—	0.0100	—	0.1735	0.1034	0.0029	—	—	—	—

Table 13 Element ratios contributed from corrected fatty acids in selected foods.

	$(H/C)_c$	$(O/C)_c$	Content in wastewater	Relative H/C	Relative O/C
cow's milk	1.862	0.154	18.0%	0.335	0.028
egg	1.846	0.109	18.0%	0.332	0.020
bacon	1.860	0.110	3.0%	0.056	0.003
beef	1.864	0.111	7.0%	0.131	0.008
lamb	1.870	0.111	7.0%	0.131	0.008
pork	1.859	0.110	7.0%	0.130	0.008
chinken	1.844	0.110	7.0%	0.129	0.008
turkey	1.840	0.110	7.0%	0.129	0.008
ham	1.855	0.110	3.0%	0.056	0.003
pork sausage	1.857	0.110	3.0%	0.056	0.003
haddock	1.694	0.103	5.0%	0.085	0.005
salmon	1.764	0.108	5.0%	0.088	0.005
peanut	1.810	0.106	2.0%	0.036	0.002
Ice cream	1.870	0.151	2.0%	0.037	0.003
olive oil	1.827	0.107	2.0%	0.037	0.002
rapeseed oil	1.784	0.106	2.0%	0.036	0.002
sunflower oil	1.783	0.106	2.0%	0.036	0.002
			Total ratio	1.838	0.118

In summary, the generic formulae of carbohydrates, proteins and fats are listed in Table 14.

Table 14 Generic formulae of carbohydrates, proteins and fats.

	Carbohydrates	Proteins	Fats
Generic formula	$CH_{1.826}O_{0.913}$	$CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$	$CH_{1.838}O_{0.118}$

Chapter 3. Energy of food components in wastewater

The heat of combustion (Hc) is the heat energy generated when a compound is combusted completely with O_2 under standard conditions. Quantatively, there are two Hc values, higher heating value (HHV) and lower heating value (LHV). HHV considers all the combustion products are cooled down to the original pre-combustion temperature. The generated water is considered as in liquid form. However, LHV treats H_2O products as vapor, which hence provides a smaller Hc value by the heat of vaporization of water. In the present study, Hc values of the food components are considered as the representative of gross energy. Due to the structural variation and the different DP, the molar Hc quoted directly from the database are not good for comparison between components. Instead, the specific Hc, which is the value in unit mass, are calculated by dividing the molar heats by the corresponding molecular weights and applied as parameters in further calculations.

Assembled in the induction of generic formula, the Hc values of mono-, di- and polysaccharides are evaluated separately. The estimated contributions of food to wastewater are applied to calculate an overall Hc value for carbohydrates.

Inspired by the Woodward–Fieser rules applied UV-visible spectometry[28, 29], a relationship between functional groups and the change of Hc values of proteins have been build. The generic formula of protein can be structurally deconstructed into a combination of small amino acid pieces and extra functional groups.

The averaged Hc values of saturated and unsaturated fatty acids are worked out separately, based on the linear relationship between the Hc values and the number of carbon. Then the fat with generic formula is considered as a specific combination of saturated and unsaturated fatty acids, and its Hc value is worked out collectively.

3.1 Carbohydrates

Similar to the deduction of the generic formula of carbohydrates, the specific Hc in mono-, di- and polysaccharides are considered separately. The evaluated contents of the three types in total carbohydrates are also used in energy calculation. Table 15 lists the molecular weights and molar Hc values of selected mono- and disaccharides, which are retrieved from the National Institute of Standards and Technology (NIST) database[30]. It's observed that the major C_5 and C_6 monosaccharides possess an average specific Hc of 15.61 kJ g^{-1}, while disaccharides had a slightly higher average Hc in 16.50 kJ g^{-1}. Both of the averages contains narrow variations.

E. Kienzle *et al*. measured the heats of combustion of many pet foods in adiabatic bomb calorimeters[31] and found that both cellulose and starch have average Hc of 17.25 kJ g^{-1} with narrow variations. However, higher Hc value and larger variation are obtained from lignin and other non-starch polysaccharides. Considering that cellulose and starch are the dominant polysaccharides in human food, the overall average heat of combustion of polysaccharides is set as 17.30 kJ g^{-1}.

Consequently, combining the content of three types of carbohydrates (31.7% of monosaccharides, 31.7% of disaccharides and 36.6% of polysaccharides, Table 6 in Page 22), the average energy of carbohydrates in wastewater is calculated as 16.51 kJ g^{-1}.

Table 15 Molar and specific Hc values of typical mono- and disaccharides.

Name	Formula	Molecular weight/ g mol^{-1}	Hc/kJ mol^{-1}	Hc/kJ g^{-1}
Ribose	$C_5H_{10}O_5$	150.13	2347.59	15.64
Arabinose	$C_5H_{10}O_5$	150.13	2338.80	15.58
Glucose	$C_6H_{12}O_6$	180.16	2805.00	15.57
Mannose	$C_6H_{12}O_6$	180.16	2812.67	15.61
Gulose	$C_6H_{12}O_6$	180.16	2817.30	15.64
Sucrose	$C_{12}H_{22}O_{11}$	342.30	5643.40	16.49
Lactose	$C_{12}H_{22}O_{11}$	342.30	5652.09	16.51

3.2 Proteins

The molar Hc of the selected amino acids are quoted from the NIST database[30] and listed in Table 18. Combining the molecular structures of the amino acids (Table 4 in Page 16), some rules are summarised below:

