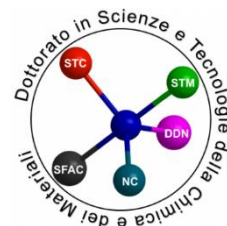




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Valorisation of sludge biomass obtained from civil and industrial wastewater treatment plants (WWTPs)

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List of abbreviations

A	Ash
AD	Anaerobic digestion
ATR	Attenuated total reflection
BSG	Brewer's spent grain
COD	Chemical oxygen demand
DM	Dry matter
DTG	Differential thermogravimetry
EDX	Energy dispersive x-ray
EU	European Union
EWC	European waste catalogue
FC	Fixed carbon
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography- mass spectrometry
GC-TCD	Gas chromatography- thermal conductivity detector
GPC	Gel permeation chromatography
HHV	Higher heating value
HPLC	High performance liquid chromatography
MC	Moisture content
PAH	Polycyclic aromatic hydrocarbon
PHA	Polyhydroxyalkanoate
PTFE	Polytetrafluoroethylene
RI	Refractive index
SCOD	Soluble chemical oxygen demand
SSD	Sewage sludge directive
TGA	Thermogravimetric analysis
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphate
TS	Total solids
VM	Volatile matter
VOA	Volatile organic acid
VS	Volatile solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant
XRF	X-ray fluorescence spectrometry

Summary

The PhD research program has been funded by Regione Liguria on the European Social Fund 2014-2020 under the project “Valorizzazione delle biomasse prodotte dal trattamento delle acque civili ed industriali attraverso processi innovativi – ARGE17-992/5/2”.

The PhD research aimed to identify and study innovative solutions for enhancing the valorisation of biomass produced from civil and industrial wastewaters. In particular, the main focus was to test and evaluate some chemical and thermal approaches to valorise sludge biomass from municipal wastewater treatment plants (WWTPs).

The production of sludge from WWTPs is a significant problem given the total annual production of biosolids which is on the rise, European legislations on effluent discharge and current strategies for sludge management. Solutions to transform this sludge into a valuable resource based on the concept of circular economy thus is necessary. Therefore, the work of the thesis was towards achieving this goal also utilizing some approaches that were mainly investigated for other kinds of biomass.

Firstly, pre-treatments were performed on sludge to break the sludge flocs and cells and to enhance solubilisation of organic matter present. Individual and combination of physical (ultrasonication), chemical (sodium hydroxide addition), low temperature thermal treatment (75 °C) was carried out. The effect of these pre-treatments on sludge solubilisation was studied and also potential for bioenergy estimated on the solid part after pre-treatments. A combination of chemical and thermal method was found to be most efficient among others.

To further improve the hydrolysis of sludge, hydrothermal treatments were done in mini autoclaves with varying conditions of temperature, pH, and reaction time to find the best operating conditions. The influence of hydrothermal tests was analysed by measuring chemical oxygen demand (COD) and carbohydrates concentrations in the liquid phase. Using the ideal operating parameters, larger batch hydrothermal treatments were performed to evaluate the effect of the different variables involved in the hydrothermal treatment, namely hydrothermal carbonization at milder temperatures, on the solubilisation of organics and the consequent reduction in sludge volume.

Utilization of sludge as a source for energy and resource recovery is a promising alternative in terms of sludge management. So, valorisation on solid and liquid fractions of hydrothermally

treated sludge were attempted. On the solid fraction, thermal valorisation by pyrolysis was performed as it is a promising technology for stabilization and resource recovery of sludge due to the ease and efficiency of operation. On the liquid fraction, preliminary studies such as biotechnological transformations with commercial enzymes, fermentation and protein precipitation were investigated.

Furthermore, valorisation of sludge that was meant for disposal into landfills was carried out. This was done by pyrolysis experiments at 500 °C and 800 °C to study the effect on the product yields. Determination of the product distribution and characterisation of char, liquid, and gaseous products were assessed to enable an efficient valorisation of this stabilised sludge.

Finally, pre-treatment and hydrolysis studies on a particular type of waste biomass, namely, brewer's spent grains (BSG), a by-product of brewing industry was investigated. This was part of brief research period at Wroclaw University of Science & Technology, Poland and was considered to be the first step in valorising this agro-industrial biomass for recovery of value-added molecules and energy.

Chapter 1: Introduction

1 Introduction

1.1 Sludge definition and characterisation

Wastewater treatment plant (WWTP) is a facility for the removal of pollutants from wastewater using physical, chemical, and biological processes. The water after the depuration treatments can be released back into the environment on the basis of the national and regional regulations. Sludge is a by-product of the wastewater treatment process in WWTP. It is a semi-solid slurry which has to be treated further before disposal. Different kinds of sludges are produced in a municipal WWTP based on the type of treatment undergone by it such as shown in Figure 1.1 [1]:

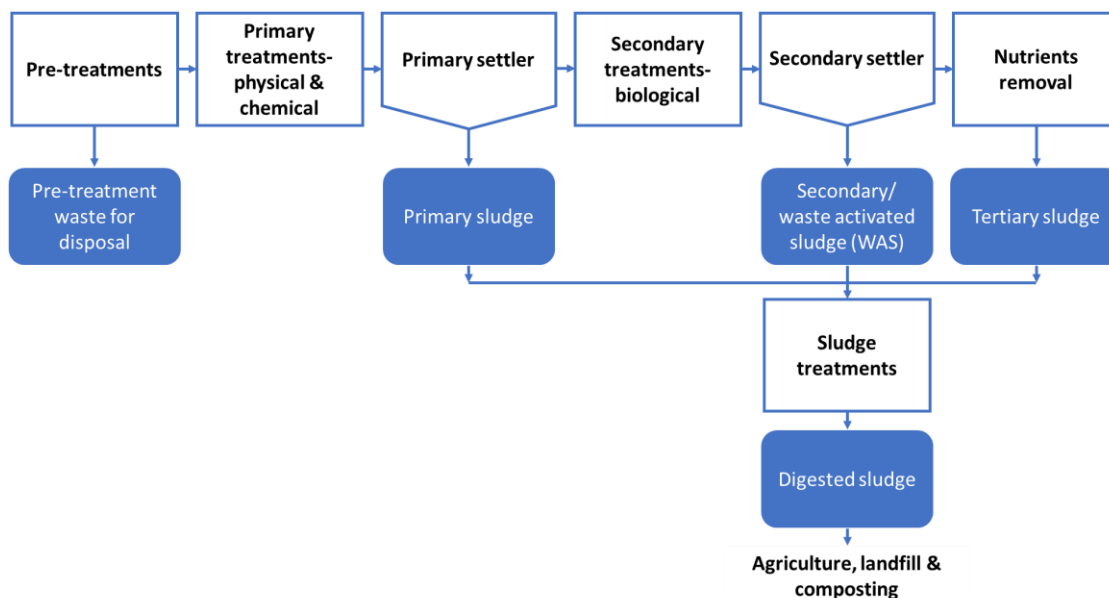


Figure 1.1. Points of sludge production in WWTP

1.1.1 Primary sludge

It is the sludge generated after the primary treatments like screening, grit removal, flotation, precipitation, and sedimentation [2]. Materials that settle or float including heavy solids, oil and grease are removed from wastewater here while the remaining liquid passes into secondary treatment. Total solid concentration in primary sludge is between 2- 9% and > 90% is water even occasionally as high as 99.5% [3].

1.1.2 Secondary sludge

This sludge also known as waste activated sludge (WAS) is produced as a result of secondary treatments. Removal of suspended and dissolved organic materials takes place here. It is usually a biological treatment stage where microbes break down the biodegradable organic matter in water. [4]. Prior to discharge of the liquid to tertiary treatment, it may be necessary to separate the microorganisms present [5]. In most of the conventional WWTP facilities the sludge separation is performed by sedimentation and recently in more efficient processes a filtration on membranes is used to recover the activated sludge. Due to the process conditions and to the continuous availability of organic substrate in the wastewater the biomass grows and therefore the excess of biomass which cannot be recycled to the oxidation tank needs to be disposed. Solids content present in the waste activated sludge is between 0.8- 3.3% and water concentration 96.7- 99.2% [6]. WAS contains high concentration of microorganisms, basically bacteria, protozoa and fungi aggregated as flocs. From the chemical point of view the sludge comprises large fraction of organic matter 59- 88% with carbon content of 50- 55%, oxygen 25- 30% and also includes nutrients like 10- 15% nitrogen and 1- 3% phosphorus and hydrogen 6- 10% and sulphur 0.5- 1.5 % [3]. The ash of secondary sludge is composed of largely of minerals like calcite, and quartz and ions like Ca, K, Mg and Fe. Also, some heavy metals like Ni, Cr, Zn, Cu, Hg, Pb and Cd can be found [7].

1.1.3 Tertiary sludge

Tertiary sludge is generated during the advanced/ tertiary wastewater treatment stage where nutrients like nitrogen and phosphorus are removed. Advanced decontamination is done using chemical or biological processes to eliminate unwanted nutrients [2].

As a final step, the sludge undergoes further treatment in order to lower the water content, for stabilization of organic material and disinfection of the end product, thereby making it available for either final disposal or reuse. This additionally treated sludge can be categorised as aerobic or anaerobic stabilized sludge depending on the treatment conditions. The treatment techniques include thickening, aerobic or anaerobic stabilization, mechanical dewatering, and thermal drying [8].

The chemical and physical properties of sludge depend on the type of pollutants present in the raw wastewater and also the kind treatment or stabilization process applied to it. Table 1.1 summarises typical properties of primary and waste activated sludges [6, 8].

Table 1.1. Common characteristics of primary and WAS sludges

Parameter		Primary sludge	Waste activated sludge
Total solids (TS)	% TS	2-9	0.8-3.3
Volatile solids (VS)	% TS	60-80	59-88
Protein	% TS	20-30	32-41
Oil and fats	% TS	7-35	5-12
Carbohydrates	% TS	-	6.1-9.8
Nitrogen	% TS	1.5-4	2.4-5
Phosphorus	% TS	0.2-2.8	0.5-2.3
Organic acids	mg/L acetate	200-2000	1100-1700
pH		5-8	6-8
Energy	MJ/kg TS	2.9-23	19-23

The concentrations of solids, organics, nutrients, and energy contents are different for both sludges. Primary sludge has higher solids content whereas secondary sludge has higher nutrient contents thereby making it more appropriate for resource recovery and use in agriculture while primary sludge is more suitable for anaerobic digestion.

1.2 Sludge production

The European Union (EU) with a population of 500 million people is estimated to have a total sludge production of about 13 million tons of dry matter (DM) as of 2020 from WWTPs [10]. As per latest data available on Eurostat, 10 million tons DM were produced across Europe in 2015 as shown in Figure 1.2 [11,12]. Data for Spain, France, Netherlands, Austria, Finland, Greece, and Estonia is taken from 2014, data for Switzerland, Latvia, Bosnia & Herzegovina refers to 2013, data for UK, Portugal, and Belgium is from 2012 and data for Italy and Denmark is from 2010 for representing in Figure 1.2. It can be seen that greater than 60% of the total sludge was generated by Germany, UK, Spain, Italy, and France. Eastern countries like Poland, Romania, Latvia, Estonia, and Lithuania together contribute to 12% of overall sludge production in EU.

From Figure 1.2, it is clear that Italy ranks among the top 5 producers of sludge in Europe. However, recent official reports on quantity of sludge produced in Italy are lacking on Eurostat. Available data reported 1.1-million-ton dry sludge generated in 2010 [12]. As per records of ISPRA, between years 2015 to 2018, yearly sludge production was between 3.07- 3.18 million

tons of wet sludge [13]. If the average concentration of dry solids is assumed to be 22.5% of wet sludge, then the estimated value of dry matter produced was 7.16 thousand tons DM in 2016 [14]. This shows a decrease in the amount of DM produced from 2010 to 2016, however, a complete comparison is not possible due to the limiting value of dry matter that was assumed in 2016. According to ISPRA data, nearly 40% of the sludge was produced in northern Italy in 2018 [13].

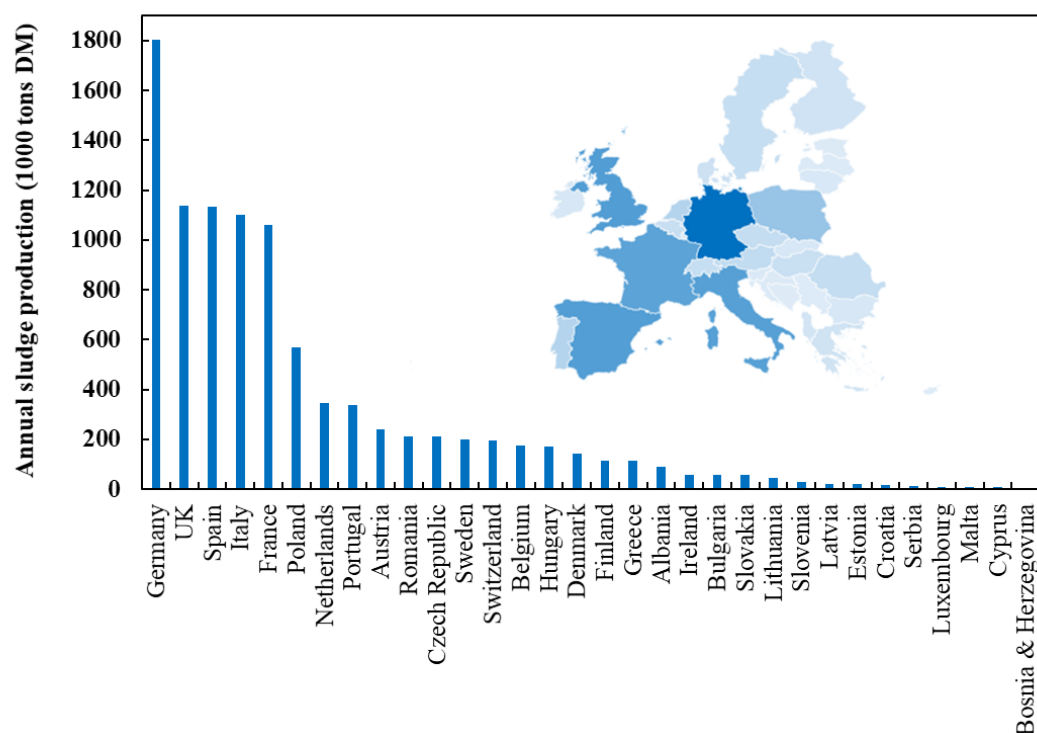


Figure 1.2. Annual sludge production in Europe in 2015 [11,12] DM- dry matter

1.3 Traditional sludge management

Figure 1.3 shows the current trends across Europe and Italy regarding sludge disposal based on Eurostat [12,14]. In Europe, use of sludge in agriculture is greatest as fertilisers or for land spreading. This is followed by 23% for incineration either directly or by mixing sludge with other wastes, then in composting by combining sludge with organic matter for use in parks. Smallest percentage is for landfilling by depositing sludge in special sites that serves no purpose. In Italy, disposal in landfill accounts for the highest utilisation of sludge. Amount of sludge dedicated for agricultural use and composting in Italy is almost same as that followed by Europe. While a really small fraction of 3% is sent for incineration as compared to 23% in

Europe. 20% is other methods that refer to different methods of disposal in different countries. Among European countries ‘others’ include storage in WWTP, soil enrichment, forestry, land reclamation and landscaping. However in Italy, others refer to blending, mixing and repackaging and using sludge as a fuel and for storage according to ISPRA [13].

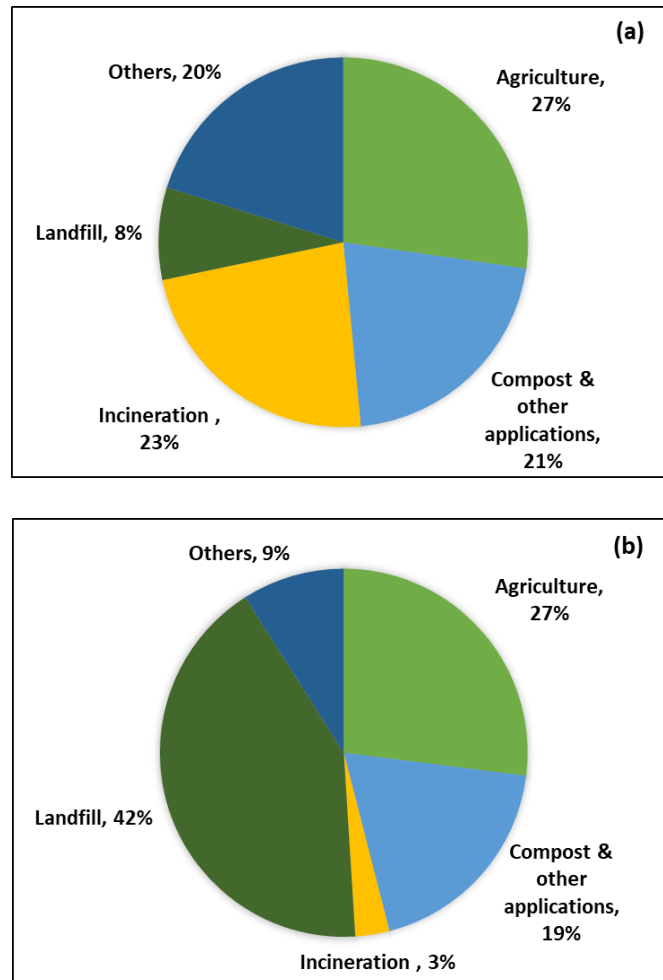


Figure 1.3. Sludge disposal methods in (a) Europe and (b) Italy

To get a better insight, the traditional sludge strategies are described below:

1.3.1 Agricultural use

Though almost 27% of sludge generated in EU is utilised for agricultural purposes, some of the EU countries have implemented individual stringent limiting values for pollutants in addition to the ones mentioned in Sewage Sludge Directive (SSD). Some of them have included some additional contaminants on the SSD list while some others have discarded this method of sludge disposal due to environmental consequences of reuse of sludge in agriculture [15]. Employing sludge in agriculture is one of the best practicable environmental options however,

it depends on the decision of the farmers ultimately. If the agricultural land is used for production of food crops, then it is necessary to perform special sludge analyses and implement appropriate procedure to prevent the leaching of the contaminants in sludge. Nevertheless, utilisation of sludge for agriculture remains the most feasible option due to the recovery of nutrients in waste sludge [16].

1.3.2 Landfill

Use of sludge in landfilling is the least preferred choice of disposal due to stricter EU regulations, negative consequences of leachate produced and carbon dioxide emissions [2]. In the last two decades, only Italy, Denmark, and Estonia have reported an increase of landfill depositing [17].

1.3.3 Incineration

Incineration allows for complete oxidation of organics at elevated temperatures. Sludge solids are combusted in the presence of excess air supply to form water and carbon dioxide with inert ash as a residue. This ash can be further used in production of building raw materials or disposed away [3]. Incineration results in reduction of sludge volume and improved energy recovery and thus it has been increasingly adopted by many EU countries like Germany and Netherlands in comparison to other sludge management techniques [18].

1.3.4 Composting

Composting of sludge converts it into a stabilised form that be used as a value-added product or as organic fertilisers [19]. The major influences on organic material stabilization and microbial activity is the moisture content, pH and carbon-to-nitrogen ratio of the sludge [20]. Under aerobic conditions, the organic matrix of sludge is converted to simple molecules by the microorganisms and enzymatic hydrolysis. Process of composting can be divided into three steps that is temperature dependent [19]. So, the limiting factors affecting composting are inadequate temperature, complexity of sludge, presence of pathogens and absence of microbes. Also, the need for composted sludge to comply with properties of organic fertilisers limits their use [16]. Despite the drawbacks, countries like France, Hungary, Sweden, Slovakia, and Czech Republic have largely adapted composting [21].

However, with more attention being given to stringent legislation and environmental protection, alternative methods for sludge minimization and disposal like sludge digestion has been on focus.

1.3.5 Anaerobic digestion

Anaerobic digestion (AD) is a significant technology as it includes production of biogas from organic solids, removal of microbes, and stabilization of sludge solids for further dewatering [22]. Co-digestion with organic matter produces methane for recovery of energy with low impact on environment making it an important cost-efficient methodology [23]. AD process consists of several biochemical processes like hydrolysis, acidogenesis, acetogenesis and methanogenesis and several pre-treatments can be applied prior to AD [24]. Pre-treatments help to improve AD performance by damaging the microbial cells to enable the release of organics for further biological transformations and lower the retention time required for AD.

1.3.6 Aerobic Digestion

It is another digestion method that takes place in an aerated reactor and is affected by reaction temperature and retention time [25]. Aerobic digestion is a sludge stabilization process that enables a rapid disintegration of sludge biomass at high temperatures and short retention times while disabling pathogenic activity. However, at temperature above 35 °C, ammonium nitrogen accumulates due to the inhibition of nitrification and de-nitrification processes that will lower the microbial activity and thus sludge stabilisation [26].