1) From the comparison between Gly, Ala, AABA, Val and Ile, every addition of methylene group on hydrocarbon chain gradually increases the molar Hc by 646.0, 633.0, 662.7 and 661.6 kJ mol^{-1}, respectively, which average 650.8 kJ mol^{-1}.
2) From the comparison between Ser and Ala, Thr and AABA, it is observed the addition of hydroxyl group unexpectedly lead to a reduction in molar Hc by 172.8 and 169.4 kJ mol^{-1}, with average of 171.1 kJ mol^{-1}.
3) A slight reduction has also been found when carboxylic acids are attached to the hydrocarbon end of amino acid. The average reduction caused by this group is 11.7 kJ mol^{-1}. However, there is a large variation of Hc change between Ala and Asp (19.9 kJ mol^{-1}), AABA and Glu (3.5 kJ mol^{-1}).
4) On the contrary, the addition of amide group contributes to the total molar Hc positively. The Hc values of Asn and Gln are 307.5 and 316.3 kJ mol^{-1} higher than the corresponding Als and AABA. So the average Hc increment is 311.9 kJ mol^{-1}.
5) The attachment of phenyl group increases the molar Hc significantly. The molar Hc of Phe increases a dramatic 3025.3 kJ mol^{-1}, compared to that of Ala.
6) No matter that sulfur atom inserts into the hydrocarbon chain to make a thioether group or adds to the end of the chain to give a thiol group, considerable molar Hc increases result in. The average increment due to one sulfur atom is 637.6 kJ mol^{-1}.
7) Cyclic structure amino acid unsurprisingly reduces the molar Hc value, compared to its corresponding straight-chain derivative. A difference of 170.5 kJ mol^{-1} is found between the Hc values of Pro and Val. Similarly, double bond is assumed to have the same effect on the molar Hc to cyclic structure.
8) Comparing the Hc values of Lys and Ile, an increase of 104.9 kJ mol^{-1} has been found after attaching an amine group to the hydrocarbon chain.

In summary, the molar Hc changes of these functional groups are listed in Table 16. Consequently, the molar Hc of other amino acids can be estimated based these structural difference of the known amino acids and the summarised Hc changes. Two examples are shown in Table 17.Table 17A Trp molecule is considered as an Ala attached to a phenyl, an amine and two methylene groups with a cyclic structure and a double bond. The estimated Hc is therefore calculated as $(1621.0+3025.3+104.9+2\times650.8-170.5-170.5) = 5711.8$ kJ mol^{-1}, which is only 1.5% higher than its measured Hc listed in the NIST database[30].

On the other hand, an His molecule is regarded as an Ala molecule added two amine and three methylene groups with two double bonds and one cyclic structure. The estimated Hc of His is $(1621.0+2\times104.9+3\times650.8-2\times170.5-170.5) = 3271.7$ kJ mol^{-1}, which is 2.1% higher than the actual Hc of His, 3205.5 kJ mol^{-1}. Therefore, the structure of the generic formula of protein can be understood as a structural stacking of various functional groups and the overall Hc value of protein is calculated by the summation of the individual Hc values.

Table 16 The summarised relationship between the Hc changes and functional groups (negative for reduction, positive for increase)

Functional groups	ΔHc/kJ mol^{-1}	Functional groups	ΔHc/kJ mol^{-1}
$-CH_2-/CH_3-$	650.8	$-S-/SH-$	637.6
$-OH$	-171.1	$-CONH_2$	311.9
Cycle/C=C	-170.5	$-COOH$	-11.7
$-NH_2$	104.9	$-C_6H_5$	3025.3

Table 17 Examples of Hc calculations of amino acids using the summerised rules.

Name	Formula	Abbr.	Est. Hc/kJ mol^{-1}	Measured Hc/kJ mol^{-1}	Relative Error%
L-tryptophan	$C_{11}H_{12}O_2N_2$	Trp	5711.8	5628.3	1.5
L-histidine	$C_6H_9O_2N_3$	His	3271.7	3205.5	2.1

Table 18 Molar Hc values of selected amino acids.

Name	Formula	Abbr.	Molar Hc/kJ mol^{-1}
Glycine	$C_2H_5O_2N$	Gly	975.0
L-alanine	$C_3H_7O_2N$	Ala	1621.0
A-aminobutyric acid	$C_4H_9O_2N$	AABA	2254.0
L-valine	$C_5H_{11}O_2N$	Val	2916.7
L-isoleucine	$C_6H_{13}O_2N$	Ile	3578.3
L-serine	$C_3H_7O_3N$	Ser	1448.2
L-threonine	$C_4H_9O_3N$	Thr	2084.6
L-proline	$C_5H_9O_2N$	Pro	2746.2
L-lysine	$C_6H_{14}O_2N_2$	Lys	3683.2
L-methionine	$C_5H_{11}O_2NS$	Met	3564.1
L-cysteine	$C_3H_{12}O_2NS$	Cys	2248.8
L-asparagine	$C_4H_8O_3N_2$	Asn	1928.5
L-glutamine	$C_5H_{14}O_2N_2$	Gln	2570.3
L-aspartic acid	$C_4H_7O_4N$	Asp	1601.1
L-glutamic acid	$C_5H_9O_4N$	Glu	2250.5
L-phenylalanine	$C_9H_{11}O_2N$	Phe	4646.3

As mentioned above (page 24), the generic formula of protein is $CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$, which is further scaled up to $C_{125.0}H_{257.9}O_{78.3}N_{35.3}S$ to maintain the amount of sulfur as unity. The primary consideration is all the N atom are derived from glycines ($C_2H_5O_2N$). That means a generic protein molecule possesses an N-containing piece equivalent to 35.3 glycine molecules and the remaining in the generic formula is $C_{54.4}H_{81.4}O_{7.7}S$. The second omitted element is O, which is considered completely from carboxylic acids. That accounts for 3.9 carboxylic acids in a generic protein molecule and leaves $C_{50.6}H_{77.5}S$ in the generic formula. In addition, the sole S atom is considered as the contribution from a thioether group. Finally, the hydrocarbon portion is a combined contribution from phenyl and methylene groups, the amount of which can be derived from a simultaneous equation based on the number of C and H atoms left in the generic formula. The step-by-step calculation results are shown in Table 19. The specific Hc is hence calculated as 18.2 kJ g^{-1}.

Table 19 Calculation of Hc values of proteins.

	C	H	O	N	S	M.W/g mol^{-1}
Generic formula	1.000	2.063	0.626	0.282	0.008	28.3
scaled-up generic formula	125.0	257.9	78.3	35.3	1.0	3535.4

	Individual Hc/kJ mol^{-1}	amount	Total Hc/kJ mol^{-1}
Gly ($C_2H_5O_2N$)	975.0	35.3	64313.4
─COOH	-11.7	3.9	
─S─	637.6	1.0	Specific Hc/kJ g^{-1}
─C6H5	3025.3	3.4	18.2
─CH2─	650.8	30.2	

3.3 Fats

Many research have been conducted on the measurement of Hc values of fatty acids and their methyl esters[32-34]. It's found that the heat of combustion of saturated fatty acids increase as the alkyl chain extends. Table 20 lists the Hc values of selected saturated fatty acids, quoted from the NIST database. The average increment of Hc is *ca.* 650 kJ mol^{-1} per methylene group. Table 20 also provides some Hc values of unsaturated fatty acids with one double bond, from which it is observed that their Hc values are slightly lower than those of the corresponding saturated fatty acids, as shown in Figure 3.

Table 20 Molar and specific Hc values of selected fatty acids.

Name	Abbr.	Fomula	M.W./g mol^{-1}[30]	Hc/kJ mol^{-1}	Hc/kJ g^{-1}	C	O
Acetic acid	C2:0	$C_2H_4O_2$	60.052	875.2	14.574	2	2
Butyric acid	C4:0	$C_4H_8O_2$	88.11	2183.5	24.783	4	2
Caproic acid	C6:0	$C_6H_{12}O_2$	116.16	3494.3	30.082	6	2
Caprylic acid	C8:0	$C_8H_{16}O_2$	144.21	4799.9	33.270	8	2
Capric acid	C10:0	$C_{10}H_{20}O_2$	172.26	6079.3	35.290	10	2
Lauric acid	C12:0	$C_{12}H_{24}O_2$	200.32	7377.0	36.826	12	2
Myristic acid	C14:0	$C_{14}H_{28}O_2$	228.37	8676.7	37.994	14	2
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.42	10028.6	39.109	16	2
Stearic acid	C18:0	$C_{18}H_{36}O_2$	284.48	11336.8	39.652	18	2
Arachidic acid	C20:0	$C_{20}H_{40}O_2$	312.53	12574.2	40.234	20	2
2-Butenoic acid	C4:1	$C_4H_6O_2$	86.09	2000.0	23.232	4	2
3-Pentenoic acid	C5:1	$C_5H_8O_2$	100.12	2676.1	26.730	5	2
3-Hexenoic acid	C6:1	$C_6H_{10}O_2$	114.14	3342.1	29.280	6	2
Oleic acid	C18:1	$C_{18}H_{34}O_2$	282.46	11160.7	39.512	18	2
13-Docosenoic acid	C22:1	$C_{22}H_{42}O_2$	338.57	13699.0	40.462	22	2

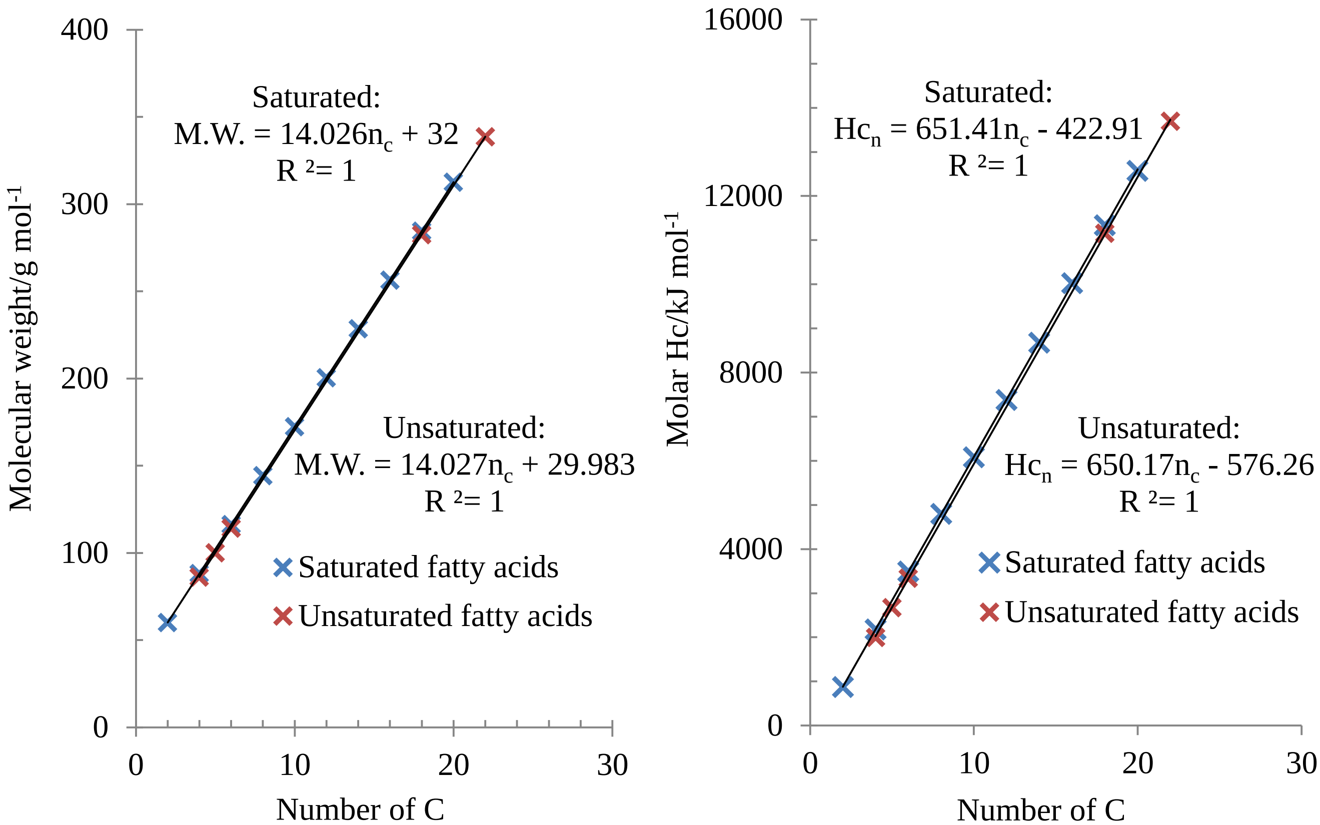


Figure 3 Relationships between molecular weight (left), molar Hc value (right) and the number of C in saturated (blue) and unsaturated (red) fatty acids.