The application of the best sludge management technique depends on the costs involved for effective operation, leaching of heavy metals into environment and suitability of sludge for land spreading [15]. Though the above methods for sludge management and minimization exists, in general effective sludge management still poses a major concern globally.

1.4 European Union and Italian National legislation on sludge management and disposal

EU legislation establishes the set of quality standards for different methods of sludge management, disposal, recycling, and alternative techniques as given below [2,15,18,27]:

- Directive 1986/278/EEC also known as Sewage Sludge Directive (SSD) is concerning environmental protection primarily on soil with reference to use of sludge in agriculture.
- Directive 1975/442/EEC also called Waste Framework Directive along with its amendments 1991/156/EEC and 2006/12/EC of the European Parliament and of the Council refers to the regulations for handling sewage sludge problems.
- Directive 1991/271/EEC with its amendment 1998/15/EEC known as Urban Water Treatment Directive is about improving wastewater treatment processes and increasing the number of already existing WWTPs. It obliges to monitor and report municipal sludge treatment and disposal method for agglomerations. Article 14 of this directive also states appropriate reuse of sludge to avoid negative consequences.
- Directive 1999/31/EEC called Landfill Directive deals with the increase in the constraints on the amount of biodegradable waste matter that can be deposited in landfills due to concern regarding production of methane under anaerobic conditions.
- Directive 2003/33/EEC on establishing criteria and procedures for the acceptance of waste at landfills; pursuant to the Article 16 and Annex II of Directive 1999/31/EC.
- Directive 2000/76/EEC also called Incineration Directive is concerning waste incineration which reports the regulations and restrictions for incineration of waste and emission standards.
- Directive 1989/369/EEC refers to the prevention of air pollution from municipal WWTPs.
- Directive 1991/676/EEC is on water protection from pollution by nitrates from agricultural sources.
- Directive 1991/689/EEC on controlled management of hazardous waste.
- Directive 2001/118/EEC about the European Waste Catalogue (EWC).
- Decision 2000/532/EC establishing a list of wastes, as amended.
- Directive 2008/98/EC regulates recycling of wastes including sewage sludge. As per this directive, sludge is a waste that must be subjected to procedures available for waste treatment. Also, its first priority is waste prevention followed by waste reuse, recycling, recovery and finally disposal.
- Fertilising Products Regulation EU2019/1009 is about encouraging a greater shift of EU from use of mineral fertilisers to organic ones and developing a circular economy for nutrients.

- National Decree 2010/75 concerns the use of fertiliser and manure produced from sludge in agriculture in Italy.
- National Decree 1992/99 as revised by Decree 2018/130 regulates the sludge stabilisation treatment and the maximum quantity of sludge that be utilised in a particular area during a period of three years. It also bans the use of sludge on flooded soil and on land dedicated for animal feed, food crops, and horticulture.
- National Decree 2010/75 governs composting rules following EU 2019/1009 regulation.

1.5 Valorisation of sludge - Circular Economy

To date, sludge is used with difficulty both in agriculture in the formulation of compost and in general on energy purposes. The application for the production of biogas is more recent, but today it poses several problems for its purification. In most cases, therefore, the sludge is brought into landfills similar to municipal solid waste or in the case of presence of toxic substances and pollutants in special landfills. Since waste sludge is based on organic material, according to the Italian and European directives as of 2020 such waste due to their organic content can no longer be disposed of in landfill other than those destined for the collection of special waste. Disposal through agriculture is always difficult as the formation of compost from sludge is limited and there is always a concern regarding metal content and for the possible presence of contaminants.

Though sludge is thought of as waste material, it could be used as a source of energy or for resource recovery thereby aiding the replacement of an equivalent quantity of non-renewable based materials or energy with significant environment effects [16]. The presence of heavy metals, toxic pollutants, organic contaminants, and pathogens in residual sludge can have considerable impacts on health and environment thereby, making effective sludge disposal and treatment methods essential [19]. However, sludge treatment is an expensive process costing about 50% of the total running cost of WWTPs and needs high amounts of energy [27]. Moreover, sludge disposal techniques represent 40% of the total greenhouse gas emissions from WWTPs [28].

It is therefore necessary to think of innovative solutions for the management and treatment of the huge quantity of sludge generated each year both by civil and industrial plants. Such

solutions in a more modern vision of "circular economy" will be utilised in such a way as to make a waste material a valuable resource. The concept of circular economy as shown in Figure 1.4 states that starting and final products of a process should persist in the economy for a long period and waste must be considered as primary or secondary raw materials for recycle and reuse, even WWTPs could be integrated into this circularity from a linear system. In this context, the sludge from the WWTPs can be considered, for example, as a raw material for the extraction of compounds of interest or for the synthesis of compounds and materials according to the principles of Green Chemistry. It will allow to valorise the treated wastewater sludge in the WWTPs using direct or indirect routes to be used not only for energy purposes but in particular as a carbon source for the production of primary and secondary raw materials.

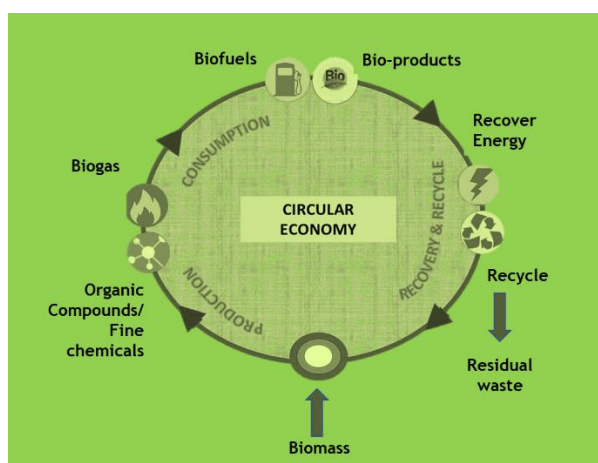


Figure 1.4. Concept of Circular Economy

1.6 Sludge pre-treatments

Pre-treatment of sludge is necessary to open up its complex heterogenous matrix to make the simple molecules available for further treatments (e.g., separation, purification, conversion). Till date, most of the WWTP sludge treatments were applied mainly with the aim of reducing the amount of sludge, but it is also possible to use some of those treatments that can be tailored as pre-treatment steps which can favour the release of interesting classes of molecules in a circular economy approach. The pre-treatment methods can be classified as thermal, mechanical/physical, chemical, and biological [29].

1.6.1 Thermal pre-treatments

Thermal treatment is an existing well-established technology which is used to increase sludge dehydration and enhance anaerobic digestion of sludge. This method is dependent on operating temperature, pressure, and reaction time. The application of heat changes the sludge structure and improves solubilisation by increasing the sludge biodegradability [30]. This technique helps to break the chemical bonds in the microbial cell walls and membranes to release the cellular molecules into the aqueous phase. It also enhances sludge dewaterability by destroying the complex sludge matrix to enable release of water and the heat from the reaction kills pathogens making the treated sludge more sterile [31]. Heat recovery during thermal treatment will help to lower the pre-treatment costs and improve energy efficiency. Depending on the range of temperature applied, thermal treatment can be classified as low temperature treatment (below 100 °C) and high temperature treatment (above 100 °C) [32].

1.6.1.1 Low temperature thermal pre-treatment

Temperature ranging between 50- 90 °C has been suggested to overcome the cons of other intensive treatments like production of toxic refractory compounds, and higher energy demands [32–34]. Low thermal treatment increases physical rupture and release of endogenous enzymes in sludge flocs resulting in improved sludge solubilisation, production of volatile fatty acids, increase in biogas generation. For instance, protease secreting bacteria was found in sludge treated at 60 °C for 1 h which proposes that since the enzymes originate from sludge itself, they are significant to solubilise sludge components [35]. However, treatment time is longer and could be from few hours to several days [36].

1.6.1.2 High temperature/ Hydrothermal pre-treatment

Hydrothermal treatment is performed by heating sludge at operating temperatures in the range of 150 to 450 °C and in the presence of an inert atmosphere [37]. During hydrothermal treatment, sludge hydrolysis takes place which increases the solubilisation of high concentrations of organic molecules in the aqueous phase [38]. Hydrolysis of proteins, lipids and carbohydrates in sludge produces amino acids, fatty acids, and sugars respectively. The residue of hydrothermally treated sludge could be utilised for their nutrient contents like nitrogen, phosphorus, and potassium in bio-fertilizers. In literature, hydrothermal treatment of sludge at 190 °C and 20 bar enhance sludge dehydration and water concentration was lowered

to 55% by mechanical dewatering [3]. Additionally, it is advantageous because improves biogas production, decreases sludge viscosity, microorganisms and odour [39].

1.6.2 Physical/ mechanical pre-treatments

During physical treatments, no chemicals, enzymes, or other extra molecules are added. They improve the surface area, bulk density and porosity of sludge and increases the chances of contact between organics in sludge and microorganisms.

1.6.2.1 Ultrasonication

With ultrasonication, sludge biomass undergoes continuous and fast compression and decompression cycles produced by ultrasonic sound waves. The bubbles generated during this method tends to collapse after attaining a specific size and forms a cavity. This rapid and violent collapsing results in the formation of hydromechanical shear forces that can destroy sludge flocs, microbial cell walls and membranes, thereby increasing the solubilisation of organics and sludge dehydration [40]. The energy supplied by ultrasonic waves for sludge hydrolysis results is utilised for generation of strong shear forces, formation of radicals, breakdown of volatile solids, and raising the process temperature [41]. The concentration of sludge total solids, specific energy and frequency applied influence the degree of disintegration. Low frequencies between 20- 40 kHz provides sufficient mechanical forces to hydrolyse organic compounds like carbohydrates and proteins that enhances methane yield [42]. However, energy consumed is high and this must be equilibrated with production of biogas to make this treatment feasible.

1.6.2.2 Microwave

Microwave treatment uses electromagnetic radiation with wavelength of 1mm- 1 m and frequencies between 0.3- 300 GHz. Microwaves elevates the kinetic energy of water dipoles promoting them to boil quickly and this shift in the orientation of polar molecules along with thermal effects breaks hydrogen bonds and ruptures the complex sludge matrix [43]. This treatment method enhances the disintegration of organics in sludge biomass, eliminates pathogens and improves biogas yields [44].

1.6.2.3 Pulsed electric field pre-treatment

This technology applies high voltage pulsating electric fields. The electric charges produced give rise to shock waves that cause rapid breakage of sludge flocs and biological membranes [41]. Thereby, improving sludge hydrolysis and making molecules available for fermentation bacteria [45]. However, this method lacks significant research in this field, high energy consumption and electrode erosion.

1.6.2.4 High pressure homogenization

This treatment relies on creation of sudden pressure gradient, high cavitation, turbulence, and mechanical shear forces to hydrolyse sludge when it is subjected to strong depressurization up to 900 bar [46]. When the external pressure becomes greater than the internal resistance of sludge flocs/ cells, both the microbial cell walls and membranes rupture thereby, allowing sludge solubilisation [47]. Low initial investment, ease of operation, and high energy efficiency are the advantages of using this treatment.

1.6.3 Chemical pre-treatments

Chemical pre-treatments are a commonly used technique for efficient break down of complex heterogeneous sludge matrix. Addition of an acid, alkali, ozone, and ionic liquids hydrolyses the biopolymers in the sludge cells making the organic molecules available for valorisation.

1.6.3.1 Acid and alkali pre-treatments

Acid and alkali treatments is an important treatment method due to high solubilisation of sludge, ease of operation, and use of simple equipment [48–50]. Acidic treatment generally utilises acids like hydrochloric acid (HCl), sulphuric acid (H₂SO₄), phosphoric acid (H₃PO₄), and nitric acid (HNO₃). While alkaline hydrolysis employs sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)₂), magnesium hydroxide (Mg(OH)₂), calcium oxide (CaO), and ammonia (NH₃). Addition of an alkali or acid prevents the need for using high temperature, thereby enabling treatment to take place at room or lower temperatures. Commonly used pH ranges for acid and alkali treatments are 1- 4 and 10- 13 respectively. Alkaline treatment is more suitable than acid for sludge hydrolysis and the alkali best suited for this is NaOH followed by KOH, Mg(OH)₂ and Ca(OH)₂ [51]. However, pH adjustment needs to be done after treatment like neutralising with an acid which increases the cost of pre-treatment. Therefore, it is vital to optimize the amount of reagent that is added to improve sludge hydrolysis.

1.6.3.2 Ozonation

Oxidation with ozone gas is used as a pre-treatment for sludge hydrolysis and increasing biogas yields. Ozonation involves subsequent decomposition reactions resulting in rupture of flocs, solubilisation of biodegradable organic matter, and therefore oxidation of the organics released [52,53]. The efficiency of this process depends on the concentration of ozone employed. Ideal ozone dosage is between 0.05-0.5 g ozone/g TS depending on sludge characteristics [24].

1.6.3.3 Fenton process

Oxidation with Fenton is a combination of hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}) catalyst to produce hydroxyl radicals with high oxidation potential at a pH between 2 and 3 [54,55]. The hydroxyl radicals destroy the solubilised organic compounds without the production of any inhibitory by products. This technique improves sludge hydrolysis, biodegradability, and dewatering and is influenced by concentration of H_2O_2 , initial pH, reaction time and temperature.

1.6.3.4 Ionic liquid

Ionic liquids with low melting point below 100 °C and non-volatile properties are used for ionic liquid pre-treatment [29]. Characteristics of ionic solvents like thermal stability, low vapour pressure, suitability for low temperature reactions makes it a viable pre-treatment. It is mostly used as a pre-treatment for bioethanol production.

1.6.4 Biological pre-treatments

Biological pre-treatment includes the involvement of microorganisms either directly or indirectly to break down the sludge matrix and is an eco-friendly method. Biological pre-treatments could be pre-digestion using anaerobic or aerobic microbes (as discussed previously in Section 1.3), enzymatic hydrolysis or addition of fungi.

1.6.4.1 Enzymatic hydrolysis

Biopolymers in the microbial cells of sludge can be destroyed by the addition of enzymes that improve sludge solubilisation to release organic molecules into the aqueous phase. Enzymes convert the cellular components into lower molecular weight molecules that can be digested easily. Each class of enzyme is responsible for a particular group of organic matter [56,57]. Proteases and cellulases are vital for the macromolecular break up of sludge to aid the release of amino acids and simple sugars respectively. Lysozymes destroy β -1,4 glycosidic linkages between N-acetylmuramic acid, N-acetylglucosamine and

cell walls resulting in microbial lysis while lipases act on the lipid fraction. Enzymatic pre-treatment is better in comparison to other chemical pre-treatments as it avoids the release of toxic by-products. However, the high cost of commercial enzymes and lower yield of solubilisation restricts its full-scale application as a sludge pre-treatment [29].

1.6.4.2 Fungal pre-treatment

Fungal treatment decomposes the biomass matrix with fungi like brown, white and soft rot. Parameters like reaction time, temperature, moisture content in sludge play an important role on the efficiency of this treatment [58]. Although fungus addition does not require other chemicals and has low energy consumption, this method has long reaction times, larger area for treatment and necessity for particular species to develop continuously. These cons have to be overcome for this pre-treatment to be more feasible.

1.7 Energy recovery from sludge

Energy recovery in waste sludge management is of significance due to its influence on world waste minimization, alternate energy production, and resource optimization. Conversion of sludge to solid/liquid fuels, syngas, heat, and electricity can be achieved using technologies like combustion, pyrolysis, and gasification.

1.7.1 Combustion

Combustion is complete oxidation that is carried out at temperatures of 850- 1000 °C and in excess supply of oxygen to produce carbon dioxide, water vapour, heat, and some trace gases. Combustion process can be applied to sludges either for primary heat generation using conventional combustion technology or for reducing sludge volume by incineration. Dewatered and dried sludge is used so that it has sufficient heating value to support auto-combustion [59]. In case of insufficient calorific value of sludge, then co-combustion is performed with substances having a higher lower heating value [60]. Most commonly used material for co-combustion with sludge is municipal solid waste.

1.7.2 Pyrolysis

Pyrolysis process is the thermal decomposition of fuel between 350- 900 °C in a non-reactive atmosphere which is the absence of oxidizing agents. During pyrolysis, sludge is not subjected

to combustion whereas, sludge floc degradation occurs due to the action of high temperature [60]. Pyrolysis generates three products that is solid char, pyrolytic liquid, and gas. Char can be utilised as solid fuel or for producing activated carbon. Pyrolysis can be performed with a focus on obtaining predominantly char, bioliquid or gas by varying the operating parameters [61]. The quality and quantity of the pyrolysis products depends on residence time, reaction temperature, pressure, and sludge characteristics. Pyrolysis process can be categorised based on heating rate, residence time and temperature as slow pyrolysis with low operating temperature of 300- 400 °C, greater residence time of 5 to 30 min and heating rate of 0.1- 1 °C/s. While fast pyrolysis is performed at higher temperature range 450- 600 °C, smaller residence time 0.1- 0.3 s and higher heating rate of 10- 200 °C/s making it more fitting for the conversion of sludge to pyrolysis liquid or gaseous products. Some of the advantages of pyrolysis technique includes decreased formation of carbon dioxide, reduction of dioxins, simple set up of the pyrolysis system, flexibility of the process and reduction of sludge residue [62].

1.7.3 Gasification

This technology allows the conversion of organic contents in sludge into ash and combustible gas like syngas (H_2 and CO) and also CO_2 , CH_4 and H_2O . Gasification takes place generally at high temperatures of 800- 1000 °C in an atmosphere of oxygen, air, carbon dioxide, steam, or a mixture of these gases [63,64]. Partial oxidation occurs as the amount of oxidizing agent provided to the process is lower than the stoichiometric value as compared to the total organic carbon present [60]. Ash produced as a result of gasification can be deposited in landfills or applied for agricultural use or in construction. While the gas product can be utilised for generation of heat and electricity, after processing as syngas or for chemical synthesis. Advantages of gasification include removal of water contents of sludge, generation of syngas to be utilised for energy recovery and decrease in the concentration of sludge volatiles.

1.8 Resource recovery from sludge

For efficient sludge management and within the concept of circular economy, sludge can be considered as a feedstock for production of bio-products and for resource recovery and this plays a vital role in the conversion of WWTPs into biorefineries.

1.8.1 Proteins

The main organic components of waste activated sludge are proteins, lipids, and polysaccharides. Proteins contribute to about 50% of dry weight of the microbial cells and so WAS can be utilised as a source of protein or amino acids [65]. Protein derived from sludge can be used in animal feed for providing nitrogen and energy after necessary removal of toxic substances and heavy metals and sterilization has been done. Thus, recovery of proteins from sludge is more beneficial than conventional protein recovery sources. The important part in protein recovery is the efficient hydrolysis of cellular components using different extraction methods which include thermal digestion, chemical methods like solubilisation and precipitation using acids, alkalis, salts and solvents, and detergent based extraction agents [66]. All these methods improve destruction of sludge flocs and biological cell lysis.

1.8.2 Short chain fatty acids

Recovery of short chain fatty acids like formic acid, acetic acid and propionic acid from WAS can be done using anaerobic digestion, thermal treatments like hydrothermal processes, wet air oxidation [67]. These recovered short chain fatty acids could be utilised as feedstock and be transformed into fuels like primary or secondary alcohols and hydrocarbons, or chemicals like carboxylic acids, ketones, esters, and ethers.

1.8.3 Enzymes

~80% of sludge organic matter is comprised of carbohydrates, proteins, and lipids [3]. So, the recovery of several enzymes like proteases, lipases glycosidase, galactosidases, aminopeptidases, dehydrogenase, catalase, peroxidase, phosphatases, α -amylase, and α -glucosidase from WAS is significant [68]. These enzymes help to convert the large macromolecular components in sludge to smaller molecules. If the WAS derived enzymes could replace the commercial enzymes in food, paper and pulp, pharmaceutical, cosmetic, and fine chemicals industries, it would lower costs because currently 30- 40% of the total production costs are related to industrial enzymes [69]. Most commonly used extraction method is ultrasonication alone or combined with detergents or ion exchange resins.

1.8.4 Nutrients

Waste activated sludge consists of substantial concentrations of nutrients like phosphorus P which is 0.5- 0.7 %TS and nitrogen N 2.4- 5.05 %TS existing predominantly in the forms of cellular proteins [3]. The decomposition and hydrolysis of WAS and its transformation to ammonia and phosphates could promote the production of magnesium ammonium phosphate (struvite). Struvite is an important plant fertilizer which can be directly applied on agricultural land [70]. P is considered to be a non-renewable resource and it is becoming necessary to recover this value-added product despite its high cost for recovery than commercial P.

1.8.5 Bio-pesticides

In recent time, *Bacillus thuringiensis* (Bt) is the best bio-pesticide in use that can produce δ -endotoxins and is largely used in forestry for pest control and agronomy for agricultural crops [66,71]. Bio-pesticides have no toxic residues and is environmentally friendly as compared to their chemical alternatives. The high cost of raw materials like a traditional fermentation medium for Bt contributes to about 40- 60% of the total cost of production thereby limiting their large-scale use. However, reuse of WAS as a nutrient rich and low-cost alternative medium for BT generation would likely resolve this problem and help with sludge management.

1.8.6 Bioplastics

Polyhydroxyalkanoates (PHAs) are biodegradable plastics made from biodegradable polyesters by microorganisms using a carbon substrate [72,73]. They are the best alternatives to conventional petroleum derived plastics as they do not pose any health or environmental issues and have applications for producing cups, bottles, packaging films, etc. Poly- β -hydroxybutyric acid (PHB) and its copolymer poly (3-hydroxybutyrate-co-hydroxyvalerate [P(3HB-co-HV)]) are the most commonly used forms of PHAs. Various microbes in WAS are capable of accumulating PHAs but the high cost of production has limiting effect on their wide use. However, the current innovations in WAS usage and bacterial fermentation are expected to enhance yield and lower the PHAs production cost [1].

1.8.7 Bio-flocculants and Bio-surfactants

Another value-added product of bacterial transformation of WAS are bio-flocculants and bio-surfactants [74,75]. Bio-flocculants, bio-surfactants, carbohydrates, lipids, cellulose derivatives are all released from microbes and are utilised in chemical fields like wastewater treatment processes, downstream processing, dredging, fermentation and food industries. Bio-flocculants and bio-surfactants have advantages over their corresponding synthetic ones due to their biodegradability, non-toxicity, and eco-friendly nature but the large production cost owing to sourcing of organic substrates limits their commercial use.

1.9 Aim of PhD work

The increasing worldwide energy consumption, higher energy requirements for future, exhaustion of fossil fuel resources and global warming have driven researchers to utilize renewable sources such as biomass to meet energy demands. The main aim of the PhD project was to explore experimental strategies to valorise the sludge biomass obtained from civil and industrial wastewater treatment plants.

As of 2020 in Europe about 13 million tons of dry solids were produced annually by WWTPs. With an increase in the number of WWTPs and stringent sewage discharge rules, sludge biosolid production rate is expected to rise in the coming years. Italy produces 1 million tons of dry matter and currently about 70% of the sludge produced in the WWTPs is considered as waste to be disposed of. Only in few cases it is valorised from an energy point of view, thereby minimizing its environmental impact.

At present, sludge management involves the disposal of sludge using conventional methods like incineration, landfilling and agricultural use. However, sludge is used with difficulty both in agriculture for the formulation of compost and in general for energy production. Moreover, stringent regulations have been imposed on sludge disposal procedures due to their socio-economic and environmental impact. It is therefore necessary to think of innovative solutions for the management and treatment of sludge. The present sludge disposal methods do not include the valorisation of sludge as feedstock for production of fine chemicals and bioproducts. In a vision of Circular Economy, valorisation of secondary sludge could be a sustainable method for producing value added bio-products like fuels and chemicals either by direct or indirect routes. To enable valorisation of waste activated sludge that is mainly

composed of microorganisms, first of all it is necessary to solubilize the organic carbon present. This organic carbon is contained in the cell walls and membranes of the microbes that will serve as raw materials for production of value-added molecules. Therefore, it is important to disintegrate the cell structures of sludge so as to enhance sludge solubilization.

Several sludge treatment methods have been explored in this work to enhance WAS hydrolysis including mechanical, chemical, thermal (Chapter 2), and hydrothermal treatments (Chapter 3). The treatments were focussed on destroying microbial cell structure and releasing organics and the effect of different treatments on sludge solubilization was investigated. Furthermore, an effort to valorise both the solid and liquid fractions of the hydrothermally treated samples were done (Chapter 4). Point of interest was to use the solid fraction to estimate bioenergy potential while on the liquid, recovery of organic molecules was attempted. In addition to this, raw sludge was attempted to be valorised by subjecting it to pyrolysis experiments (Chapter 5). A small part of the PhD research was also dedicated to studying the valorisation of brewer's spent grains (BSG) which is by product of beer manufacturing process in Poland (Chapter 6).

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Chapter 2: Effect of physical, chemical, and thermal treatments on sludge solubilisation and estimation of bioenergy potential

2 Effect of physical, chemical, and thermal treatments on sludge solubilisation and estimation of bioenergy potential

Within the vision of circular economy, valorisation of secondary biomass can offer a sustainable way to obtain useful products such as fuels and chemicals either directly or indirectly [1]. To facilitate valorisation of sludge, it is important to firstly solubilize the organic content present. Waste-activated sludge (WAS) mainly comprises microbes. The cell walls and membranes of the microbial cells contain organic carbon that can serve as raw materials for production of value-added materials [2]. However, the cell walls act as physical walls that prevent the easy release of these organics [3]. Hence, it is necessary to pre-treat WAS to hydrolyse the cell structure and, therefore, enhance sludge solubilisation [4].

Several treatment techniques have been investigated in the past to improve sludge hydrolysis. These include mechanical [5,6], chemical [7,8] thermal [9,10] and biological [11] methods. Mechanical methods such as ultrasonication improve the solubility of organic molecules by disrupting sludge flocs through cavitation phenomena. Alkali treatment, as a chemical treatment method, involves increasing the sludge pH to 12 with the addition of an alkali and maintaining it for a specified amount of time. This can increase the solubilisation of complex organics by destroying cells and flocs [12]. In thermal treatment, cell hydrolysis of sludge takes place because of a pressure difference. The most utilized temperature range for solubilizing organics varies from 60 °C to 180 °C [13]. Low-temperature treatment (<100 °C) requires longer reaction times than high-temperature treatment, but they can both be used to efficiently improve the production of biogas from secondary sludge [14].

Additionally, combined treatments (e.g., thermal–chemical [15], thermal–chemical–ultrasonic [16], ultrasonic–chemical [17], and thermal–ultrasonic [18]) have been studied previously on WAS. A summary of results from coupled treatments is shown in Table 2.1, suggesting that the combination of different treatments can improve sludge hydrolysis and the solubilisation of organics.

Table 2.1. Summary of combined treatment methods

Treatments	Conditions	Results	References
Thermal-chemical	130 °C, pH 12, 5 min	Enhanced biodegradability and solid solubilization	[15]
Thermal-chemical	50-90 °C, pH 9-12, 5-180 min	80 °C, pH 11 optimum	[16]
Thermal-chemical-ultrasonic	80 °C, pH 11, 20 kHz, 5-180 min	Improved COD solubilization and suspended solid reduction	
Alkali-ultrasonic	Ambient temperature, 40 meq/L NaOH, 20 kHz, 120 W, 24 h	Enhanced production of volatile acids	[17]
Ultrasonic	20 kHz, 1 h	Efficient hydrolysis on WAS	[18]
Thermal-ultrasonic	Thermal: 30-90 °C, 0-3 h Ultrasonication: 20 kHz, 20 min	Improved hydrolysis for thermal-ultrasonication compared to ultrasonic method	
Thermal-alkali	80 °C, 80 min, 0.1 M NaOH	Improved solubilization of organic matter in thermal-alkali method	[12]
Thermal	80-170 °C, 30 min-12 h		

This study focused on treating WAS to disintegrate its cell structure and release organics such as polysaccharides and proteins by using ultrasonication, chemical, thermal, ultrasonication–chemical, ultrasonication–thermal, chemical–thermal, and ultrasonication–chemical–thermal methods. Thus, in addition to single treatments, two or three of these individual methods were also combined and experiments carried out. The main objective of this chapter was to study the effects of different single and coupled treatments on the solubilisation of secondary sludge obtained from a municipal wastewater treatment plant. A thorough search of the relevant literature did not yield a similar compilation of these seven treatments performed on waste-activated sludge from municipal WWTPs all in a single study. Therefore, the work here was to give better insight into the disintegration efficiency of organics in secondary sludge after the abovementioned treatments for their further resource recovery, biotechnological transformation, or energy exploitation. Concentrations of total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), carbohydrates, proteins, and total nitrogen were measured. The solid fraction of sludge after treatments was further subjected to thermogravimetric analysis to determine the higher heating value (HHV) for estimating the bioenergy potential.

2.1 Materials and Methods

2.1.1 Materials

A municipal WWTP located in Genoa, Italy provided the raw WAS used here. Samples obtained were taken after the secondary settler unit of the WWTP. Sludge was stored at 4 °C, and the experiments were carried out with a living sludge. The major composition of raw WAS is given in Table 2.2. Prior to the treatments, sludge was thickened to a TS concentration of 15 g/L with a VS fraction of 12.2 g/L. Chemicals such as sodium hydroxide (NaOH) used for the tests was purchased from Sigma Aldrich and used as received.

Table 2.2. Characteristics of raw waste-activated sludge (WAS)

Parameter	Mean Value \pm Standard Deviation
pH	~ 7.0
Total solids (TS)	4.8 ± 0.1 g/L
Volatile solids (VS)	3.7 ± 0.2 g/L
Soluble chemical oxygen demand (SCOD) ¹	500 ± 10 mg/L
Soluble carbohydrates ¹	143 ± 5 mg/L
Total nitrogen ¹	230 ± 35 mg/L

¹Evaluated on the liquid fraction of sludge

2.1.2 Treatments

Physical (ultrasonication), chemical (alkali addition), and thermal (heating) methods, as well as their combinations, were applied to WAS. Each treatment was done using 80 mL of thickened sludge in 100 mL Pyrex bottles for a reaction time of 1 h.

Ultrasonication (U) was performed using an ultrasonicator bath (Bandelin Sonorex Digitec DT 52 H) operating at a constant and continuous frequency of 35 kHz and heating power of 140 W. The pH of sludge was not controlled, and only a negligible change in pH was observed after ultrasonication. A rise in temperature is expected during sonication due to the cavitation phenomenon [14]. However, during treatment, the sample was maintained at ambient temperature 25 °C by circulating cold water for a reaction time of 1 h. These ultrasonic parameters were selected following the work by Tiehm et al. [19] and Zhang et al. [20]. It was suggested that a low frequency (below 40 kHz) and longer sonication were optimum for sludge degradation [21]. Lowering the frequency produced cavitation bubbles large enough to collapse

and permit initiation of strong shear forces in the sludge sample. Furthermore, increasing the sonication time resulted in the disintegration of microbial cell walls and enhanced the solubilisation of organic matter [22].

The alkali (chemical) treatment (A) was carried out using 5M NaOH to set the initial pH of the thickened WAS to 12. NaOH was chosen as it was found to better influence sludge solubilisation [23]. The sample was stirred continuously at 200 rpm at room temperature for 1 h.

For thermal treatment (H), a heating temperature below 100 °C was considered. Microbial cell lysis was effectively studied between 60 °C and 100 °C in the lower temperature range [24]. Here, a median temperature of 75 °C was selected and, after heating WAS to the set temperature, the reaction continued for 1 h with constant heating and stirring at 200 rpm. The pH of the sample was not adjusted and remained at 7 before and after treatment.

In addition to the abovementioned individual treatments, combined treatments (ultrasonication–alkali (UA), ultrasonication–heating (UH), alkali–heating (AH), and ultrasonication–alkali–heating (UAH)) were performed to study the effect of the different treatments on the solubilisation of organic matter in WAS.

The UA method was a combination of ultrasonication and the addition of alkali at a fixed sludge pH of 12. The total time of treatment was 1 h. After NaOH addition, WAS was mixed and then sonicated, maintaining the batch experiment at room temperature by circulating cold water.

In the UH method, ultrasonication and thermal treatments were combined. The temperature of the sonicator bath was set to 75 °C, and sludge was ultrasonicated for a total treatment time of 1 h. The initial pH of the sludge was not modified.

The AH treatment combined the addition of NaOH to set the pH of sludge to 12 and subsequent low-temperature thermal treatment. WAS was heated at 75 °C with constant stirring at 200 rpm for a reaction time of 1 h.

The UAH method was the combination of ultrasonication, alkali addition, and thermal treatments. This treatment was performed analogously to the UH method with the difference being that the sludge pH was adjusted to 12 before the start of the experiment using 5M NaOH. After pH adjustment, sludge was sonicated for 1 h at 75 °C.

2.1.3 Sample analyses

After the treatments, the solid fraction was separated from the liquid phase by centrifuging (Neya 16, Italy) at 7000 rpm for 15 min. The aqueous samples were filtered using 0.2 μm cellulose acetate syringe filters (Merck, Darmstadt, Germany) before the determination of soluble composition. The Merck Spectroquant COD Cell Test (catalog number 114541 from Darmstadt, Germany) and the Merck Spectroquant Nitrogen (total) Cell Test (catalog number 114537 from Darmstadt, Germany) were used to measure SCOD and total nitrogen (TN), respectively. Proteins were estimated by multiplying organic nitrogen concentration by a factor of 6.25 [25]. The soluble carbohydrates were determined using Dubois's colorimetric phenol–sulfuric acid method with d-glucose as a standard solution for calibration [26]. Total solids (TS) and volatile solids (VS) measurements were gravimetrically done on the treated WAS samples as per Standard Methods for the Examination of Water and Wastewater 21st edition [27]. For TS calculations, samples were dried to a constant weight at 105 °C and weighed. This sample was then allowed to undergo combustion at 600 °C for measuring VS. All the experimental runs were performed in duplicate, and the results were shown as means \pm standard deviation. The percentage of solubilized TS and VS were calculated as follows:

$$\% \text{ solubilized TS} = \frac{\text{TS}_i - \text{TS}_f}{\text{TS}_i} \times 100, \quad 2.1$$

$$\% \text{ solubilized VS} = \frac{\text{VS}_i - \text{VS}_f}{\text{VS}_i} \times 100, \quad 2.2$$

where TS_i and VS_i refer to TS and VS before treatment, and TS_f and VS_f refer to values after treatments. Sludge pH was read using a pH 209 bench top pH meter (Hanna Instruments Italia, Ronchi di Villafranca Padovana, Italy).

2.1.4 Estimation of higher heating value by thermogravimetric analysis

The energy potential of sludge can be determined from its HHV. Most commonly, a bomb calorimeter is used as a standard to obtain this value; in the absence of this instrument, HHV can be estimated from ultimate and proximate analysis. However, a special instrument is required to carry out ultimate analysis. Although proximate analysis can be done using more

commonly used instrumentation, the time needed for the experimental runs is higher and strongly dependent on the operator's skill. Thus, thermogravimetric analysis (TGA) has been successfully used in the past to determine the main analytical parameters such as moisture content (MC), volatile matter (VM), fixed carbon (FC), and ash (A) required for estimating HHV [28]. Furthermore, TGA allows faster analysis with smaller quantities of samples. Here, TGA was done using a thermal analyzer (TGA/DSC 1 Mettler Toledo, Milan, Italy) to estimate the HHV of the sludge sample as per ASTM Standard E1131-20 [29]. The experiments were done on the solid part of the sludge obtained after centrifugation before and after all treatments. Approximately 7–8 mg of solid samples pre-dried at 60 °C were placed in an alumina crucible for the analysis. In the first segment of the experiment, the sludge sample was heated at 10 °C /min in an inert environment (N₂ gas) from ambient temperature to 110 °C with a hold time of 5 min. Then, the sample was heated to 950 °C with the same heating rate and an isothermal period of 7 min at this temperature. After this, at 950 °C, carrier gas was switched to O₂ with a dwelling time of 10 min to ensure complete combustion of the sample. N₂ and O₂ flow was maintained at 80 mL/min for the complete run. Prior to the sample run, blank experiments with an empty crucible were performed to obtain baseline correction. For accuracy of results, experiments were done in duplicate and reproducibility of the data was assured. The thermogravimetric (TG) curve was plotted using OriginPro software.

The following proximate parameters were evaluated as per ASTM Standard E1131-20 [29]: MC and VM were calculated in the nitrogen atmosphere; MC was measured as the weight loss between room temperature (starting temperature) and 110 °C; VM was considered to be the difference in mass at 110 °C and 950 °C, assuming that complete devolatilization took place before the combustion stage; FC was evaluated as the mass loss during the isothermal period at 950 °C when the N₂ carrier gas was substituted by O₂; Ash (A) was considered to be the residual mass at 950 °C in the oxidative atmosphere after combustion.

2.2 Results

The treated sewage sludge can be used as primary or secondary raw material to produce fuels or biomolecules such as amino acids, proteins, biosurfactants, short-chain fatty acids, and monomers for bioplastics. Here, the focus was to explore the ability of different treatments and their combinations to release organic molecules in the liquid phase, thereby enhancing

valorisation efficiency. Furthermore, the work was specially oriented toward a reduction in total solids, SCOD, carbohydrate solubilisation, and protein estimation from nitrogen content.

2.2.1 Solubilisation of TS, VS

Figure 2.1 shows the percentage of solubilized TS and VS after the different treatments calculated as per Equations 2.1 and 2.2 in Section 1.1.3. The concentrations of TS and VS in g/L after treatments are also represented in Figure 2.1. In general, for all the treatments, the percentage of TS solubilisation was lower than VS solubilisation. This is because, in addition to organic matter, TS is also composed of mineral solids which cannot be easily broken down [30]. Among the individual treatments, alkali addition with TS 6.2% and VS 11.2% seemed better than ultrasonication (TS 2.0% and VS 5.4%) and thermal (TS 2.5% and VS 7.0%) treatments at solubilizing total and volatile solids.

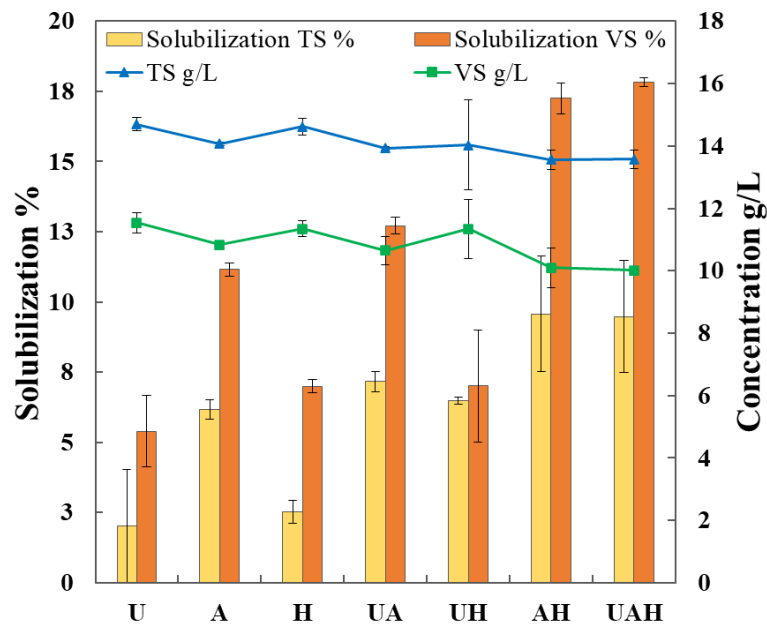


Figure 2.1. TS and VS in terms of percentage solubilized and g/L after various treatments. U, A, and H represent ultrasonication, alkali, and heating, respectively

Overall, combining at least two or more of the treatment methods improved the sludge solubilisation compared to the corresponding individual treatments. However, combining the ultrasonication method with heating had the least effect on solubilisation in terms of both TS (6.5%) and VS (7.0%) in comparison to other combined treatments. WAS solubilisation improved when chemical treatment was combined with ultrasonication (TS 7.2%, VS 12.7%)

and the values increased greatly for the combined alkali–thermal treatment to TS 9.6% and VS 17.2%. When all three single treatments were coupled, the solubilisation of TS 9.5% and VS 17.8% was similar to that of the alkali–thermal method. This suggests that combining ultrasonication with the alkali–thermal treatment method did not significantly impact sludge solubilisation. Nevertheless, improving WAS solubilisation helps to increase the concentration of organic molecules released into the soluble phase.