According to Figure 3, linear relationships have been established for both saturated and unsaturated fatty acids between the molecular weights, molar Hc values and their number of C atoms. Therefore, the relationship between the specific Hc and the number of C for saturated fatty acids is calculated as follow:

$$\textbf{specific Hc (saturated)} = \frac{\textbf{molar Hc}}{\textbf{M.W.}} = \frac{\mathbf{651.41n_c - 422.91}}{\mathbf{14.026n_c + 32}} = \mathbf{46.443} - \frac{\mathbf{1909.086}}{\mathbf{14.026n_c + 32}} \quad (7)$$

Similarly, the corresponding relationship for unsaturated fatty acids is written as:

$$\textbf{specific Hc (unsaturated)} = \frac{\mathbf{650.17n_c - 576.26}}{\mathbf{14.027n_c + 29.983}} = \mathbf{46.351} - \frac{\mathbf{1966.011}}{\mathbf{14.027n_c + 29.983}} \quad (8)$$

Krisnangkura has estimated the heat of combustion of triglycerides[34] and given the equation as:

$$\textbf{heat of combustion}\ (\mathbf{kcal\ g^{-1}}) = \frac{\mathbf{1896000}}{\mathbf{SN}} - \mathbf{0.6} \times \mathbf{IV} - \mathbf{1600} \quad (9)$$

where SN stands for the saponification number, which is a measure of the average hydrocarbon chain length of fatty acids in triglycerides, and IV represents iodine value, a parameter reliant on the amount of unsaturation. It's seen that the unsaturation has a negligible effect on the Hc value, which is supported by the data listed in Table 21.

Table 21 Molar Hc values of selected triglycerides.

Triglyceride formula	Composed fatty acids	Molecular weight /g mol^{-1}	Molar Hc /kJ mol^{-1}	$\frac{1}{3}$ ×Formula	$\frac{1}{3}$ ×Molar Hc /kJ mol^{-1}	C	$\frac{1}{3}$ ×C
$C_{27}H_{50}O_6$	C8:0	470.68	15259.0	$C_9H_{16.667}O_2$	5086.3	27	9
$C_{33}H_{62}O_6$	C10:0	554.84	19861.4	$C_{10}H_{20.667}O_2$	6620.5	33	11
$C_{39}H_{74}O_6$	C12:0	639.00	23731.6	$C_{12}H_{24.667}O_2$	7910.5	39	13
$C_{45}H_{86}O_6$	C14:0	723.16	27643.7	$C_{14}H_{28.667}O_2$	9214.6	45	15
$C_{51}H_{98}O_6$	C16:0	807.32	31605.9	$C_{16}H_{32.667}O_2$	10535.3	51	17
$C_{57}H_{110}O_6$	C18:0	891.48	35806.7	$C_{18}H_{36.667}O_2$	11935.6	57	19
$C_{63}H_{122}O_6$	C20:0	975.64	39467.7	$C_{20}H_{40.667}O_2$	13155.9	63	21
$C_{69}H_{134}O_6$	C22:0	1059.80	43208.2	$C_{22}H_{44.667}O_2$	14402.7	69	23
$C_{36}H_{62}O_6$	C11:1	596.92	21401.2	$C_{11}H_{20.667}O_2$	7133.7	36	12
$C_{51}H_{92}O_6$	C16:1	801.27	31179.2	$C_{16}H_{30.667}O_2$	10393.1	51	17
$C_{57}H_{104}O_6$	C18:1	885.43	35099.6	$C_{18}H_{34.667}O_2$	11699.9	57	19
$C_{63}H_{116}O_6$	C20:1	969.59	39020.0	$C_{20}H_{38.667}O_2$	13006.7	63	21
$C_{69}H_{126}O_6$	C22:1	1051.73	42802.3	$C_{22}H_{42.667}O_2$	14267.4	69	23

The generic formula of fat was known to be $CH_{1.838}O_{0.118}$ (Page 28), which is derived from the formulae of fatty acids with an average correction of glycerol, $CH_{0.667}$. This generic formula can scale up to $C_{16.949}H_{31.153}O_2$ to maintain the number of O as 2. It is also known that the generic formulae of saturated and one double-bond unsaturated fatty acids are $C_nH_{2n}O_2$ and $C_nH_{2n-2}O_2$, respectively. Applying the "average correction" to them, the corrected formulae of both fatty acids are obtained as $C_{n+1}H_{2n+0.667}O_2$ and $C_{n+1}H_{2n-1.333}O_2$. Given the number of C in the formulae of saturated and one double-bond unsaturated fatty acids identical to the value of the generic formula of fat (16.949), the corrected generic formulae of both acids are $C_{16.949}H_{32.565}O_2$ and $C_{16.949}H_{30.565}O_2$. Following the simultaneous equations below:

$$a \times 32.565 + b \times 30.565 = 31.153$$

$$\mathbf{a + b = 1} \tag{10}$$

where a and b are the fractions of saturated and one double-bond unsaturated fatty acids. It can be considered that the entire group of fat consists of 29.4% saturated fatty acids and 70.6% one double-bond unsaturated fatty acids.

One-third of C numbers and molar Hc values of fat are accounted to keep the number of O as 2. The linear relationship between the molar Hc and the number of C are still well maintained, as seen in Figure 4. The molar Hc of both fats at the C number of 16.949 are calculated respectively, which are 10486.4 and 10357.4 kJ mol^{-1}. Combining the considered percentages of both types of acids, the overall molar Hc of fat is calculated as

$$29.4\% \times 10486.4 + 70.6\% \times 10357.4 = 10395.3 \text{ kJ mol}^{-1}$$

Divided by the molecular weight of the scaled generic formula of fat (267.02 g mol^{-1}), the specific Hc of fat is obtained as 38.93 kJ g^{-1}. All the calculation results are summarised in Table 22.

Table 22 Calculation of the Hc value of fats.