2.2.2 Solubilisation of SCOD and carbohydrates

The SCOD and soluble carbohydrate concentration are shown in Figure 2.2, which indicates that all the single and grouped treatments had a significant impact on the SCOD measurements as compared to the untreated sludge sample with an SCOD of 500 mg/L. The release of total carbohydrates into the soluble phase of WAS improved with the treatments but did not have a strong dependence on the type of treatment, varying in the range of 500–800 mg/L. The highest SCOD of 5235 mg/L was achieved by a combination of alkali and heating treatments. In general, combined treatments gave better SCOD values of about 3600–5235 mg/L as compared to individual tests with SCOD values in the range 1770–3600 mg/L. This was also confirmed by previous studies [31,32]. Coupling of single treatments gave a trend similar to solubilisation of TS and VS. The effect of alkali addition proved to be pivotal for effectively solubilizing COD, which is agreement with previous studies [33,34]. Chemical treatment alone resulted in an SCOD of 3654 mg/L, which was slightly increased to 4081 mg/L when combined with ultrasonication and improved significantly to 5235 mg/L when combined with thermal treatment. Alkali treatment was the most efficient among individual treatment methods, as well as when combined with ultrasonication, heating, or both, at helping to enhance COD solubilisation. Ultrasonication had a lower impact on solubilizing COD in comparison to other single treatment methods. The reason being that ultrasound treatment was effective enough for disrupting the sludge flocs but not for the breakup of microbial cells. Also, adding ultrasonication to the most impactful alkali–heating treatment only slightly changed the SCOD and soluble carbohydrate concentration. This could be because alkali and heating resulted in the lysis of cells, which then released soluble materials such as proteins but however hindering the propagation of ultrasound waves that could not reach intact cells. Overall, the results suggest that all the different treatment methods elevated the release of carbohydrates and COD. However, combined treatments with alkali addition solubilized greater quantities of organic molecules in the soluble phase.

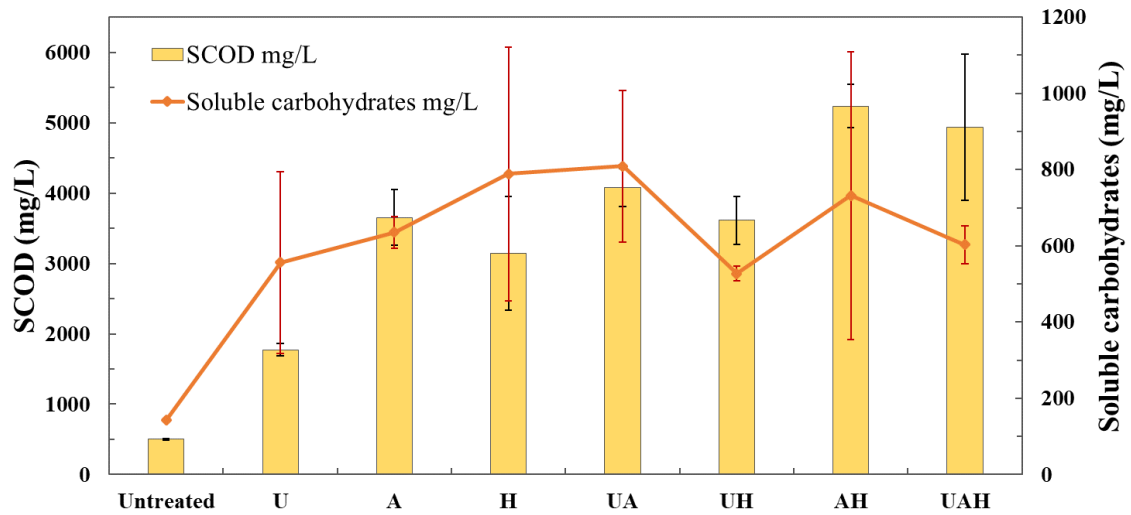


Figure 2.2. Comparison of different treatments in terms of SCOD and carbohydrates

2.2.3 Total Nitrogen and Soluble Proteins

One of the significant constituents of the microorganisms found in WAS is represented by proteins, which are comprised of carbon, nitrogen, oxygen, and hydrogen. Disruption of microbial cells releases proteins into the aqueous phase of WAS. The concentration of nitrogen in protein is historically assumed to be 16% [35]. Thus, the concentration of proteins in WAS was calculated as 6.25 times the total nitrogen [33]. Here, only TN concentration was measured, and total proteins were estimated by assuming that all the nitrogen released was in an organic form. The concentrations of TN and proteins are shown in Figure 2.3. The concentration of proteins present in WAS was comparatively similar to the trend observed in SCOD, with a higher quantity of proteins resulting from combined treatments than each separate treatment. Ultrasonication alone had a limited effect on protein release; however, when coupled with other treatments, it improved the solubilisation of proteins. Alkali treatment resulted in a protein concentration of 1969 mg/L which gradually rose when combined with ultrasonication, heating, or both, ranging from 2281 mg/L to 3031 mg/L. It was found that increasing the pH of sludge increases protein solubilisation, whereas the protein release accounts for a higher value than the corresponding carbohydrate solubilized [36], as seen here. Alkali with thermal treatment at 75 °C was seen to be the most effective among treatments in terms of COD solubilisation, as well as carbohydrate and protein transfer, into the soluble phase. Similar results were obtained by Uma Rani et al. [37], who suggested that a low temperature and pH of 12 were important parameters of treatment for enhanced solubilisation

of organic matter. Generally, treatments involving the alkali method were found to better solubilize both lower- (below 20 kDa) and higher-molecular-weight (above 20 kDa) proteins compared to other treatments due to the enhanced removal of protein nitrogen at pH 12 [38].

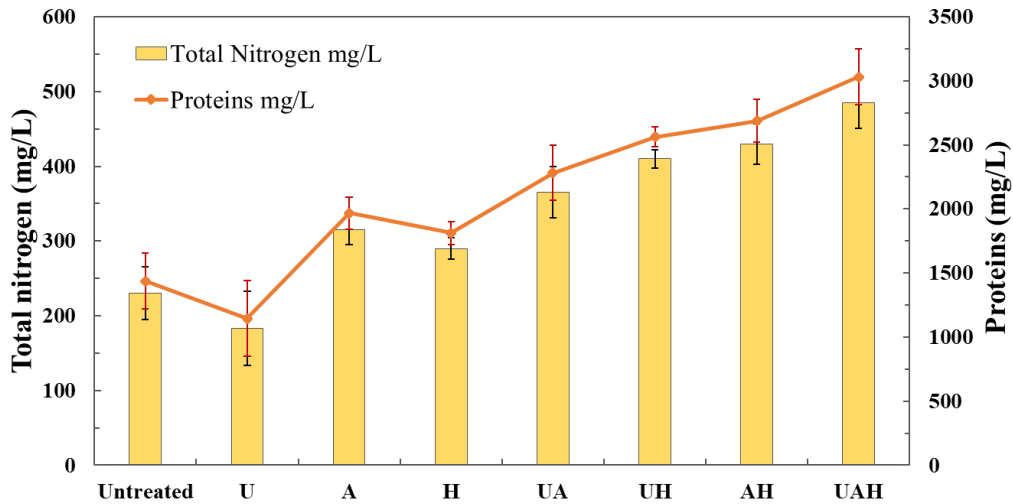


Figure 2.3. Effect of treatments on the concentration of total nitrogen and protein release

2.2.4 Effect of alkali addition on sludge solubilisation

The effect of adding NaOH on solubilizing the organic fraction of sludge was compared for different treatment methods, as shown in Figures 2.2 and 2.3. Figure 2.2 compares the extent of solubilisation of COD and carbohydrates, while Figure 2.3 shows the proteins (estimated from concentration of total nitrogen) for both individual and combined treatments. The pH sludge treated with alkali was adjusted to 12 using 5M NaOH solution. Among the individual treatment methods, alkali treatment had a significant influence on WAS disintegration with respect to ultrasonication, as it resulted in higher concentrations of soluble COD, carbohydrates, and proteins. Similar to these results, Tyagi et al. [39] also found that an alkali pH of 12 was better for the release of organics in the soluble phase. Thermal treatment at low temperature with an SCOD of 3141 mg/L was comparable to the soluble COD of 3654 mg/L obtained after alkali addition. However, this value of 3141 mg/L was improved to 5235 mg/L when thermal and alkali treatments were combined. The concentration of soluble proteins was also enhanced from 1813 mg/L to 2688 mg/L after AH treatment. In general, the type of treatment performed had a very limited effect on the solubilisation efficiency of carbohydrates. In the combination of ultrasonication and alkali methods, at the end of 1 h, the values of soluble

COD, carbohydrates, and proteins was almost doubled with respect to the corresponding initial concentrations after ultrasonication treatment. During the UA method, ultrasonication created hydromechanical shear forces that helped to break the WAS flocs. The more opened floc structure resulted in better interaction of the microbial cell, with OH^- ions promoting better disintegration. Hence, the sludge solubilisation efficiency of UA was higher than individual ultrasonication and alkali methods. Upon combining alkali, ultrasonication, and thermal methods, there was significant WAS disintegration due to the synergetic effects of the three different treatments. However, in terms of the release of soluble COD and carbohydrates, the concentrations were quite comparable to the alkali–thermal method, with only a negligible increase in protein concentration with respect to AH. As a result, the effect of alkali addition on sludge solubilisation was most prominent when alkali treatment was coupled with low-temperature thermal treatment.

2.2.5 Thermogravimetric analysis

TGA was used for the mass loss experiments to estimate the HHV of the sludge sample before and after treatments. TGA analysis was carried out on the solid part of the sludge samples to understand their behaviour in terms of energy potential. The results of this analysis can give better insight into the usage of sludge for energy even after treatments and the resource recovery phase. MC, VM, FC, and A were calculated from TGA curves as outlined in Section 2.1.4, and an example of this can be seen in Figure 2.4 (a), showing the TGA curve of the untreated sample. Figure 2.4 (b) shows the TGA graphs before and after all treatments.

For all TG curves, it can be observed that there was an initial mass loss attributed to water volatilization, expressed as MC. In the second stage, there was large weight loss up to 950 °C due to the decomposition of the main volatile constituents of the sludge sample, represented as VM. Significant sludge pyrolysis took place between 200 °C and 550 °C. Biodegradable organic matter was disintegrated in the temperature range of 200–400 °C, whereas most of the nonbiodegradable organics decomposed at 400–550 °C. A comparatively lower loss of weight was noticed from 550 °C to 950 °C, which contributed to the remaining volatilization of organics. At 950 °C, sludge was allowed to completely oxidize in the O_2 environment, and FC and A were measured at this stage. The FC of samples was also measured as the difference in mass percentage between the sample and sum of masses of MC, VM, and A. These results were

found to be fully in agreement with those calculated from the TG curves. The TG curves for all alkali-treated samples were observed to be steeper in the 550 °C to 950 °C region. In almost all treated sludge samples, especially the alkali ones, FC was found to strongly decrease with respect to the untreated sludge.

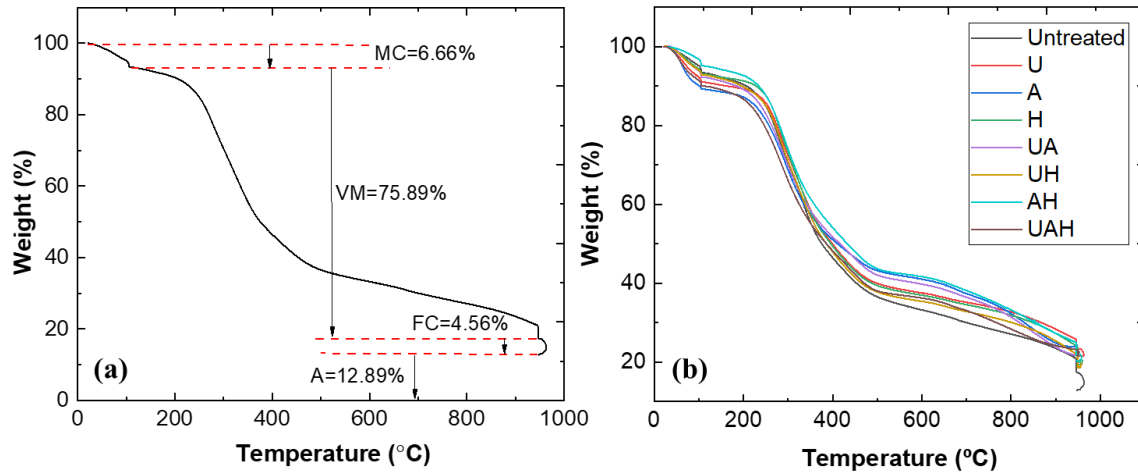


Figure 2.4. TGA graphs of (a) untreated sludge with proximate parameters shown, and (b) sludge before and after all treatments

HHV was estimated here from thermogravimetric analysis using an empirical equation from the literature [40].

$$\text{HHV} = 0.3536\text{FC} + 0.1559\text{VM} - 0.0078\text{A}, \quad 2.3$$

where FC, VM, and A are the weight percentages on a dry basis yielding the HHV in MJ/kg. This correlation was selected as it can be useful for computing HHV from proximate analysis for any kind of biomass material. Moreover, it had an average absolute error and bias error of 3.74% and 0.12%, respectively, highlighting the good relationship between calculated and measured HHV. The HHV results for all the samples are shown in Table 2.3. The estimated HHV values according to Parikh et al.'s correlation of the sludge samples after treatments using proximate values from TG curves were in the range of 10–12 MJ/kg, while the untreated sludge had an HHV of 13.34 MJ/kg.

Table 2.3. Results of HHV from thermogravimetry

Sample	HHV MJ/kg
Untreated	13.34
U	10.90
A	10.00
H	11.44
UA	10.88
UH	11.47
AH	11.82
UAH	10.58

It is the VM that mainly contributed to the heating value of sludge. From the literature, municipal sludges with VM 23–53% had an HHV of 4.3–13.9 MJ/kg (measured using a bomb calorimeter) [41]. The percentage of VM obtained in this study was around 65–75%, which is comparatively higher than the above values, with an HHV between 10 and 14 MJ/kg. Due to variability found in FC, a clear comparison was not possible among the different pre-treated samples. However, the proximate analysis using the TG curve gave a reasonable estimation of HHV in case of the absence of expensive equipment, which could be used for quick and preliminary energy studies. Moreover, it can be seen that the HHV value of sludge after treatments did not largely differ with respect to the HHV of untreated sludge. Thus, this suggests that, even after treatment, there is a possibility to utilize this sludge for further energy recovery.

2.3 Conclusions

This paper evaluated the effect of different treatments on WAS solubilisation, which is the first step for a biorefinery exploiting WAS as a source. The treatments resulted in an increase in the concentration of dissolved organics. Among individual treatments, the alkali addition method was the most significant. The conversion of volatile solids in general was higher for alkali-treated samples, ranging from 11 to 18%. Upon comparing all treatments and all tested parameters, the combined alkali–heating treatment was found to be the most effective. The highest solubilisation of both TS (9.6%) and VS (17.2%) was obtained for the alkali–heating treatment method. Higher SCOD values showed that the treatment could be more effective in improving the release of organic soluble molecules in the liquid phase, and that a combination of treatments was the most effective. Solubilisation of carbohydrates was independent of the

treatments performed. The amount of nitrogen release was higher for the combined treatments involving the alkali step. On the other hand, the higher SCOD compared to the concentration of carbohydrates and estimated proteins revealed the presence of other classes of soluble compounds. Identification of the major contributors to the SCOD is necessary to efficiently valorise WAS. Therefore, alkali-coupled treatments could be further explored to produce an aqueous liquor with organic constituents which can undergo subsequent biotechnological steps to produce value-added chemicals/materials. For all the treatments, the HHV values were approximately 10–11.82 MJ/kg and did not greatly vary with comparison to the untreated sample. The HHV of the sludge after treatments was reduced to a maximum of 10 MJ/kg from 13.34 MJ/kg (untreated sample), which shows that, after resource recovery, the solid part of sludge can be further utilized for its energy properties.

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Chapter 3: Effect of hydrothermal treatments on sludge solubilisation

3 Effect of hydrothermal treatments on sludge solubilisation

Waste sludge is the biological material produced as surplus of a biological reactor. When it is produced by an activated sludge process from wastewater treatment is referred as waste activated sludge. The excess of sludge is removed for the activated biological sludge process to keep the desired ratio between the organic substrate and the biomass in the biological reactor. The waste sludge contains high concentration of microorganisms, basically bacteria, protozoa and fungi aggregated as flocs. From the chemical point of view the sludge is mainly composed of proteins, carbohydrates, and humus that can be usefully utilised as a source of biodegradable renewable matter [1]. However, these organic molecules are present in a complex matrix in the sludge flocs [2]. It is therefore necessary to break the floc structure and open the microbial cell walls and membranes to facilitate solubilisation and release of the components and molecules which than can be recovered or eventually converted into other interesting molecules [3]. Among the several different techniques employed to enhance sludge solubilisation, thermal hydrolysis is considered to be a feasible choice as it destroys microorganisms and aids with reduction of sludge volume which is considered beneficial from the point of view of the wastewater treatments process [4]. This last point is highly relevant for the management of wastewater treatment plants since the high volume of waste sludge generated yearly consistently affects the economy of the wastewater facilities. Nevertheless, really high composition of water over 95% in waste activated sludge can hinder the effective application of standard thermal methods [5].

However, in recent years, hydrothermal treatment which is a particular kind of thermal method using water as both reactant and solvent has been gaining attention [6,7]. Hydrothermal method can be classified based on the operating temperature: hydrothermal carbonization at 180- 250 °C, hydrothermal liquefaction at 250- 400 °C and hydrothermal gasification at 400- 600 °C [8]. Hydrothermal carbonization, liquefaction and gasification produces solid fuel, biofuels, and hydrogen rich biogas respectively that can be recovered. In addition to generation of energy products from dewatered sludge, hydrothermal treatments also improves anaerobic digestion process to produce biogas from sludge that has not been dewatered [9].

Hydrothermal treatments can be influenced by several parameters like reaction temperature, sludge pH and reaction time. Between 130- 270 °C, hydrothermal treatments have been found to show enhanced performance for both primary and secondary sludges from wastewater

treatment plants [6,10]. Acidic and alkaline hydrothermal treatments has been previously studied to destroy sludge floc and cell structure thereby enhancing solubilisation [11,12]. Although different alkalis like sodium hydroxide (NaOH), potassium hydroxide, calcium hydroxide and magnesium hydroxide have been utilised for alkaline treatment, it is NaOH addition that provides highest rate of solubilisation and is most commonly used [13]. While chemicals like hydrochloric acid (HCl), sulphuric acid and phosphoric acid are frequently used for acid hydrothermal treatments.

Hydrothermal methods are catching growing interest among the researchers as it can be observed from Figure 3.1 through a search on the Scopus database.

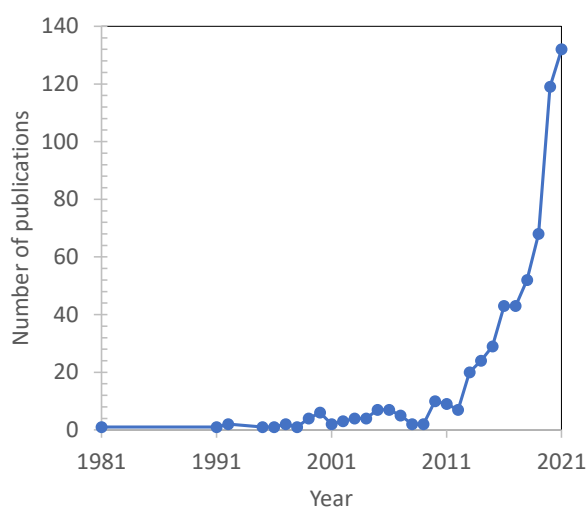


Figure 3.1. Number of publications indexed by Scopus with the query “hydrothermal AND waste AND sludge” in the fields title, abstract and keywords

Nevertheless, this technology is still not enough mature for a wide application on an industrial scale and further studies are still necessary to address its features, characteristics of the products, applicability, strength, and weakness on the different types of sludges.

In this chapter, work relating to two sets of batch low temperature hydrothermal treatments on municipal waste activated sludge have been discussed. At temperatures below 210 °C on the basis of literature the release of several type of monomers (e.g. oligo and monosaccharides, proteins and amino acids, fatty acids) through a dissolve degradation mechanism is expected [14]. First a set of preliminary hydrothermal treatments were performed by varying hydrothermal temperature, sample pH and reaction time in mini-autoclave reactors with 12 ml

capacity. Chemical oxygen demand (COD) and carbohydrates on the filtered samples were measured to determine the best operating parameters for sludge solubilisation. Using the best operating conditions, next set of hydrothermal treatments were done using a 300 ml reactor. The solid and liquid fractions were separated for the large batch experimental runs and in-depth quantitative analyses carried out on both fractions. The aim of this work was to study the effect of the different variables involved in the hydrothermal treatment, namely hydrothermal carbonization at milder temperatures, on the solubilisation of organics and the consequent reduction in sludge volume.

3.1 Material and Methods

3.1.1 Materials

Waste activated sludge used in this section was collected from the oxidation tank of a municipal wastewater treatment plant in Genoa, Italy. Some chemical characteristics of the waste activated sludge have been determined beforehand. The raw sludge sample contains 4.8 g/L of total solids TS (measured on as received basis), 3.7 g/L of volatile solids VS (measured on dry basis) and 1.1 g/L of mineral solids (measured as difference and on dry basis). The sludge was preserved at 4 °C to prevent any degradation till it was used for the batch experiments.

3.1.2 Batch experiments for hydrothermal treatments

Hydrothermal treatments of activated sludge were performed as batch experiments using reactors of two different sizes and varying treatment conditions. First set of preliminary experiments were conducted in a stainless-steel vessel of maximum capacity of 12 ml using sludge thickened to biomass concentration of 11.5 g/L (measured on wet basis), volatile solids of 9.2 g/L (dry basis) by decantation. The purpose of these small batch experiments was to help select the best operating conditions for the bigger batch experiments. The hydrothermal conditions were varied as follows: temperature of 75 - 250 °C; pH of 6 - 14; and reaction time of 0.5 - 3 h. Initially, about 8 ml of concentrated sludge was added into the 12 ml reactor and purged with nitrogen gas for 3 mins. The autoclave was then heated in a furnace as per the desired operating condition. After the set reaction time of experiment, the autoclave was removed from the furnace and cooled using water. The treated sludge sample was filtered with a 0.45 µm cellulose acetate syringe filter to separate the organic liquid and solid fraction.

From the results of the above small batch experiments, using the optimised operating parameters, further hydrothermal treatments using increased quantity of sludge in 300 ml stainless steel vessel was performed. 80 ml of sludge concentrated to TS of 15 g/L and VS of 12.2 g/L was added to the reactor, purged with nitrogen, and heated on a hot plate using an aluminium insert as shown in Figure 3.2(a). For experiments that were done at low and high pH conditions, a PTFE liner was inserted inside the reactor before use as shown in Figure 3.2(b).

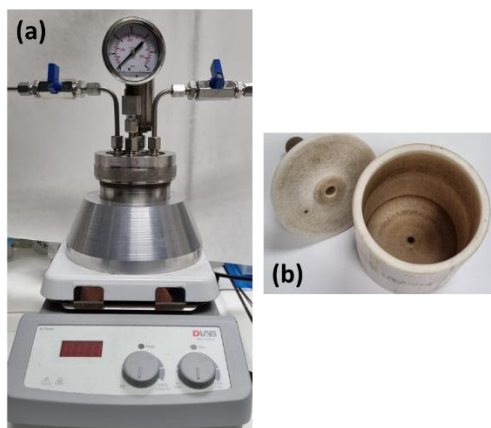


Figure 3.2. (a) 300 ml reactor in aluminium insert on heating plate (b) PTFE liner used

Experimental runs with constant temperature and reaction time while varying the pH were performed. The pH was adjusted to 3 using hydrochloric acid (HCl) and set to pH 12 using sodium hydroxide (NaOH) addition. After the treatments the solid fraction was separated from a liquid phase by centrifuging at 7000 rpm for 15 minutes. The samples were filtered using 0.2 μm cellulose acetate syringe filters (Merck, Italy) before the determination of soluble composition.

3.1.3 Sample Analyses

Merck Spectroquant analytical cell test kits (Merck, Italy) were used to measure soluble chemical oxygen demand (COD), total nitrogen (TN), total phosphates (TP), total organic carbon (TOC) and volatile organic acids (VOA). Protein concentrations were determined using total protein kit- Micro Lowry, Onishi & Barr modification kit from Sigma. The soluble carbohydrates were determined by phenol-sulfuric acid method with D-glucose as standard [15]. Total solids (TS) and volatile solids (VS) were measured as per standard methods [16]. The composition of the soluble compounds in the liquid fraction was expressed as mg/g and

was calculated by dividing the concentration of soluble parameter (mg/L) with concentration of thickened sludge (g/L).

3.2 Results

3.2.1 Effect of varying hydrothermal conditions on sludge solubilisation- preliminary tests

Hydrothermal parameters such as temperature, pH and reaction time were found to influence the solubilisation of sludge as reported also in the literature [2,10,17]. The effect of hydrothermal treatments by varying the operating parameters on the solubilisation of organics was done here. The thickened sludge used had a biomass concentration of 11.5 g/L and VS of 9.2 g/L. Initially only one reaction condition was changed, and other parameters kept constant. For the first set of preliminary reactions, the effect of the different hydrothermal conditions on sludge solubilisation was studied by evaluating the concentrations of soluble COD and total carbohydrates.

Figure 3.3 shows the results obtained at different temperatures for a reaction time of 1 h and it can be seen that soluble organic compounds like carbohydrates were released as a result of hydrothermal treatments. The pH of the samples was not altered and was measured to be 6.2 before the hydrothermal runs. Increasing the temperature, increased the COD concentration from 87 mg/g at 75 °C to a maximum of 917 mg/g at 200 °C. Generally using COD measurements, it is difficult to accurately calculate the concentration of soluble organics due to the interference from several inorganic ions like chlorides and ammonium that are present in the soluble phase [18]. However, COD content provides a preliminary information on the influence of hydrothermal treatments on sludge solubilisation. In the past, COD has been used as a simple measurable variable for determining organic concentration in the absence of other analytic instruments [19,20].

Furthermore, carbohydrates concentration was also measured that helped to supply some additional information on the solubilisation of organics by hydrothermal treatments. The carbohydrate content increased from 12 mg/g at 75 °C to 92 mg/g at 200 °C but this value was lowered to 67 mg/g at 250 °C which could be due to probable disintegration of carbohydrates into other volatile molecules at 250 °C. The ideal operating temperature depends on the type of

sludge and its properties. In this case, as temperature increased from 75 °C to 200 °C, the increase in COD was almost ten times, which further decreased slightly as temperature reached 250 °C. A similar behaviour was shown by total carbohydrates results. The above results indicate that best reaction conditions in terms of COD is alkaline pH, and the temperature range of interest is about 150- 250 °C.

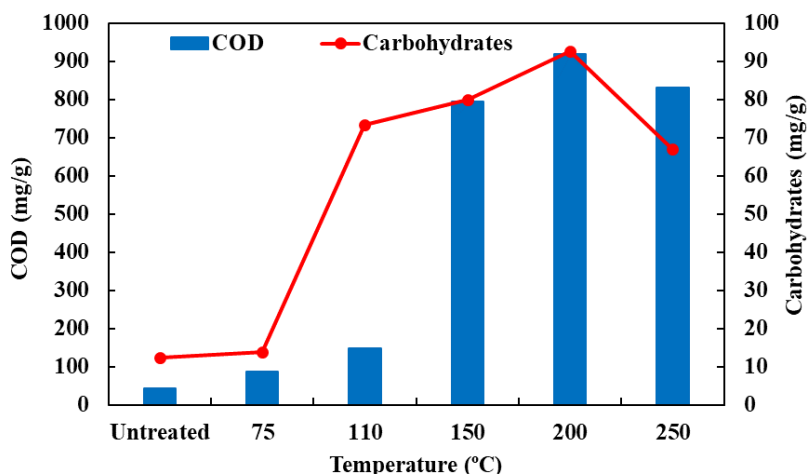


Figure 3.3. Composition of soluble chemical oxygen demand (COD) and carbohydrates at different temperature between 75- 250 °C

From results shown in Figure 3.3, optimum temperature range was selected as 150- 250 °C. Therefore, for this temperature range, pH was changed using NaOH addition in the range from 10 to 13 for an operation time of 1 h in the hydrothermal reactor. Alkaline pH conditions were chosen based on the studies done in Chapter 2 which indicate that in basic environment the biomass matrix is better disintegrated [21]. Hydrothermal tests were performed at 150, 200, and 250 °C as shown in Figure 3.4.

At 150 °C, as the pH increased even the COD contents increased to a maximum value of 1626 mg/g at pH 13 from 795 mg/g at pH 6. The solubilisation of COD was largely improved from pH 12 and above. Carbohydrate concentration was enhanced from 80 mg/g at pH 6 to 144 mg/g at pH 13 and showed an increasing trend similar to that of COD.

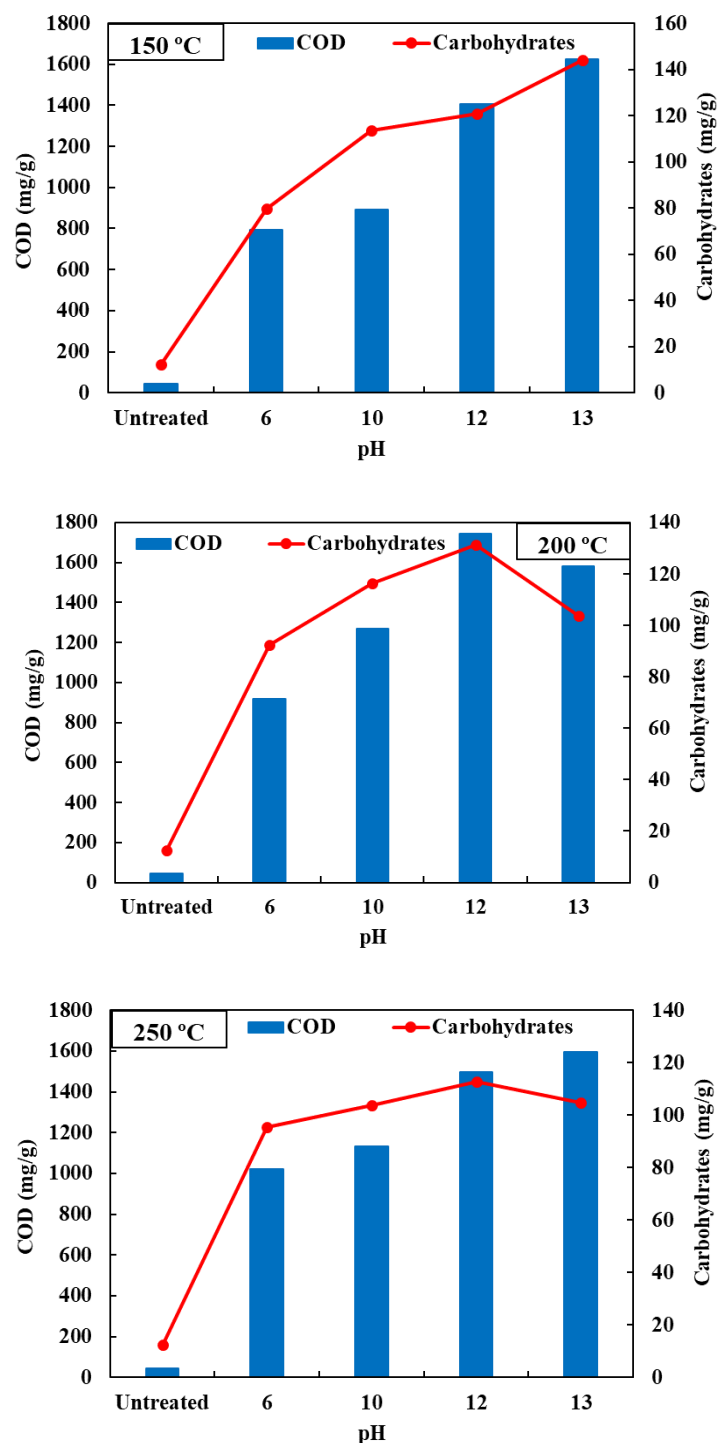


Figure 3.4. Impact of pH on solubilisation of COD and carbohydrates at 150 °C, 200 °C and 250 °C

The COD of the untreated sludge of 43 mg/g increased to 917 mg/g at 200 °C for sample at pH 6. Adding NaOH increased the COD content further to a maximum value of 1743 mg/g at pH 12 which decreased on increasing the pH to 13 and above. A similar trend was also observed in the case of carbohydrates released with concentration increasing 10 times from 12 mg/g for

untreated sample to 131 mg/g for hydrothermal treatment at pH 12 which was reduced to 104 mg/g at pH 13. In literature too, an alkaline medium with pH 12 was found to be effective for destroying the sludge flocs and breaking the cell membranes to increase the availability of organic molecules [2].

The COD solubilisation trend at hydrothermal temperature of 250 °C was in the increasing order and similar to that at 150 °C. At pH 12, COD released improved significantly to 1495 mg/g while increasing the pH further to 13 enhanced the COD contents only by a smaller concentration to 1595 mg/g. Soluble carbohydrate concentration was elevated to 112 mg/g at pH 12 from 95 mg/g at pH 6. However, at pH 13 there was a drop in the carbohydrate content to 104 mg/g and this behaviour was similar to that at 200 °C. A probable reasoning for this could be the degradation of carbohydrates to other organic molecules at more severe treatment conditions of pH 13 and temperatures of 200 °C and higher. Hence, from Figure 3.4 it can be seen that an alkaline pH is optimum to open the biomass matrix and at higher temperatures the pH has to be within pH 12 for efficient solubilisation.

From the previous experimental runs, 200 °C was chosen to be the best temperature and 12 to be the best pH in terms of highest concentrations of COD and carbohydrates that were released into the aqueous phase of sludge after hydrothermal treatments. At these best identified conditions, the effect of reaction time on sludge solubilization was studied in the range 0.5- 3 h as it was the final parameter selected in addition to temperature and pH affecting solubilisation. Also, to better understand the results, hydrothermal treatments at pH 6 and 200 °C were also performed. Figure 3.5 shows the influence of reaction time on solubilisation of COD and carbohydrates at pH 6 and 12. It can be observed that at pH 6, there was an increase in COD from 766 to 1013 mg/g from 0.5 to 3 h while in case of carbohydrates, the increase was from 80 to 93 mg/g from 0.5 to 1 h and lowered to 83 mg/g after 3 h. There was an increasing trend at pH 12 in terms of COD content from 1548 to 1826 mg/g. Carbohydrate concentration increased 131 mg/g at 1 h which dropped to 120 mg/g after 3 h. This behaviour was same at pH 6 and 12. A possible reasoning could be as hydrothermal treatment progresses there is solubilisation of organics including carbohydrates and also simultaneous decomposition of these dissolved organics.

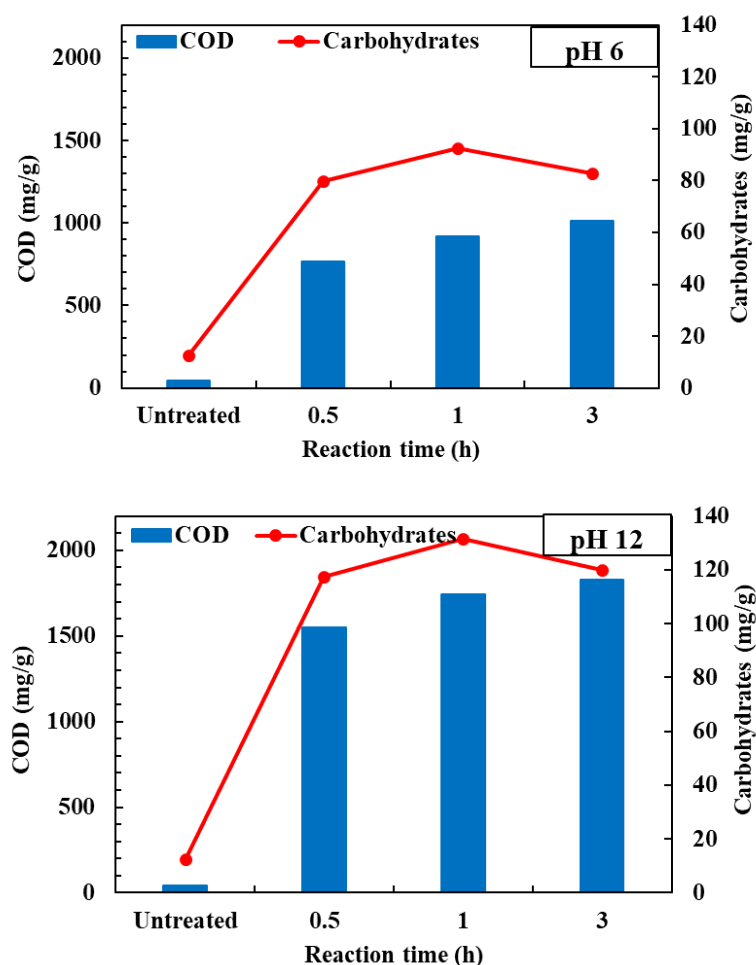


Figure 3.5. Impact of reaction time on solubilisation of sludge at 200 °C and pH 6 and 12

3.2.2 Effect of best conditions on sludge solubilisation

Using the preliminary hydrothermal test results in the previous section, the best operating temperature was 200 °C but it was seen that any temperature in the range between 150- 200 °C were comparable and suitable. So, the conditions selected for big batch runs were 180 °C, 1 h reaction time and pH 12. As the previous hydrothermal experiments were performed only in alkaline environment, it was also important to see the effect of acid addition on solubilisation of organics and so treatments were done at pH 3 in addition to pH 12 and one reaction at original pH of sludge that is 6 for comparison. The hydrothermal treatments were done using 80 ml of thickened sludge with 15 g/L solid biomass and VS 12.2 g/L at 180 °C and reaction time of 1 h. Figure 3.6 gives the composition of volatile and mineral solids in the solid and liquid fractions after treatments while Figure 3.7 shows the composition of the soluble compounds in the liquid phase.

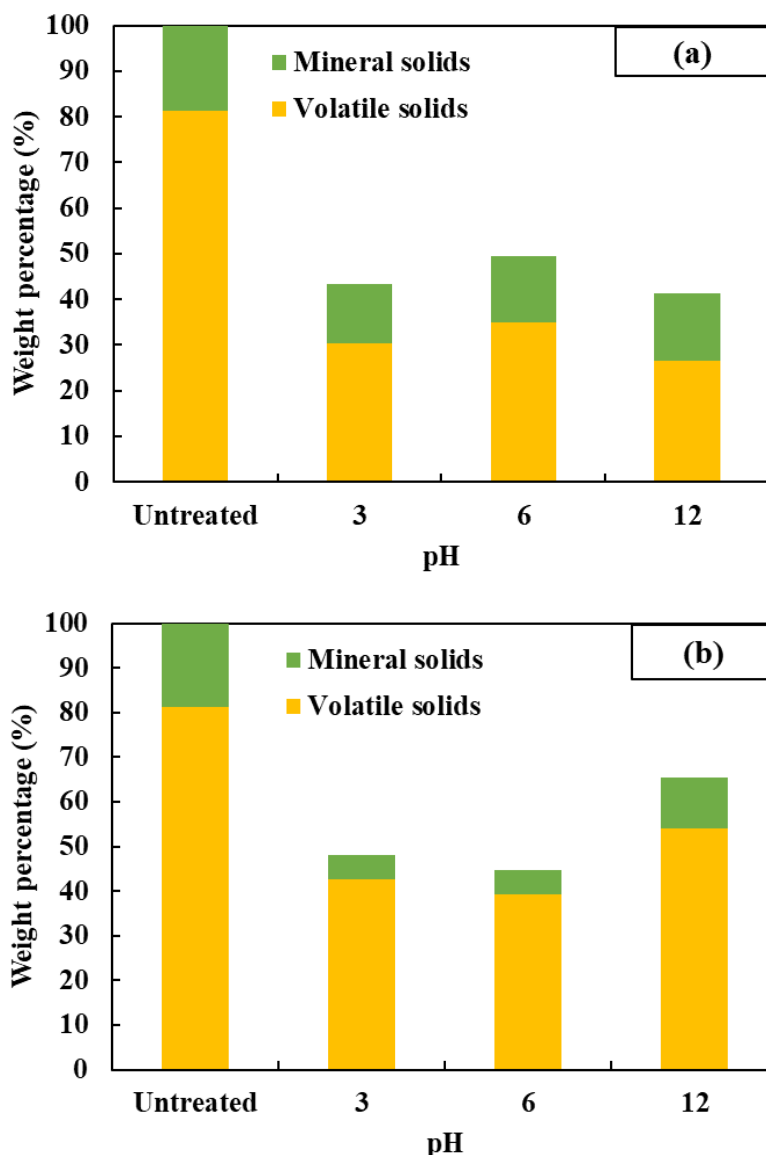


Figure 3.6. Composition of (a) solid and (b) liquid fractions in terms of volatile and mineral solids

The volatile solids concentration in the solid phase was lower than in the liquid fraction for all the pre-treatments as shown in Figure 3.6 implying better solubilisation of sludge and improved release of organics into aqueous phase. It can be seen that for all hydrothermal treatments, percentage of volatiles solubilised into aqueous phase was more than 50% of the untreated sample. For instance, about 43, 40, 54% of volatile matter was found to be released at pH 3, 6, 12 respectively into the liquid part starting from the volatile concentration in untreated sludge which was 81%. Mineral solids content was 19% in untreated, and this was lowered to 12-15% in solid fraction after treatment. The concentration of mineral solids/ ash was higher in the solid part than liquid, however, pH 12 in the liquid phase had comparatively higher ash at 11% due to the presence of salts from NaOH addition.