	Corrected formula	Considered percentages in fat	Molar Hc/kJ mol^{-1}
Saturated fatty acid	$C_{16.949}H_{32.565}O_2$	29.4%	10486.4
Unsaturated fatty acid	$C_{16.949}H_{30.565}O_2$	70.6%	10357.4

	Generic formula	Scaled generic formula	Molecular weight/ g mol^{-1}	Overall molar Hc /kJ mol^{-1}	Specific Hc/ kJ g^{-1}
Fat	$CH_{1.838}O_{0.118}$	$C_{16.949}H_{31.153}O_2$	267.02	10395.3	38.93

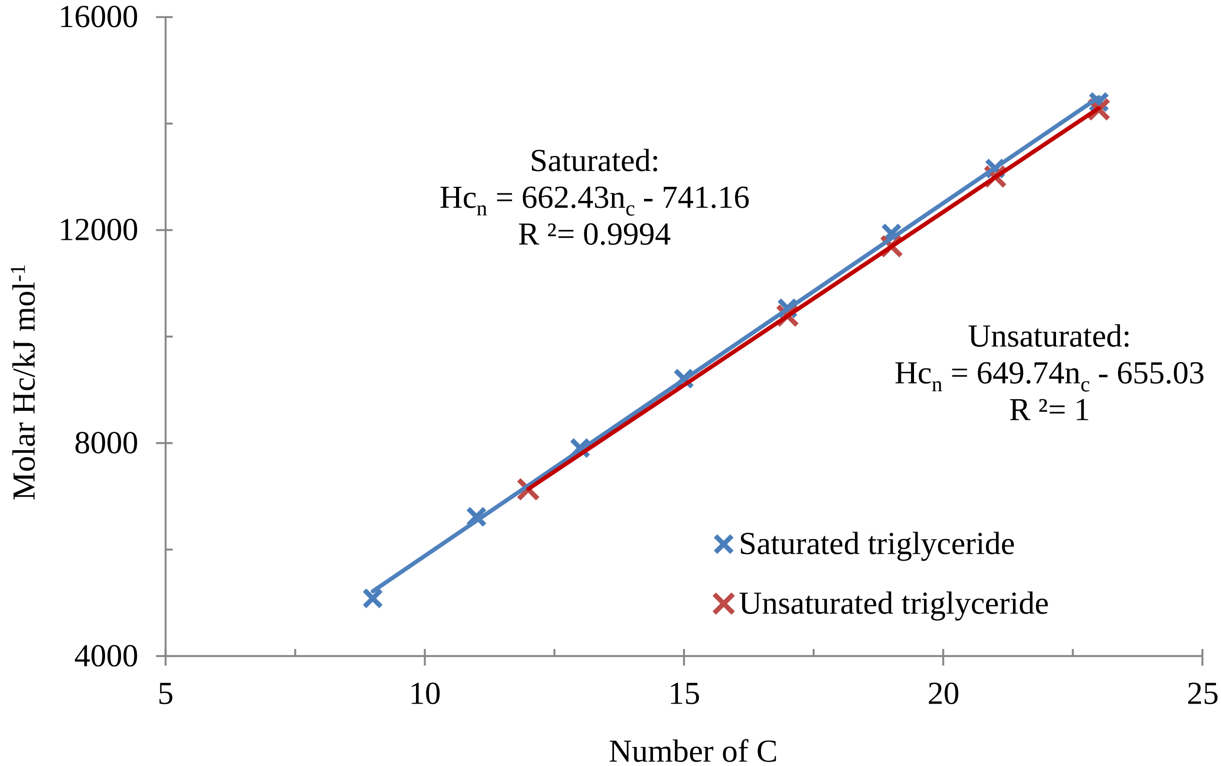


Figure 4 Relationships between molar Hc value and the number of C in saturated (blue) and one double-bond unsaturated (red) triglycerides.

Chapter 4. Applications

In this chapter, application of the generic formulae and specific energies of carbohydrates, proteins and fats, which are deduced in the previous two chapters, in wastewater analysis are provided. The COD values in selected wastewater samples are estimated based on the generic formulae of the three components and their contents in samples. The estimated results are also compared with the experimental measurements and the estimations based on generic formulae reported by Henze *et al*[1].

The heat of combustion of one commercial food, NESCAFÉ® Coffee-Mate Original, was measured experimentally. According to the nutritional data on the product's label, the specific energy contained in the product is calculated, using the specific energies of carbohydrates, proteins and fats. Both of the energies are compared to the data provided by the manufacture. The food sample was also dissolved into water. After the removal of the solvent, the heat of combustion of the dried sample was measured again and compared with the results above.

4.1 Estimation of COD

COD, short for chemical oxygen demand, is a common measure to indirectly evaluate the total amount of organic compounds in water. A COD test is based on the assumption that all C, H and N atoms in organic compounds are oxidised to the compounds in highest valence states, that is, CO_2, H_2O and NO_3^-, respectively.

If the formula of a compound is known, given O_2 as the oxidant, the required amount of oxygen to oxidise one mole of organic compound is calculated stoichiometrically as $(n + \frac{a}{4} - \frac{b}{2} + \frac{5}{4}c)$, based on the generic reaction below:

$$C_nH_aO_bN_c + \left(n + \frac{a}{4} - \frac{b}{2} + \frac{5c}{4}\right) O_2 \rightarrow a\,CO_2 + \left(\frac{a}{2} - \frac{3c}{2}\right) H_2O + c\,NO_3^- + cH_2O^+$$

The oxygen demand calculated from the stoichiometric calculation for $C_nH_aO_bN_c$ is named as theoretical oxygen demand, or ThOD for short.

Due to the incomplete oxidisation by dichromates and the dismissed volatile chemicals in the COD test with standard method[35], the actual COD value is considered to be smaller than ThOD. However, it is reported that the ratio of COD to ThOD exhibits a mixed beta-normal distribution around unity, ranging from 0.1 to 1.3, depending on the chemical properties of organic chemicals[36].

In practice, potassium dichromate is used as the oxidising agent and the residue dichromate after reaction with wastewater is titrimetrically measured with ferrous ammonium sulfate (FAS). The COD is calculated as

$$\mathrm{COD}(\mathrm{mg\ l^{-1}}) = 8 * 1000 * \mathrm{M} * (\mathrm{V_b} - \mathrm{V_s}) \tag{11}$$

Where *M* is the molar concentration of FAS in mol l^{-1}, V_b and V_s are the titres of FAS in the blank test and the water sample mixed with equal volume of $K_2Cr_2O_7$, 8 is a quarter of the molecular weight of O_2, which is required to oxidise one mole of FAS, and 1000 is used to balance the unit of the equation.