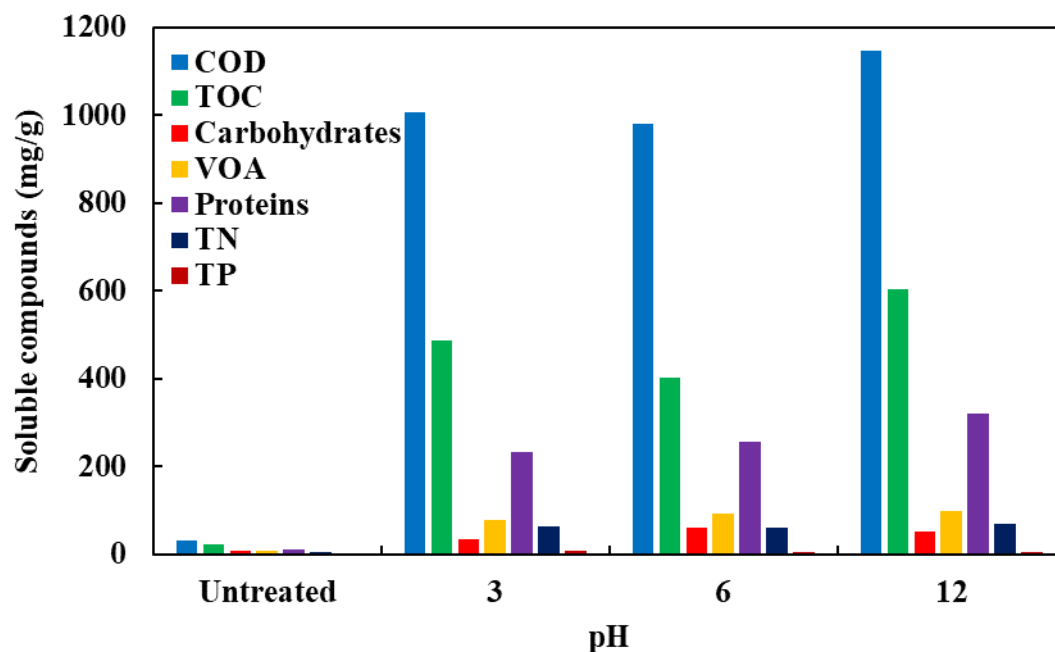


Figure 3.7. Composition of soluble compounds in liquid fraction at 180 °C and 1 h at pH 3, 6 and 12

All the treatments at pH 3, 6 and 12 was able to release enhanced concentrations of organic compounds into soluble phase, however, alkaline treatment seems to be more effective as seen in Figure 3.7. With respect to untreated raw sludge, the increase in COD was from 33 mg/g to 1007, 980 and 1147 mg/g at pH 3, 6, and 12 respectively after hydrothermal reactions. The improved solubilisation at alkaline conditions was alike that of existing literature where there was higher release of organics in the aqueous phase in comparison to acidic environment and use of same chemicals to achieve the desired pH [2,22]. The slightly lower solubilisation effect at acidic and neutral pH was maybe due to the less efficient break up of cell membranes as compared to sludge floc lysis. Total organic carbon was measured to have a better understanding of the actual organic concentration in soluble phase. Much higher values of COD to TOC can be explained by the fact that COD measurements include inorganic oxidisable ion concentrations together with organic molecules. TOC was highest for pH 12 at 603 mg/g followed by 488 mg/g at pH 3 and 403 mg/g at neutral pH. Results obtained suggest that highest sludge disintegration was in an alkaline medium with greater damage to cell membranes and walls and this was in accordance with Guo et al. [23].

The destruction of sludge cells leads to higher concentrations of proteins, carbohydrates, volatile organic acids to be solubilised [24]. Therefore, the availability of soluble proteins, carbohydrates and organic acids were evaluated. The highest concentrations of these organic compounds were at pH 12 followed by pH 6 and 3. Among the several soluble organic

molecules released, proteins presented the highest fraction after all hydrothermal treatments at 320 mg/g in alkaline pH, and 257, 234 mg/g at pH 6 and 3 respectively. VOA solubilised was between 78- 99 mg/g making them the next bigger class of organics to be released in terms of concentration after proteins followed by carbohydrates ranging from 34- 60 mg/g. Furthermore, concentrations of total nitrogen and phosphates were also investigated. TN ranged between 61- 69 mg/g and TP between 5- 8 mg/g for all the three treatments. So, TN and TP values were quite comparable and did not significantly vary at acidic, neutral, and alkaline pH conditions. Examining these results indicate that all the hydrothermal treatments were very effective in solubilisation of organics into aqueous phase. However, a comparison among the three suggest that alkaline treatment is more efficient than acidic and neutral hydrothermal conditions. This opinion was in line with work done by previous researchers who summarised that though using acidic hydrothermal conditions was able to solubilise sludge, it was the alkaline treatment which proved to be the most effective [25,26]. In conclusion, comparatively improved efficiency at pH 12 made alkaline hydrothermal treatment to be a better choice for solubilisation of sludge and reduction in sludge volume.

3.3 Conclusions

The first half of the work was focused on the preliminary screening of hydrothermal process conditions for enhanced solubilisation of waste activated sludge using small batch reactions. The impact on sludge solubilisation was evaluated by examining the soluble COD and carbohydrates concentrations in the treated aqueous phase of sludge. The effect of reaction temperature, pH and time was studied individually. All the parameters had a significant influence on sludge solubilisation and the optimum values selected for big batch reactions were temperature between 150- 200 °C, alkaline pH and reaction time of 1 h. The best results obtained for COD and carbohydrate solubilisation were 1743 mg/g and 131 mg/g respectively at 200 °C, pH 12 and 1 h. It would also be interesting to consider a chemometric study in the future to understand the combined effect of the variables on hydrothermal treatments. Based on the results of the preliminary experimental runs, further hydrothermal reactions on a larger scale were carried out at 180 °C, pH 3, 6 and 12 for 1 h of reaction. The effect of acidic medium was also studied in addition to optimised alkaline environment. Results showed that hydrothermal treatments at pH of 3, 6 and 12 were all effective in solubilising waste activated sludge. To better understand the results, qualitative analyses like TOC, proteins, VOA, TN, TP

were conducted on the liquid part of treated sludge in addition to COD and carbohydrates. While on the solid part, percentage of volatile and mineral solids were measured. There was enhanced reduction of solids of about 50-60% and increased solubilisation of organics of 57-67% after the treatments. Highest release of COD and TOC was at pH 12 and measured to be 1147 and 603 mg/g respectively. Also, solubilisation of organics like proteins, carbohydrates and VOA were comparatively improved at alkaline pH with highest fraction of protein solubilised at 320 mg/g. TP and TN release was quite comparable for all treatments. Here, the effect of different hydrothermal treatments on WAS solubilization was evaluated which is the first step for a biorefinery exploiting sludge as a source. The treatments resulted in reduction of the sludge volume and increase in the concentration of dissolved organics with the most efficient treatment being in alkaline medium. Further work was aimed at valorising the solid and liquid fraction after hydrothermal treatments which will be described in Chapter 4.

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Chapter 4: Valorisation of hydrothermally treated sludge

4 Valorisation of hydrothermally treated sludge

Utilization of sludge as a source for energy and resource recovery is a promising alternative in terms of sludge management respecting the regulation requirements and the circular economy principles. This chapter is an attempt to valorise the solid and liquid fractions of hydrothermally treated sludge obtained from Chapter 3. On the solid fraction, pyrolysis will be performed as it is a promising technology for stabilization and resource recovery of sludge due to the ease and efficiency of operation [1]. Pyrolysis will be explained in detail in the next Chapter. To date, only limited number of works have been carried out regarding pyrolysis of hydrothermally treated sludge and most of them investigated concentration of heavy metals, nitrogen polyaromatic hydrocarbons (PAHs) in char [2–4]. A recent publication has tried to exploit the use of pyrolysis liquid from hydrothermally treated sludge in energy applications [5]. Using bench-scale pyrolysis, they obtained higher heating value (HHV) of 38.46 MJ/kg from bioliquid while the potential to use char for bioenergy still remains unexplored. This motivated the study here to apply pyrolysis to hydrothermal treated solid sample to study the properties of biochar for energy or resource recovery. Additionally, the focus was also to try to valorise the complex heterogenous liquid phase obtained after hydrothermal treatments. The combination of hydrothermal and biological techniques like enzymatic hydrolysis and fermentation can be advantageous for valorisation to overcome the complex nature of sludge for specific platform biochemical production [6]. Review by Bing Song et al. studies hydrothermal treatments with subsequent enzymatic hydrolysis on lignocellulosic biomass for producing fermentable sugar monomers [7]. Previous research on ethanol production showed that about 41- 47 g/L of bioethanol was formed during fermentation of process water of hydrothermal treated lignocellulosic biomass showing the possibility to produce bioethanol even from hydrothermally treated sludge samples [8,9]. Protein precipitation in sludge takes place by thermal, chemical, biological and physical pre-treatments followed by protein separation and purification. Protein separation is done using acids like hydrochloric acid, sulphuric acid, and trichloroacetic acid, inorganic salts like ammonium sulphate, sodium chloride and organic solvents like acetone and ethanol [10–12]. 7.2 g/L of proteins were precipitated using ammonium sulphate from the liquid fraction of hydrothermally treated sludge at 160 °C [13]. Thus, this chapter tries to explore the usability of solid fraction for energy and agricultural purposes and the liquid fraction for recovery of value-added molecules.

4.1 Materials and Methods

4.1.1 Materials

Hydrothermally treated waste activated sludge (WAS) samples from Chapter 3 were used. The characteristics of the WAS before and after hydrothermal treatments are previously described in detail. It was thickened sludge with 15 g/L total solids (TS) and volatile solids (VS) 12.2 g/L concentrations subjected to hydrothermal treatments at 180 °C and reaction time of 1 h at pH 3, 6, and 12 (Section 3.1.2). The solid and liquid phases were separated by centrifugation process (Neya 16, Italy) for 15 mins at 7000 rpm.

4.1.2 Pyrolysis

The pyrolysis system used is constituted of a tubular reactor containing a crucible with a fixed bed of biomass as presented in Figure 4.1. The reactor is in turn housed in a tubular furnace whose heating is managed by an Ascon M3 temperature controller via a K thermocouple, which is inserted into the furnace, outside the reactor at the middle of the oven in correspondence of the crucible. At the outlet of the reactor there is a Liebig condenser cooled by tap water at about 14°C followed by a trap cooled by an ice bath with addition of sodium chloride, where the volatile condensable products of pyrolysis are collected. At the junction between the reactor and the condenser, a heating band controlled with a Pixsys ATR144 temperature controller with a thermocouple is inserted which further favour the condensation of volatile products in the condensation area. After the trap, there is a sampling point, where the gas is sampled with a syringe (gas syringe, Hamilton) for gas chromatographic analyses. The last component of the system is a bubble flow meter to monitor the flowrate of the outgoing gaseous stream. The pyrolysis experiments were done under atmospheric pressure with an inlet nitrogen flow rate of 200 ml/min and with a temperature increase rate of 20 °C/min. The solid fraction after hydrothermal treatments were initially dried at 50-60 °C in a ventilated oven and about 5 grams of solid material was placed in the crucible for each experimental run. The reactor was heated from room temperature to 500 °C and held at this temperature for 1 h (used as the reference residence time). After the experiment, the biochar and bioliquid fraction yields were quantitatively determined by weight while biogas yield was calculated by weight difference.

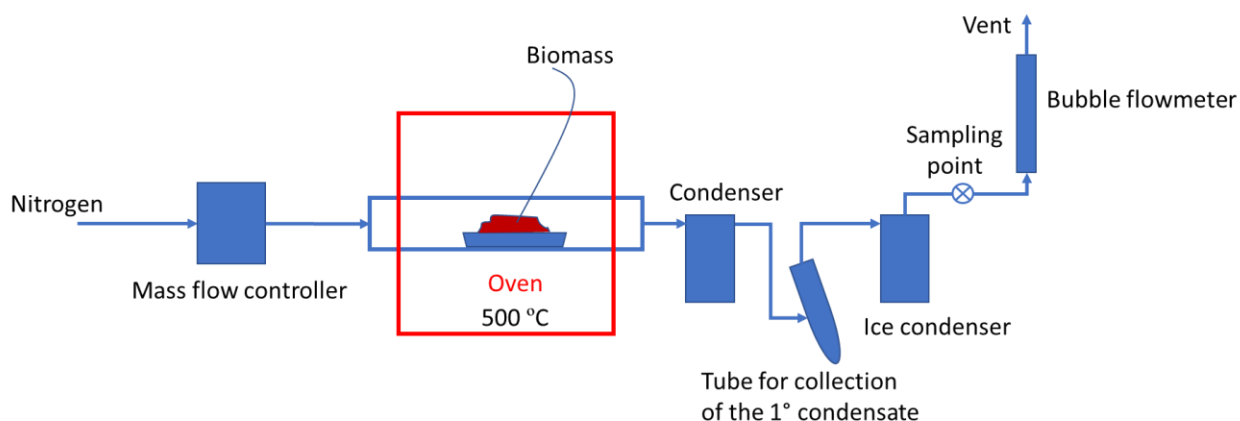


Figure 4.1. Pyrolysis system utilized

4.1.3 Sample analyses on pyrolyzed sludge

Thermogravimetric analysis (TGA) was used to estimate higher heating value (HHV) of sludge before and after pyrolysis to determine its suitability as solid fuel. The method followed is the same as explained in Chapter 2 with the following TGA experimental conditions. In N_2 gas, the sample was heated from room temperature to 800 °C at 20 °C/min. Then the environment was switched to O_2 at 800 °C with a hold time of 10 min for complete combustion of sludge. Field emission scanning electron microscopy (FESEM) analysis was carried out with a Carl Zeiss AG - SUPRA 40VP FE-SEM equipped with an energy-dispersive X-ray (EDX) detector. The accelerating voltage was fixed at 20 kV and the images were collected using a conventional secondary electron detector.

4.1.4 Biotransformation using commercial enzymes

The sludge after hydrothermal treatments with varying pH conditions were separated into solid and liquid fractions. This liquid fraction mainly comprises of proteins, volatile organic acids, carbohydrates, and fats. Biotransformation using commercial enzymes were attempted so as to convert the existing bigger complex matrix into more simpler molecules that could be available for further separation or extraction. The category of the enzymes has been chosen considering the main organic components expected in the liquid fractions. The commercial enzymes used for the trial were Cellulase from *Aspergillus niger*, Protease from *Aspergillus oryzae*, Lipase from porcine pancreas, Lysozyme from chicken egg white and Papain from papaya latex. All the enzymes were purchased from Sigma Aldrich and used as received. The trials were done

in 100 ml glass jars at the optimum operating conditions of temperature and pH for the different enzymes in a commercial domestic incubator. After the selected time period, samples were injected in high- performance liquid chromatography (HPLC) operating with different gel permeation chromatography (GPC) columns. The HPLC system comprises of a high precision HPLC pump (Dionex- P680), refractive index (RI) detector (Shodex RI-101), photodiode array detector (Dionex UVD 340U) and column oven (Peltier PCO 200). GPC columns used were TSK gel G6000PW GPC column (Tosoh Bioscience GmbH) and Rezex RPM-Monosaccharide Pb+2 column (Phenomenex). Data acquisition was performed using Chromeleon chromatography management system.

4.1.5 Qualitative studies on liquid fraction

Some qualitative tests were carried out the liquid fraction of hydrothermal treated samples to define the routes for valorisation. Fermentation with yeast was done using 50 ml of hydrothermally treated liquid and 0.2 g of commercial yeast in a commercial domestic incubator for 24 h at 35 °C. After fermentation the vapours in the head space of each fermentation jar were injected in gas chromatography with flame ionization detector (GC-FID). The gas chromatograph used is the Perkin Elmer Auto-System with helium as a carrier gas, equipped with a capillary column SPB-5, 30 m long. The method used involves an injection temperature of 250 °C, while that of the detector is 300 °C. The temperature ramp consists of an isothermal section at 60 °C lasting two minutes, followed by heating at a rate of 5 °C/min up to 230 °C. 0.5 µL of sample was injected for analysis.

Protein precipitation using hydrochloric acid HCl (32%) in the ratio of 1:1 was done in the liquid fraction at pH 1.5 [11]. The solution was mixed in a beaker overnight at room temperature and precipitated proteins separated by centrifugation and dried at 80 °C. Fourier transform infrared spectroscopy (FTIR) analysis was performed on the precipitated proteins using Bruker Vertex 70 spectrophotometer with the ATR (Attenuated Total Reflection) technique.

Solubility tests using solvents like n-hexane, toluene, chloroform, acetone, acetonitrile, ethanol, methanol of analysis grade quality was performed. The liquid fraction of treated sludge was dried using rotavapor (Buchi) to remove the water content and the residue was mixed with the solvents to check for their solubility.

4.2 Results

4.2.1 Thermal valorisation of the solid fraction

4.2.1.1 Pyrolysis of sludge

Pyrolysis experiments at 500 °C were performed on the solid fraction of the hydrothermally treated samples at pH 3, 6, 12, to study the significance of energy recovery from sludge. Hydrothermal treatment will produce a more stable solid fraction that could be a final treatment step before subjecting the sludge for disposal or energy conversion using pyrolysis. Table 4.1 shows the yield in weight percentage of char, liquid (oil and aqueous fraction combined together) and gas after pyrolysis experiment.

Table 4.1. Product yields after pyrolysis in weight % of untreated and hydrothermally treated sludge at pH 3, 6, 12

Sample	Char	Liquid	Gas
Untreated	41.8	37.3	20.9
pH 3	47.0	23.2	29.8
pH 6	49.1	34.4	16.5
pH 12	52.2	23.0	24.8

The char yield for sludges before and after hydrothermal treatments seems to be in the range of 41-52 % whereas the liquid and gas yields change significantly for most of the samples. It was observed that after treatments char and gas yields increased (except for pH 6 where gas yield was lower) while yield of pyrolysis liquid decreased with respect to raw untreated sludge. The hydrothermal treatment with no pH change produced larger amount of liquid product than samples with a chemical addition. It is noted that concentration of mineral solids produced after hydrothermal treatments was in the increasing order of acid < neutral < alkali and this pattern is similar to the char yield of these three sludge samples. This can be explained by considering that addition of sodium hydroxide at pH 12 increased the mineral contents of the sludge thus making the mineral/ ash concentration and char yield comparatively larger. The lower yield of bioliquid for hydrothermally treated sludge could be based on the degree of degradation of sludge during hydrothermal treatments. In particular, the greatest variations are found in pH 3 and 12 treated sludge samples which correspond to samples with higher solubilisation of

organics into aqueous fraction after hydrolysis (from Chapter 3). Overall, addition of either acid or alkali to sludges during hydrothermal treatments did not significantly change the pyrolysis product yields as they were quite comparable. However, the liquid yield of sludge at pH 6 increased and gas yield was lowered with respect to acid/ alkali treated sludge.

4.2.1.2 Thermogravimetry and estimation of the higher heating value (TGA-HHV)

Thermogravimetric analysis of the sludge samples before and after pyrolysis were carried out using the method described in Section 4.1.3. Figure 4.2 shows the TGA curves obtained (a) before and after hydrothermal treatments at different pH conditions and (b) char produced after pyrolysis experiments of hydrothermally treated sludge while (c) and (d) shows the differential thermogravimetric (DTG) before and after pyrolysis respectively. It is observed that from Figure 4.2 (a) and (c), the first weight loss zone below 150 °C is due to the loss of moisture content [14]. The major weight loss in the region from 200 °C to 400 °C is due to the volatilisation of medium molecular weight compounds like biodegradable aliphatic components [15]. In the zone between 400- 600 °C took place the decomposition of non-biodegradable organics like fats and lipids [16]. Above 600 °C was the degradation of inorganic matter in sludge [17]. Whereas in Figure 4.2 (b) and (d), there was an initial mass loss zone below 150 °C similar to non-pyrolyzed samples, at the temperature range of 200- 600 °C the weight loss is only about 11- 16% as the organics that were present in Figure 3 a, c was converted into liquid and gas products during pyrolysis. Finally, above 550 °C there was mass loss zone owing to decomposition of inorganic matter in char. Also, the fixed carbon which is the combustible residue remaining after all the organics have been volatilised has increased in all the pyrolyzed sludge samples. Pyrolysis experiments have resulted in an increase in the fixed carbon concentration which improves the char yield.

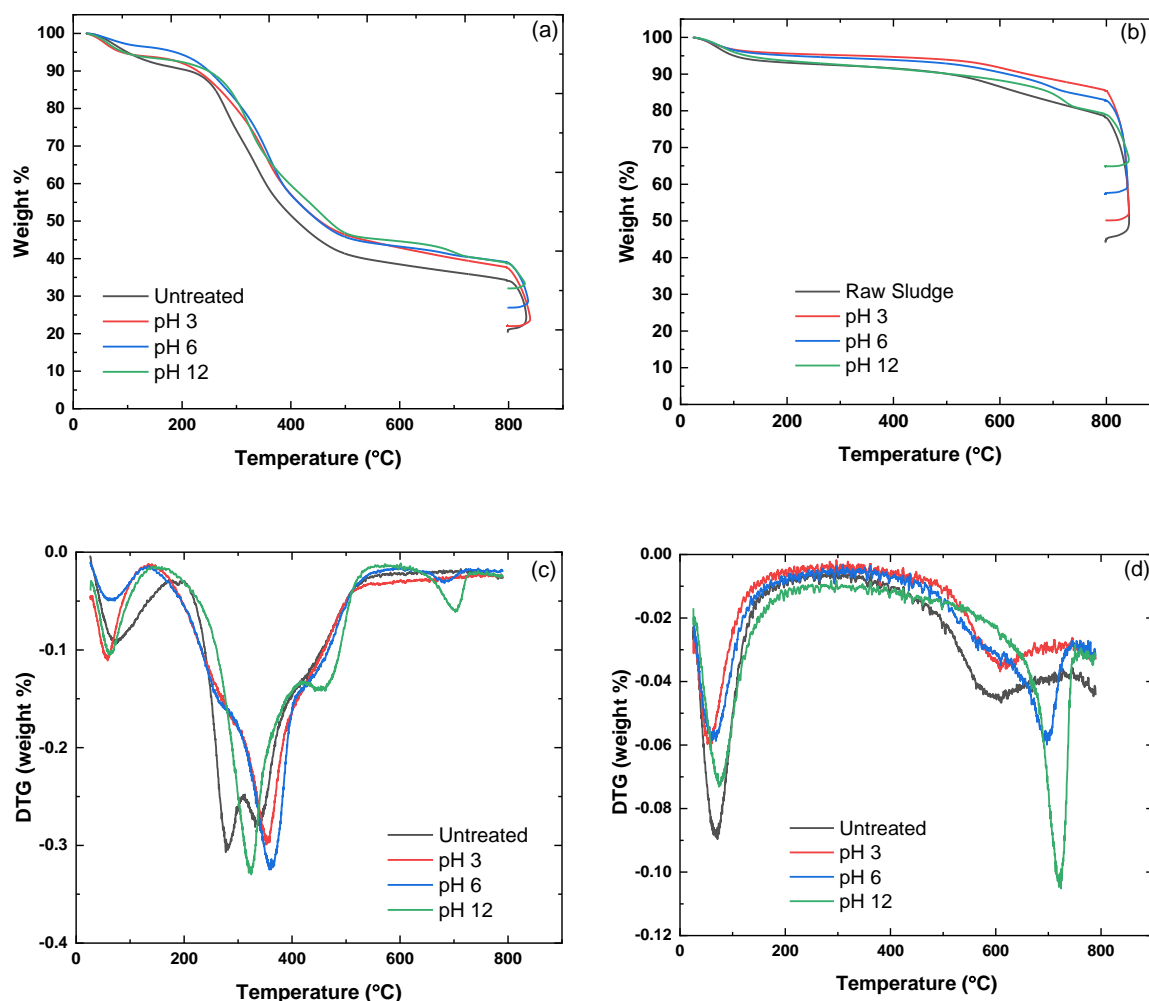


Figure 4.2. TGA curves of untreated and hydrothermally treated sludge (a) before pyrolysis and (b) after pyrolysis (char) and DTG curves (c) before pyrolysis and (d) char

Higher heating value of the sludge samples before and after pyrolysis are compared in Table 4.2. They were estimated as explained in Chapter 2-Section 2.2.5. It was seen that the HHV values were between 10-14 MJ/kg for the hydrothermal treated samples before undergoing pyrolysis. These values then shifted to around 7-14 MJ/kg after pyrolysis experiments. The hydrothermal treated samples had HHV which is quite comparable to raw sludge. After pyrolysis, the HHV of raw sludge remained almost the same while those of treated samples were observed to have decreased. This could be attributed to the fact that it is the volatile matter that mainly contributed to HHV and as mentioned previously in the pyrolyzed sludge the percentage of volatile matter was significantly lower than the non-pyrolyzed samples which resulted in lower values of HHV.

Table 4.2. HHV values of sludge before and after pyrolysis for untreated and hydrothermally treated sludge

Sample	HHV of non-pyrolyzed sludge	HHV of char
	MJ/kg	MJ/kg
Untreated	14.10	14.27
pH 3	14.13	13.81
pH 6	13.06	10.68
pH 12	10.78	7.07

As per literature, sludge with a low HHV of 5.1-8.8 MJ/kg was considered not suitable for energy generation due to their higher ash content [18]. Here except for pH 12 pyrolyzed sample, all the other sludge samples have a higher HHV above 10 MJ/kg. Thus, the solid fraction of the hydrothermal treated sludge with and without pyrolysis has a better opportunity to be used for further energy recovery.

4.2.1.3 FESEM-EDX analysis

FESEM-EDX was done to see if the pyrolyzed sample had higher concentrations of elements that can be useful for nutrients recovery or to be utilized as fertilisers. Figure 4.3 shows FESEM images of untreated sludge before pyrolysis and also its char. The diverse particle sizes in sludge are due to their heterogenous nature. There was a reduction in the volume of the char with respect to the initial volume of sludge load in the pyrolysis crucible and from the microscopy images it can be observed the formation of bigger char particles containing inorganic particles. From the visual inspection the char samples have a dark brown colour indicating the presence of a carbon residue.

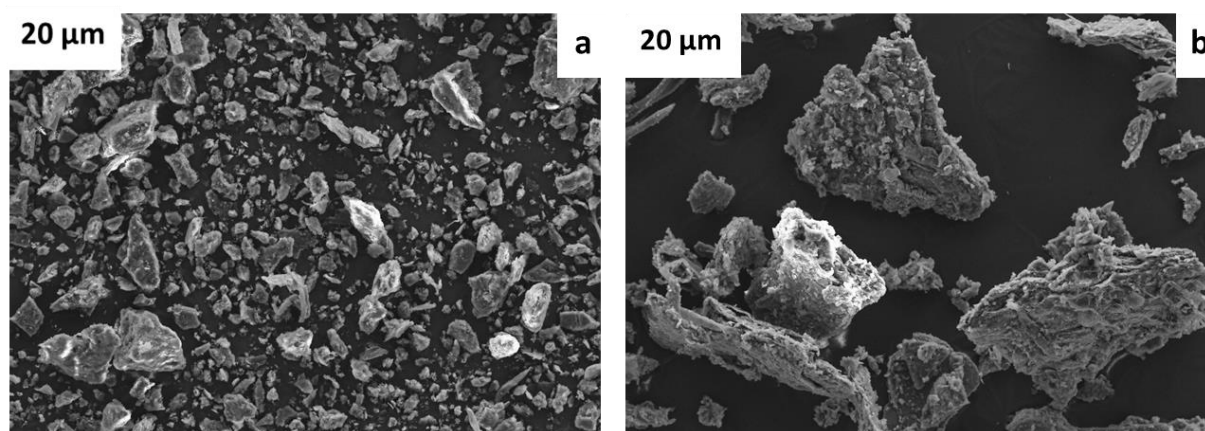


Figure 4.3. SEM of (a) untreated sludge and (b) its char

SEM EDX elemental analysis of untreated sludge before and after pyrolysis is shown in Figure 4.4. The concentrations of inorganic elements increased with pyrolysis. The highest available element was Ca followed by Si, P and Na in the pyrolyzed char. Phosphorus is an essential macronutrient for all living organisms and a component of an important class of fertilizers. It is expected that there will be a shortage of phosphorus-based fertilizers in near future [19]. The 6% of P found in the char suggest that it can be used as a source of this element or that the char can be directly used for soil amendment. In this last applicative option, the higher concentration of Ca with respect to the one of Na seems beneficial to the intended use.

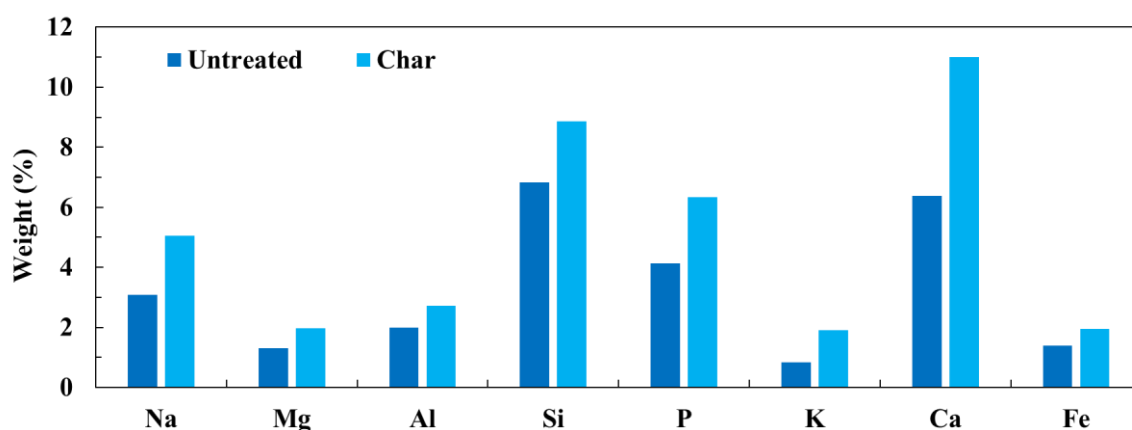


Figure 4.4. Comparison of percentage of nutrients present in untreated (non-pyrolyzed) and char (pyrolyzed)

4.2.2 Valorisation of the liquid fraction

4.2.2.1 Biotechnological transformations using commercial enzymes

4.2.2.1.1 TSK gel G6000PW GPC column

This GPC column is suited for the analysis of water-soluble polymers based on molecular weight separation up to 8×10^6 Da. The mobile phase is water with flow rate 1.0 ml/min, and the column is calibrated using EasiVial PEG/PEO standard having molecular weight cut-off between 106 and 442800 Da. Among the various different trials done using enzymes including varying the enzyme loading, only a few selected results are shown here in Figure 4.5 for which even the molecular weight was estimated using molecular weight distribution. Figure 4.5 shows

the HPLC graphs for a) liquid fraction of hydrothermally treated sludge at pH 6 before enzyme hydrolysis while the remaining graphs shows the sample after enzyme hydrolysis with 20% w/w enzyme/ total solids loading after 48hrs b) with cellulase at 40 °C and pH 5 c) with lipase at 40 °C and pH 7 and d) with protease at 55 °C and pH 5.

From these plots that were obtained using a RI detector, it can be seen that for all the samples after the reaction with enzymes, the appearance of peaks at elution time of 30- 40 mins which can be assigned to molecules of lower molecular weight of 200- 400 Da. Also, for lipase and protease the first peak at around 20 mins which represent higher molecular weight molecules in the range of 10,000- 100,000 Da seems to have significantly changed with respect to the sample before hydrolysis. It can be observed that with the use of enzymes, there is a breakdown of larger molecules into smaller ones which could be attributed to the depolymerisation of molecules like conversion of proteins to amino acids, polypeptides, or conversion of carbohydrates to reducing sugars, polysaccharides.

The basic idea that we would like also to explore in the use of different types of enzymes is the possibility of being able to possibly separate different classes of compounds by depolymerizing the corresponding macromolecules. Preliminary separation tests based on molecular weight were attempted using dialysis tubes with 2000 Da and 100- 500 Da cut off and also using the Amicon ultrafiltration cell with membranes of two different molecular weight cut-offs (GE series TFM-1000 MWCO PEG and Nadir UP005- 5000 MWCO PES) but these results are not included here as they were still not very clear, and a further study is still necessary to identify the suitable membranes. One of the major obstacles encountered in this explorative phase of the research is due to the amount of liquid to be treated still too small to conduct adequate screening tests with different membrane processes as well as ultrafiltration, nanofiltration and reverse osmosis.

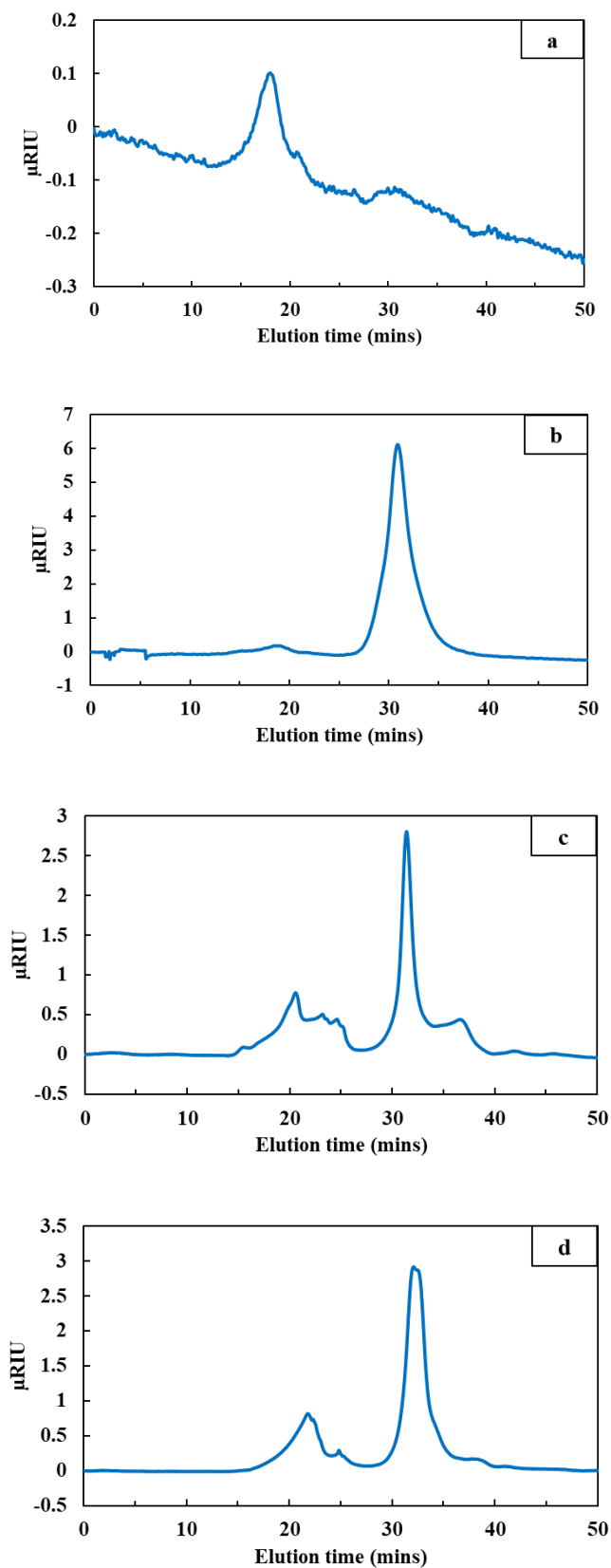


Figure 4.5. HPLC plots a) before enzymatic hydrolysis b) with cellulase c) with lipase and d) with protease

4.2.2.1.2 Rezex RPM-Monosaccharide Pb+2 column

This column is used for the separation of monosaccharides and sugar alcohols. The mobile phase is water with flow rate 0.6 ml/min, and the column is calibrated using a standard comprising maltose, glucose, mannose, fructose, and mannitol. From the older trials using the previous column, cellulase hydrolysis seemed to be better among other enzymes. So hydrothermal treated sludge samples followed by cellulase hydrolysis were injected into Rezex RPM column. Among several trials performed, one significant result is shown in Figure 4.6 which gives the graphs from RI detector of a hydrothermal treated sample at pH 6 that was subjected to cellulase hydrolysis for 48hrs at 40 °C along with the graph of glucose standard.

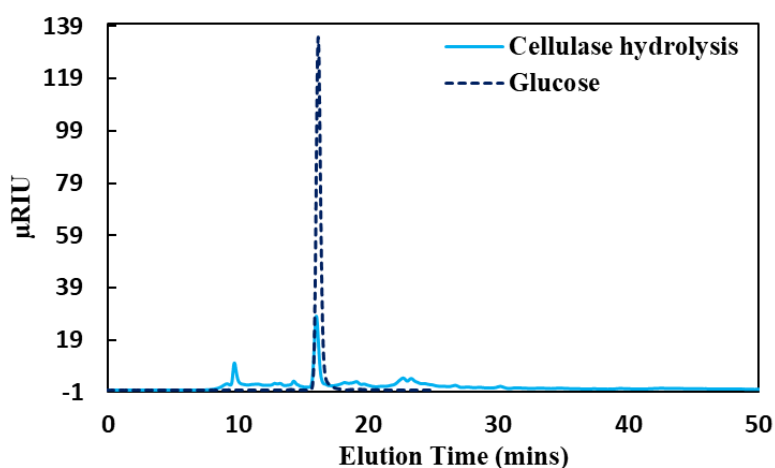


Figure 4.6. HPLC plots comparing glucose standard and cellulase hydrolysed sample

The peak at 16 mins for cellulase hydrolysed sample almost coincide with the glucose peak which shows the conversion of carbohydrates to glucose with cellulase action. This gives a better possibility to further explore the effect of cellulase on other treated samples and also efficient separation of the glucose produced.

4.2.2.2 Liquid fraction- qualitative analyses

A few of the interesting and significant results from some qualitative analyses on liquid fraction of treated sludge are described here. In addition to enzyme hydrolysis, fermentation with yeast was also tried on the hydrothermally treated sample at pH 6. After 24 hrs of fermentation, the head-space vapours were injected into GC-FID. The results in Figure 4.7 showed that vapours

were mainly comprised of ethanol. This qualitative analysis done showed further scope for improving the fermentation experiment to get improved results.

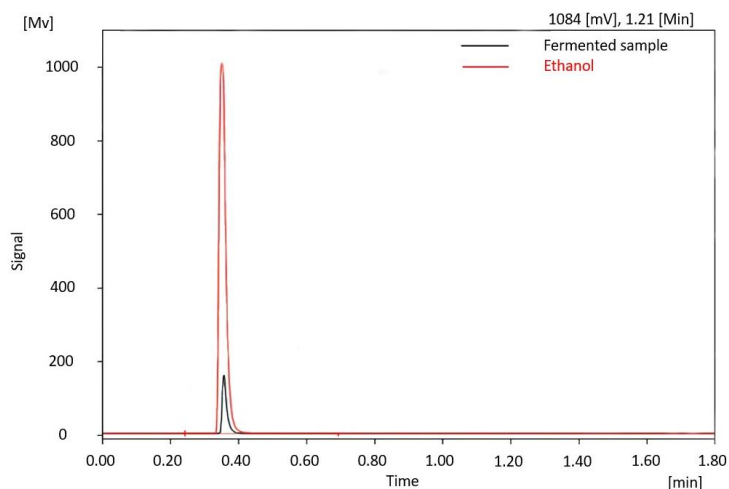


Figure 4.7. Chromatogram comparing hydrothermally treated sample at pH 6 after fermentation with ethanol peak

Figure 4.8 gives the FTIR result on the precipitate obtained after protein precipitation on the liquid fraction of pH 6 hydrothermal treated sample. Peaks at 1623 cm^{-1} , 1533 cm^{-1} and 1229 cm^{-1} represent amide I, II and III stretching vibrations respectively confirming the presence of proteins. Further, peaks at 3276 cm^{-1} suggest N-H stretch, at 2923 cm^{-1} aliphatic C-H stretching vibration, 1443 cm^{-1} deformation of C-H bond and 1033 cm^{-1} C-O stretching vibration of the polysaccharide [20–22]. Therefore, these results show that proteins were precipitated using HCl after hydrothermal treatment which can improve the rupture of the biomass matrix.

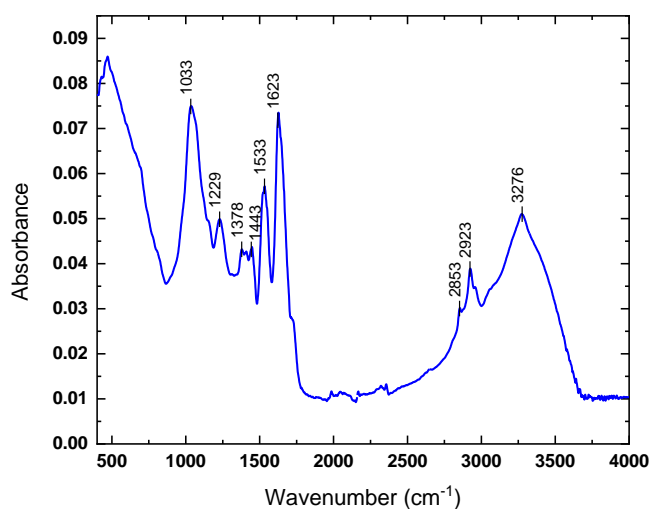


Figure 4.8. FTIR showing amide I, amide II, amide III peaks of proteins

Solubility tests using different solvents were done to have better understanding of the class of organic molecules present in the liquid phase. The liquid fraction of hydrothermally treated sample at pH 6 was dried and the residue was mixed with the solvents. Table 4.3 gives the results denoted with + for being soluble and – for insoluble in a particular solvent. The solvents in the table are presented in the increasing order of polarity. It can be seen that the residue is completely insoluble in n-hexane and fully soluble in water with greater solubility towards polar solvents. This could be because this fraction contains higher fraction of polar molecules like carbohydrates, alcohols, organic acids, proteins, and lipids which is in accordance with the solubilisation results obtained in Chapter 3.