The ThOD values which are derived from the generic formulae of the three food components in this project have been compared with those based on the generic formulae reported by Henze *et al.*[1], as shown in Table 23. A dimensionless factor is calculated based on the generic formulae and reaction. Multiplied the molecular weight of O_2 and divided by that of the organic compound, the required weight of O_2 per gram organic compound is obtained (in unit of g O_2 g^{-1} organic matters). Consequently, it enables to work out the ThOD for a specific water sample by multiplying the mass contents of the organic compounds in the sample and summate.

Table 23 A comparison between calculated ThOD values of carbohydrate, protein and fat based on this work's and Henze's result[1]. (the effect of sulfur in the work's result for proteins is neglected)

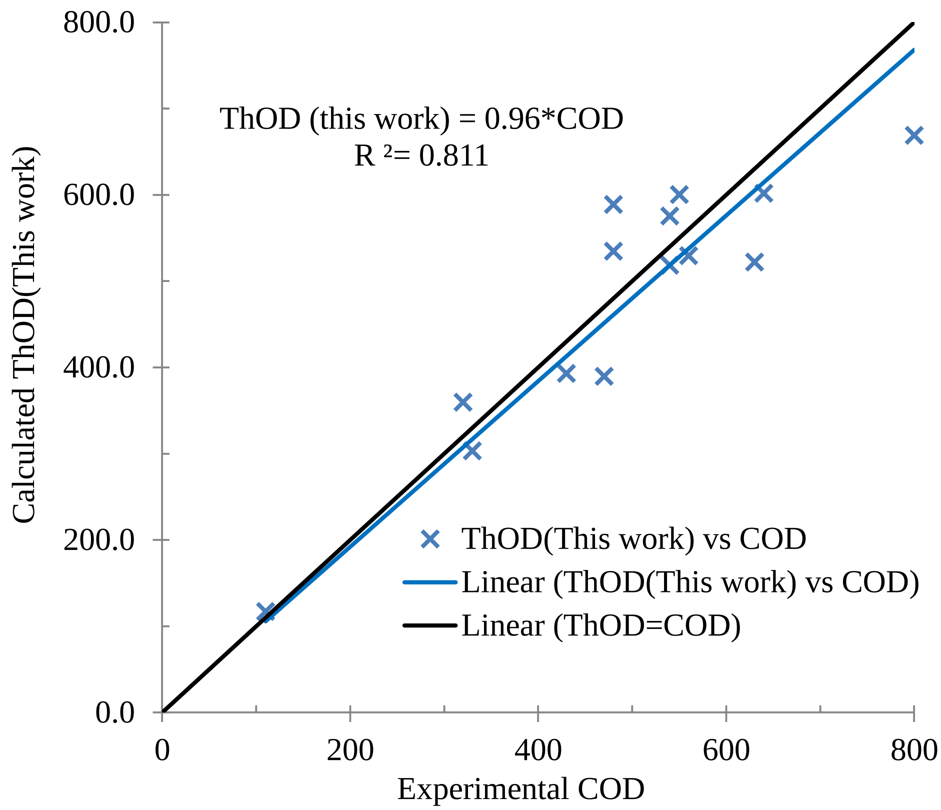
	This work's results			
	generic formula	M.W./g mol^{-1}	ThOD	ThOD/g O_2 g^{-1}OC
carbohydrate	$CH_{1.826}O_{0.913}$	28.43	1.00	1.13
protein	$CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$	28.03	1.56	1.78
fat	$CH_{1.838}O_{0.118}$	15.73	1.40	2.85
	Henze's results			
	generic formula[1]	M.W./g mol^{-1}	ThOD	ThOD/g O_2 g^{-1}OC
carbohydrate	$C_{10}H_8O_9$	282	10.0	1.13
protein	$C_{14}H_{12}O_7N_2$	320	16.0	1.60
fat	$C_8H_6O_2$	134	8.50	2.03

Raunkjaer *et al*. collected wastewater samples from four treatment plants at different time in a day and measured the COD values and the contents of carbohydrates, proteins and fats in the wastewater samples[21]. The ThOD values are hereby calculated, using the generic formula deduced in this project and by Henze. It is observed from the comparison in Table 24 and Figure 5 that using the generic formulae of carbohydrates, proteins and fats deduced in this project provides closer ThOD values of wastewater samples to the experimental measurements than those calculated from Henze's generic formulae. The ThOD/COD ratios by this work's method range from 0.829 to 1.227 with the centre of 1. The average ratio is given as 0.990. However, larger variations of ThOD by Henze's method from the experimental results have been found.

Figure 5 compares the experimental COD with the calculated ThOD using the generic formulae in this work and Henze'method. Using the generic formulae deduced in this project, the linear slope of the ratio give 0.96, which indicates that the calculated ThOD is highly consistent with the experimental results. However, results based on Henze's formulae strongly underestimated the organic compounds present in wastewater samples, giving the slope of linear trendline as 0.84, further away from unity. It is concluded that the generic formulae of carbohydrates, proteins and fats deduced in this project is able to provide an accurate and reliable prediction to the COD values of wastewater samples.

Table 24 COD and contents of carbohydrates, proteins and fats of domestic wastewater sample reported by Raunkjaer *et al*.[21] with the calculated ThOD results based on this work's and Henze's methods.

Location	Time	COD / mg l^{-1}	Carbohydrates / mg l^{-1}	Proteins / mg l^{-1}	Fats / mg l^{-1}	This work's method		Henze's method	
						ThOD / mg l^{-1}	$\frac{ThOD}{COD}$	ThOD / mg l^{-1}	$\frac{ThOD}{COD}$
Aalborg West	10.00	540	148.0	116.0	63.9	575.5	1.066	482.5	0.894
	15.00	800	128.8	171.4	86.9	669.3	0.837	596.2	0.745
	21.00	630	76.3	110.9	91.9	522.2	0.829	450.2	0.715
	4.00	330	46.6	77.8	46.7	303.4	0.919	271.9	0.824
Hjallerup	10.00	560	76.7	140.6	82.5	529.5	0.946	479.1	0.856
	15.00	470	60.1	94.3	62.1	389.8	0.829	344.8	0.734
	21.00	430	59.2	95.0	63.5	393.0	0.914	347.8	0.809
	4.00	540	79.0	123.0	84.1	518.4	0.960	456.8	0.846
Aalborg East	10.00	480	86.7	124.3	84.6	534.9	1.114	468.6	0.976
	15.00	480	48.4	119.5	129.3	588.9	1.227	508.4	1.059
	4.00	550	94.1	139.1	97.1	600.4	1.092	526.0	0.956
Aabybro	15.00	640	97.9	150.2	90.9	601.9	0.941	535.5	0.837
	21.00	320	66.1	73.5	56.0	359.7	1.124	306.0	0.956
	4.00	110	25.7	33.9	11.7	117.1	1.065	107.0	0.973
				Average ThOD/COD			0.990		0.870



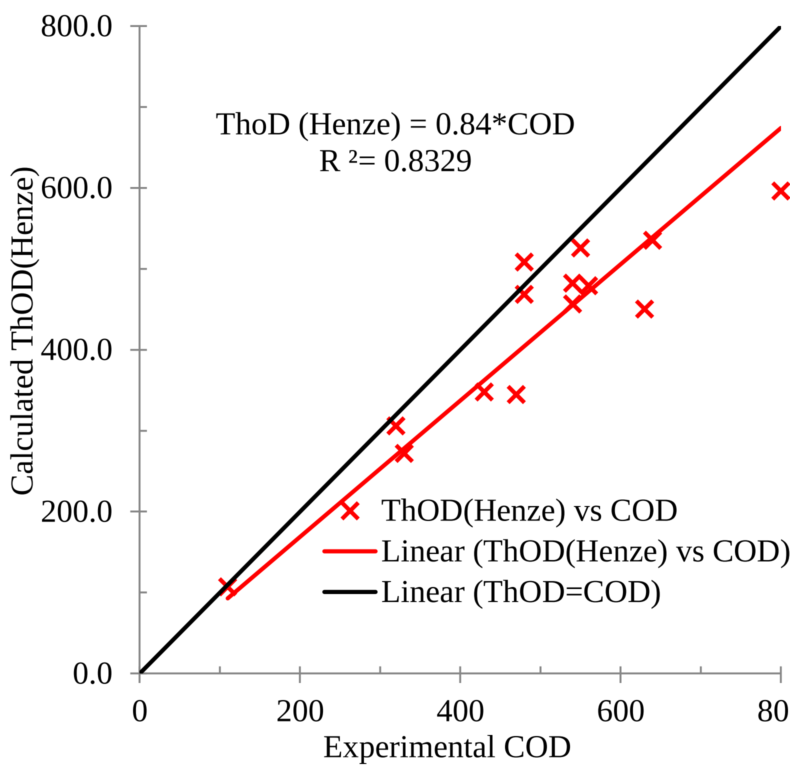


Figure 5 Comparison between experimental COD values and calculated ThOD based on this work's (top) and Henze's (bottom) methods[1].

4.2 Calculation of the heat of combustion

The heat of combustion of NESCAFÉ® Coffee-Mate Original sample is measured in a Parr® 6200 isoperibol oxygen bomb calorimeter. Sample of known weight is held in a metal cup, which is placed in a complex high-chromium-nickel alloy bomb, which is filled with O_2 to 30 atm to ensure a complete reaction. After a thermal stablisation for 20 minutes, samples are heated by platinum fuse wire and combust. The generated heat is transferred to the calorimeter bucket filled with 2.0 L de-ionised water embracing the bomb. The temperature rise of the water bucket after a complete combustion is recorded and the generated heat is calculated through multiplying the temperature rise by the heat capacity of the system. Consequently, the Hc in unit of kJ g^{-1} is worked out by dividing the generated heat by the weight of sample. The measurement for the sample was done twice in order to provide an accurate and reliable result.

Another batch of coffee mate sample is dissolved in water and dried before measuring the Hc value in the calorimeter. In a typical experiment, *ca.* 10 g coffee mate powders were dissolved in 200 mL de-ionised water in a beaker at ambient temperature. The formed suspension was then heated on a hot plate to the boiling point with moderate magnetic stirring. As the solvent evaporated, the suspension turned to be a brown, sticky, caramel-like paste. The agitation was driven by a glass rod manually for a further 10 min. The paste was then moved to an 80 ℃ oven for 1 hour and cooled down in a fume cupboard for 14 days to produce a dried sample for the heat measurement in a calorimeter.

This treatment is designed to mimic the experiment of measuring the Hc of components in wastewater. It is a reasonable consideration as the content of components in wastewater is normally low, sometimes in unit of ppm. The mass of water had to be removed before measuring the Hc value. The most common method is to heat the wastewater sample to evaporate water, which inevitably gets rid of volatile components which should have been considered in the measurement of Hc value. Another approach is to remove the water at low temperature. Although it maintains the complete compositions of the original wastewater sample, it usually takes weeks to finish the evaporation, which makes the operation unavailable in the analysis of wastewater[18].

The measured heat of combustion for original coffee mate and dissolved-dried samples are listed in Table 25. The two measurements for both samples give close results, indicating the experiments were of satisfactory success. The nutrient energy provided by the manufacture on the product label falls between the Hc values of two samples. The measured heat of the dissolved-dried coffee mate sample is 9.0% lower than that of the original sample. This discrepancy shows the sample contain the light components which was removed during the heating. Moreover, the solid sample after evaporation turned brown colour, indicating a partial oxidation occurs during the process of drying, which will cause the measurement of Hc lower than its original value. If the sample is contained in wastewater, the traditional treatment before measurement probably leads to an underestimation of the Hc value.

Table 25 Measurements of heats of combustion of original and dissolved-dried coffee mate samples.

Samples		Nutrient energy or heat of combustion/kJ g^{-1}
On product label		22.86
Original coffee mate	Measurement 1	23.70
	Measurement 2	23.66
	Average	23.68
	Standard deviation	0.0283
Dissolved-dry coffee mate	Measurement 1	21.60
	Measurement 2	21.52
	Average	21.56
	Standard deviation	0.0566

Using the calculated heat of combustion of carbohydrates, proteins and fats, the energy contained in a wastewater sample can be conveniently estimated by multiplying these values by the percentage in the wastewater sample. The estimated heat of combustion for the example of coffee mate is given in Table 26. The contents of carbohydrates, proteins and fats are provided on the label of the product's nutrient data. It is observed that the estimated total heat of combustion is 1.7% lower than the experimental result of the original sample, which means that using the calculated specific heat of combustion is able to give a trustable estimation to the experiment. Another benefit from the estimation is that it also includes the contributions from the volatile components. This indicates that using the estimated heat of combustion deducted in this project is more likely to give an accurate value of the wastewater sample than the experimental result obtained through the heat treatment.

Table 26 An estimation of the heat of combustion of coffee mate sample using this work's method.

	Contents on product label /g per 100 g coffee mate	Specific heat of combustion/kJ g^{-1}	Heat of combustion proportion/kJ g^{-1}
Carbohydrate	57.3	16.51	9.46
Protein	2.1	18.20	0.38
Fat	34.5	38.93	13.43
		Total heat of combustion/kJ g^{-1}	23.27

Chapter 5. Conclusion

5.1 Work summary

In conclusion, a method has been proposed which enables the determination of the elemental composition, ThOD and heat of combustion of a simulated wastewater sample, based on the analysis of its carbohydrates, proteins and fats components. The results are summarised in Table 27 below. Carbohydrates are classified into monosaccharides, disaccharides and polysaccharides, of which the generic formulae are studied separately. On the other hand, due to the structural similarity in carbohydrates, the Hc values of compounds in the three types are chosen to calculate the average values for their corresponding categories. In addition, the contents of the three types of carbohydrates in typical foods are retrieved from the handbook of food components. Empirical proportions of these foods in human diet are also considered. Collectively, the generic formula of carbohydrates is $CH_{1.826}O_{0.913}$ and its heat of combustion is 16.51 kJ g^{-1}.

Table 27 A summary of generic formulae and specific heats of combustion of carbohydrate, protein and fat.

	generic formula	Specific heat of combustion/kJ g^{-1}
carbohydrate	$CH_{1.826}O_{0.913}$	16.51
protein	$CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$	18.20
fat	$CH_{1.838}O_{0.118}$	38.93

On the contrary, the contribution of each protein to the generic formula of proteins has to be examined individually because of their structural variations. 18 amino acids are selected and their element ratios are calculated based on their chemical formulae. The contents of the amino acids in typical foods are referred to the handbook and the element ratios of these foods from the contributions of different proteins are worked out. Combining with the proportions of foods in human diet, the generic formula of proteins is given as $CH_{2.063}O_{0.626}N_{0.282}S_{0.008}$. Moreover, a structural analysis on proteins has been implemented. The Hc values of amino acids are referred to the NIST database and compared extensively between each other. A relationship between change of the Hc value and the difference on functional groups has been established. Therefore, an imaginary 'protein' with the generic formula is formed by a combination of fundamental amino acids and extra functional groups. Consequently, the Hc value of the 'protein', that is, of the generic formula of proteins, is calculated as 18.20 kJ g^{-1}.

The generic formula of fats is derived in a quite similar way to that of proteins. For simplicity, fats, or precisely, triglycerides are represented as a fatty acid with a correction group, $CH_{0.667}$. Multiplying the contents of fatty acids in typical foods, the generic formula of fats is obtained as $CH_{1.838}O_{0.118}$.

Fatty acids are sub-grouped into saturated and unsaturated. Based on the formulae and the Hc values of both types, linear relationships between molecular weights, molar Hc values and the number of carbon have been built up. The generic formula of fats is thought as a combination of saturated and one double-bond unsaturated fatty acids with correction groups. When setting the number of C as a constant, the molar percentages of both corrected fatty acids can be calculated in a simultaneous equation, based on the number of H. Actually, more Hc values of fats are available in the NIST database and references. One third of the Hc values of selected fats are taken. It is found that linear relationship is well maintained between molar Hc and the number of C for both types of fats. Combining the percentage of the two types, the molar Hc of fats can be calculated. Dividing the molecular weight of the generic formula, the specific Hc of fats is given as 38.93 kJ g^{-1}.

5.2 Prospect of applications

Many reagents have been developed to detect the amount of carbohydrates, proteins and fats accurately, such as anthrone reagent[37] and phenol reagent[38] for carbohydrates, Blue G-250[39], Folin-Ciocalteau phenol (FCP) reagent[40] and bicinchoninic acid (BCA) reagent[41] for proteins, chloroform-methanol extraction for fats. Nowadays, the development of analytical instrument allows a fast and accurate determination of the three components.

The method developed in this project yields the key information for the subsequent, rigorous calculation of mass and energy balances throughout wastewater treatment facilities. With the generic formulae and specific Hc values of carbohydrates, proteins and fats deducted in this project, it is possible to provide a quick and reliable estimation of the organic components, or even an *in situ* determination, which reduce the chemical engineers' time to analyse the wastewater and conduct a prompt and suitable treatment.

Two examples have been given in this project. The ThOD values of selected wastewater samples is reasonably estimated by the generic formulae of the three components. The generic formulae in this project exhibit a closer estimation of ThOD to the experimental measurements, giving the averaged relative ratio of 0.96. However, the calculation based on generic formulae those proposed by Henze *et al*., significantly underestimates the ThOD values in wastewater samples, giving the average ratio of 0.84.

In another case, the heat of combustion of a coffee mate sample has been accurately evaluated by using the calculated Hc values of the three components. The calculated heat of combustion has only a 1.7% different from the original measured aloric value. Moreover, water had to be removed prior to the measurement of heat of combustion for wastewater samples, which inevitably drives off the removal of volatile compounds contained in wastewater and cause the constituents partially oxidised, which leads to an underestimation on the energy possessed by the organic compounds in samples. Using the simulation avoids any actual removal of organic components and therefore is able to provide an accurate energy value to the wastewater samples.

5.3 Limitation and Outlook

Due to the limitation of time and budget in this project, not many examples have been examined for the availability of the generic formulae and the heats of combustion. Secondly, it is only considered that the dominant components in wastewater is those from food residue and ignores the influence of industrial production or excreta. So strictly speaking, the research object is the so-called 'greywater' instead of 'blackwater' which put human excreta. Nevertheless, this is still a reasonable assumption as in many developed countries, greywater and blackwater are transported separately and treated differently. Greywater is normally treated by the wastewater treatment plants which are the main attention in this project.

In summary, the proposed generic formulae and specific heats of combustion of carbohydrates, proteins and fats enable chemical engineers to have an initial estimation of the wastewater for the following treatments, not only fast, but also with more confidence.

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