Table 4.3. Results of solubility tests

Solvent	Solubility
n-Hexane	- - - - -
Toluene	++ - - -
Chloroform	++ - - -
Acetone	+++ - -
Acetonitrile	+++ - -
Ethanol	++++ -
Methanol	++++ -
Water	+++++

+ soluble
- insoluble

4.3 Conclusions

Valorisation of solid and liquid fractions of hydrothermally treated sludge samples were explored and studied here. On the solid fraction, pyrolysis experiments at 500 °C were done. Char yield increased after all hydrothermal treatments and was about 47- 52% making them the highest fraction of pyrolysis products obtained. On the char, HHV values were estimated using the proximate parameters obtained from TGA and compared with sludge samples before pyrolysis (i.e., only after hydrothermal treatments). It was found that HHV of char was lower than the non-pyrolyzed sample but still sufficient to be utilized for energy studies. SEM elemental analysis showed that pyrolyzed sample had greater concentration of phosphorus than non-pyrolyzed sludge. Thus, the char from the hydrothermally treated sludge is suitable either for their bioenergy potential or to be used for agricultural purposes in organic fertilizers. On the liquid fraction, different tests were carried out with an aim to valorize it. Biotechnological transformations using commercial enzymes resulted in the decomposition of larger molecules

into smaller molecular weight ones. Also, with the use of specific HPLC columns and cellulase enzyme, it was possible to obtain simple sugar like glucose which could be subjected to further separation. With GC-FID, production of bioethanol after fermentation test with yeast was confirmed and FTIR used for qualitative estimation of proteins that were precipitated using HCl acid. All the results on the liquid suggest a possibility to further explore this fraction for extraction, separation of a class of organic molecules or raw materials for fine chemicals production. An important issue that has emerged during all my valorisation experiments concerns the separation of the variety of molecules present in the hydrolysate and in the broth after the enzymatic conversion. From the point view of a laboratory or a small production, a preparative chromatography could be a proper choice. However, for an eventual industrial application the separation of the downstream solution can be critical, so an efficient and affordable solution still needs to be investigated.

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Chapter 5: Valorisation of stabilised filter pressed sludge

5 Valorisation of stabilised filter pressed sludge

Among the main crisis faced by today's society, is the efficient management and disposal of wastewater sludges that adheres to environmental laws. The amount of sludge production is significantly rising across the world and in Europe [1]. Traditional disposal techniques include landfilling, incineration, and reuse in agriculture by land-spreading [2,3]. However, landfilling is hazardous to the environment and needs a lot of disposal area [4]. Although incinerating sludge can help with reduction in volume and recovery of energy, carbon dioxide and eventually harmful emissions are released during the process to air, water, and land [5]. Moreover, recycling in agriculture tends to increase the concentration of heavy metals in the soil that poses a threat. Alternative technologies being currently used include sludge pyrolysis, gasification, and wet oxidation process. Pyrolysis has several advantages over the conventional processes [6]. Products of pyrolysis like char, oil and gas can be useful to fuel generation or as raw materials for production of typical petrochemicals. Pyrolysis is the thermal degradation of matter in the absence of an oxidative environment (inert atmosphere) at operating temperatures of 350- 900 °C [7]. The process temperature determines the pyrolysis product formation where an increase in temperature decreases char yield. Thus, at lower temperatures and with longer residence time promotes char generation while higher temperatures with short or long residence time enhances liquid and gas production respectively [8]. The decomposition of sludge takes place at different stages during pyrolysis due to its complex heterogeneous nature. At temperatures below 300 °C, degradation of minor decomposable organics like dead microorganisms and lipids take place while below 700 °C, decomposition of biopolymers, proteins and non-biodegradable organics follow [9]. Pyrolysis product biochar has several potential applications as solid fuel after combustion, agricultural use, or adsorbents for catalytic purposes depending on their properties like heavy metal, nutrients and energy contents [10]. Bioliquid can be utilised as liquid fuel for generation of heat and electricity or to produce synthesis gas for chemical synthesis. Likewise, biogas can also be used as fuel or processed to synthesis gas that can be upgraded to liquid fuel or chemicals production [11]. Since all the pyrolytic products can be utilised, pyrolysis is thought to be a zero-waste technique which has great potential for production of sustainable energy that caters to environmental, economic, and social problems. In this part of the study, pyrolysis on sludge that was meant for disposal into landfills was performed. Pyrolysis was investigated at 500 °C and 800 °C to study the effect on the product yields. The main objective was to determine the product distribution and to

characterise the char, liquid, and gaseous products to enable an efficient valorisation of this stabilised sludge. Thermogravimetric analysis was done on char to estimate their fuel properties. X-ray fluorescence and leaching test to check for the utilisation of this biochar in agriculture was additionally performed. On the liquid product, gas chromatography-mass spectrophotometry, FTIR and solubility tests are carried out to identify the class of molecules present.

5.1 Materials and Methods

5.1.1 Materials

Sewage sludge was received from Novi Ligure (AL) a municipal wastewater treatment plant (WWTP) operating in Genoa, Italy. The stabilised sludge obtained was taken after the final treatment step of the WWTP just before disposal in landfills. This sludge was subjected to primary, secondary treatments and finally to filter press dewatering. Sludge was dried in an oven at 50-60 °C for 24 h, crushed and stored in airtight containers before pyrolysis experiments. The characteristics of the raw sludge are given in Table 5.1 and the parameters were gravimetrically measured according to Standard Methods with total solids (TS) and volatile solids (VS) measurements at 105 and 600 °C respectively [12].

Table 5.1. Physical properties of sludge-proximate analysis (weight %)

Parameter	Value
Moisture ^a	80.2 %
Total solids ^a	19.8 %
Volatile solids ^b	77.4 %
Fixed solids ^{b,c}	29.2 %

^aon received basis

^bon dry basis

^cby weight difference

5.1.2 Pyrolysis of sludge

Pyrolysis at 500 °C was done using a tubular reactor as described and represented in Chapter 4 while the pyrolysis system with quartz reactor used at 800 °C is presented in Figure 5.1. For

the run, about 5 g of dried sludge at 50- 60 °C were used and subjected to a furnace temperature ramp of 20 °C/min from room temperature up to 900 °C with a residence time of 15 min. The thermocouple was placed inside the crucible, in the center of the sample mass and reached temperature of 800 °C. The atmosphere was kept inert by feeding helium with a flow rate of about 200 mL/min. By measuring the variation in the flow rate during the process using the bubble flow meter, it was possible to determine the total volume of the developed gases. Analyses of the gases produced during the process were carried out with the Agilent 490 micro-GC, equipped with 3 channels used for qualitative and quantitative analysis of the main gaseous products obtained from the process, i.e., H₂, CO₂, CH₄, C₂H₄, and C₂H₆. Micro-GC sampling was automated and occurred every 3 minutes. In this way, it was possible to analyze gas formation in relation to the process time, therefore to the temperature of the sample. After cooling the system down, it was possible to determine the yield of char, consisting of the residue left inside the crucible, and bioliquid products, condensed in the rest of the plant and biogas yield by weight difference.

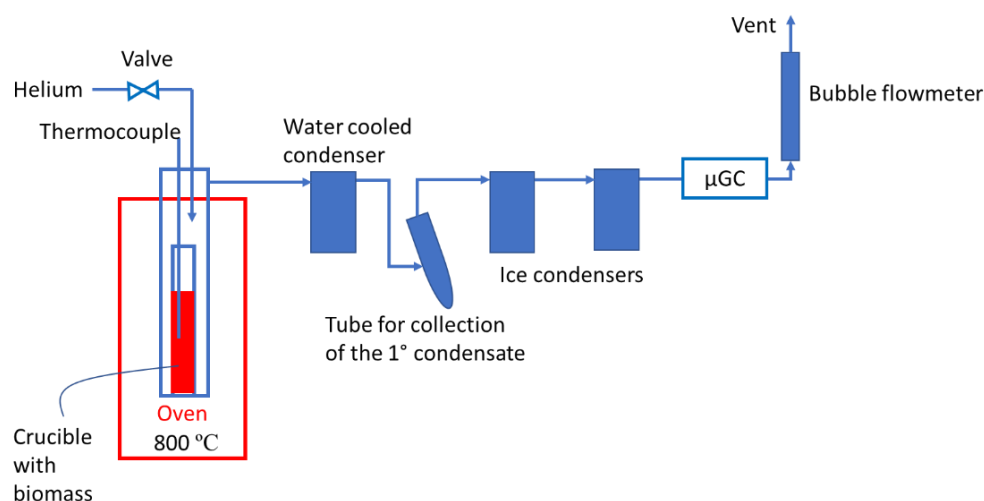


Figure 5.1. Pyrolysis system utilised

5.1.3 Characterisation of biochar

5.1.3.1 Thermogravimetric analysis (TGA) - higher heating value (HHV)

Higher heating value (HHV) is used to find the bioenergy potential of sludge by thermogravimetric analysis (TGA). The methodology adopted is as per ASTM standards and

previously described in Chapter 2. The conditions for TGA experimental runs are shown in Figure 5.2 below.

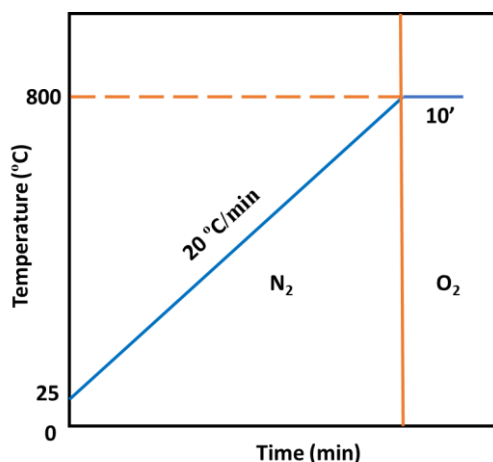


Figure 5.2. TGA experimental parameters

5.1.3.2 X-ray fluorescence spectrometry

X-ray fluorescence (XRF) analysis was kindly carried out by Italiana Coke (Cairo Montenotte, SV) to determine the inorganic composition of char produced using Shimadzu XRF-1800, Japan.

5.1.3.3 Leaching Test

In order to determine the concentration of phosphates contained in the biochar, a leaching test using acetic acid as an extracting solution was performed as per Water Research Institute (IRSA) method [13]. This method is used to simulate the behavior of a waste matrix subjected to the action of rainwater. It uses an aqueous solution of acetic acid as an extracting medium to assess the behavior of a waste material in a mixed landfill with both organic and inorganic compounds. The test is conducted by placing the biochar in contact with milli-Q water for 24 hours under mild stirring, keeping the pH of the solution at about 5 by addition of dilute acetic acid. The analyses are conducted on the liquid phase by filtration of the solid part. Here, about 1 gram of char was put in a closed vessel with 16 ml of 0.5M acetic acid and agitated for 24 h. After the extraction, the solution was filtered and injected in an ion chromatograph (Dionex DX-120) to determine the phosphate concentration in the leachate. The same procedure was repeated with a further addition of hydrogen peroxide solution to the filtered leachate so as to convert the free phosphorus to phosphates that can be detected again by Dionex. The

percentage of phosphates released from char is calculated by the equation 5.1. The total mass of phosphates in the sample was calculated from the XRF analysis.

$$\% \text{ Phosphates released} = \frac{\text{mass of phosphate released (mg)}}{\text{total mass of phosphate in char (mg)}} \quad 5.1$$

5.1.4 Characterisation of bioliquid

The liquid product was separated into aqueous and oil fraction by centrifugation and the yields determined by phase separation by weight. The residual pyrolysis bioliquid from the condensers was recovered by rinsing with small amount of acetone. With gas chromatography- mass spectrometer (GC-MS) it was possible to characterize the liquid products obtained from pyrolysis. The GC-MS analysis were kindly executed by Ireos Laboratori (Genoa) using an Agilent 5973 inert GC/MS system

Solubility tests were performed by dissolving the two fractions of the liquid product in different organic solvents to identify the class of molecules present.

Fourier transform infrared spectroscopy (FT-IR) analyses were performed for the characterization of both the dried sludge and liquid product. They were carried out using the Bruker Vertex 70 spectrophotometer using the ATR (Attenuated Total Reflection) technique. For each measurement, 128 scans were performed, while the resolution was set to 2 cm^{-1} . The samples were used as they were obtained.

5.1.5 Characterisation of pyrolysis gas

For pyrolysis at 500°C , two gas chromatography systems were used. The first one is a Perkin Elmer AutoSystem, with helium as a carrier gas. The temperature of the injector is 150°C , that of the detector 250°C . The method involves an initial isothermal stage at 60°C , 1 min long, followed by heating at a rate of $20^\circ\text{C}/\text{min}$ up to 80°C , a dwell time of 90 seconds, then heating to 150°C at a rate of $45^\circ\text{C}/\text{min}$, followed by final isothermal stage 150 secs long. In each determination, $100 \mu\text{L}$ of sampled gas was manually injected with a syringe, and it was possible to perform a run every 13 minutes. The second gas chromatograph used is the Agilent 7820A, equipped with a Shin Carbon ST 80/100 column 2 meters long. The temperature of the injector is 250°C , that of the detector 300°C . The temperature ramp consists of heating from 60 to 80

°C to 20 °C/min, followed by an isotherm lasting 90 seconds and a subsequent heating 150 °C to 75 °C/min. In this case the carrier is argon, and the sensitivity is greater, particularly regarding the quantification of hydrogen. In each stroke, 150 µL was manually injected with a syringe. With this tool it was possible to carry out an analysis every 10 minutes. In addition, the gas analyses of sludge at 800 °C was done using a micro GC (model 490, Agilent).

5.2 Results

5.2.1 Sludge pyrolysis

Sludge pyrolysis was performed at 500 °C and 800 °C by flowing nitrogen and helium respectively over a fixed bed of sludge into a crucible. The temperatures for pyrolysis were selected on the basis of preliminary TGA results obtained in Chapter 4. Also, they are in agreement with previous works that states significant sludge pyrolysis was observed at temperatures ranging between 450- 800 °C [14]. In Figure 5.3, the yields of the different pyrolysis products at two different temperatures are presented.

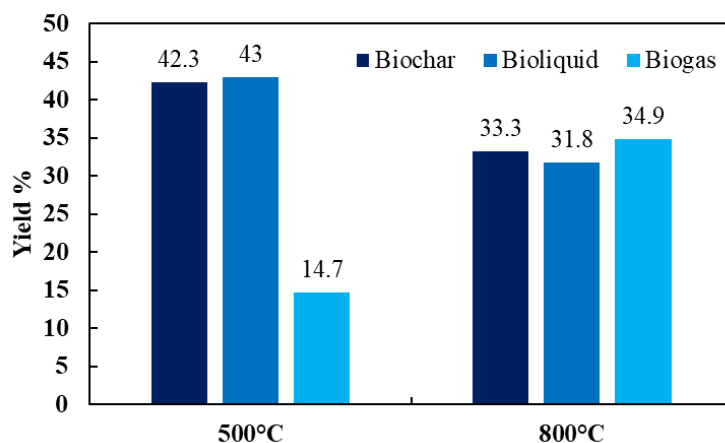


Figure 5.3. Percentage yields of pyrolysis products (on dry feedstock mass)

The yields of biochar and bioliquid were quite comparable at both pyrolysis temperatures with yields ranging about 42-43 % of dry feedstock mass at 500 °C and between 31-33% at 800 °C. The yields of the pyrolysis products change with the different operating temperatures as expected. Increasing the pyrolysis temperature to 800 °C has decreased the yield of char and liquid while increasing the gas yield. This trend noticed in the product yields at 800 °C could

be due to the generation of gaseous products formed as a result of cracking reactions. From literature too, a decrease in char yield with respect to increasing pyrolysis temperature was found that was attributed to either improved disintegration of the initial feedstock material or further reactions of the residual solid [15].

The pyrolysis at 500 °C was of greater interest due the fact that here more focus was on the solid and liquid products primarily. Pyrolysis at 800 °C gave a good comparison on the product yields at higher temperature. So henceforth, the results from only 500 °C pyrolysis experiments will be discussed.

5.2.2 TGA-HHV

Thermogravimetric analyses were done on the non-pyrolyzed dried sludge and pyrolyzed char samples with the specific aim of determining the higher heating value and the results are presented in Figure 5.4 (a) and (b). TGA and differential thermogravimetric (DTG) graphs give information on the starting and final degradation temperatures of sludge. In case of dried sludge, significant degradation of volatile matter of about 64% on dry basis was in the temperature range of 200- 600 °C. This could be attributed to the disintegration of higher concentration of organic molecules present in non-pyrolyzed sludge. Whereas there was only 14% on dry basis of organic matter that were decomposed in the char. This lower value is because organic matter in the dried sludge got converted into liquid and gaseous products during pyrolysis. The weight loss during combustion was 10% and 30% for dried sludge and char respectively. A decrease in the percentage of volatile matter and increase in fixed carbon value of char is due to the stable carbonization of sludge that took place during pyrolysis. This further explains the higher amount of residual ash of about 50% in char with comparison to the dried sludge having only 21% on dry basis of ash.

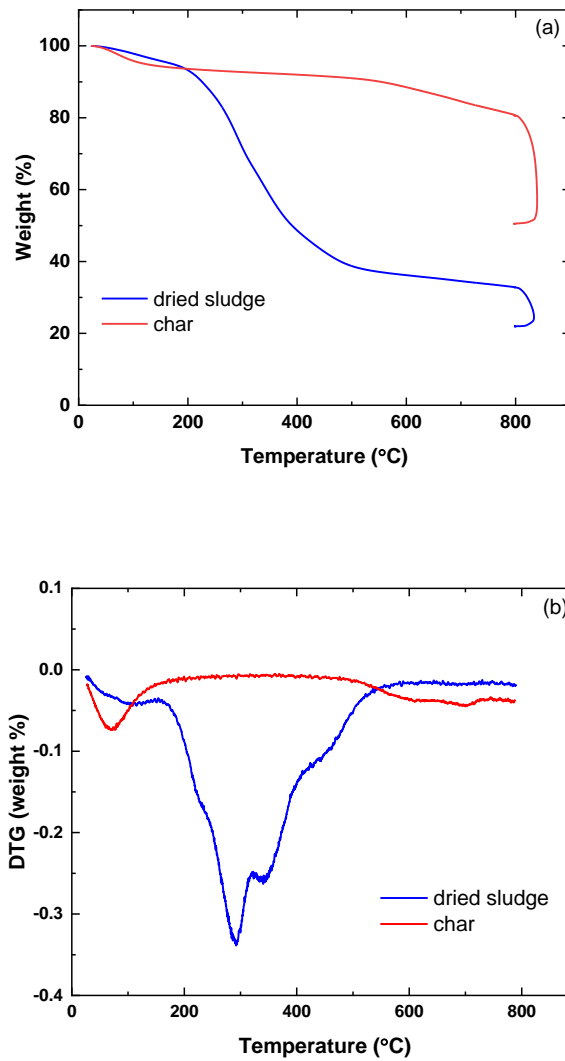


Figure 5.4. (a) TGA and (b) DTG curves of dried sludge (before pyrolysis) and char (after pyrolysis)

HHV is an important parameter for evaluating the properties of solid biofuel. The HHV values for dried sludge and char are 13.71 and 12.53 MJ/kg respectively. As previously mentioned, they were calculated from the proximate values explained in Chapter 2. In general, heating value of sludges is between 11- 17 MJ/kg [16]. Slightly lower HHV of char than dried sludge could be because of higher ash content in the carbonised char after pyrolysis. However, both their HHVs are quite comparable and as per literature it is possible to consider both the dried sludge and char for conversion to solid fuel.

5.2.3 XRF and leaching test results

The mineral composition of char was examined by X-ray fluorescence. Figure 5.5 shows the inorganic matter identified in char expressed by weight percentages. High concentration of C 31.9% remaining in char could be utilised for energy by combustion or for carbon sequestration. The major inorganics that were present were oxides of Si, Al, P, Ca, and Fe similar to results found in literature [17]. High presence of Si in sample can be assigned to sand and soil. Fe, Ca, Mg, K are also present and they can play the role of a catalyst during some pyrolysis reactions [17]. Approximately 10% on dry weight basis of P was found that was of more interest as it could be a probable supplement to be used in agriculture.

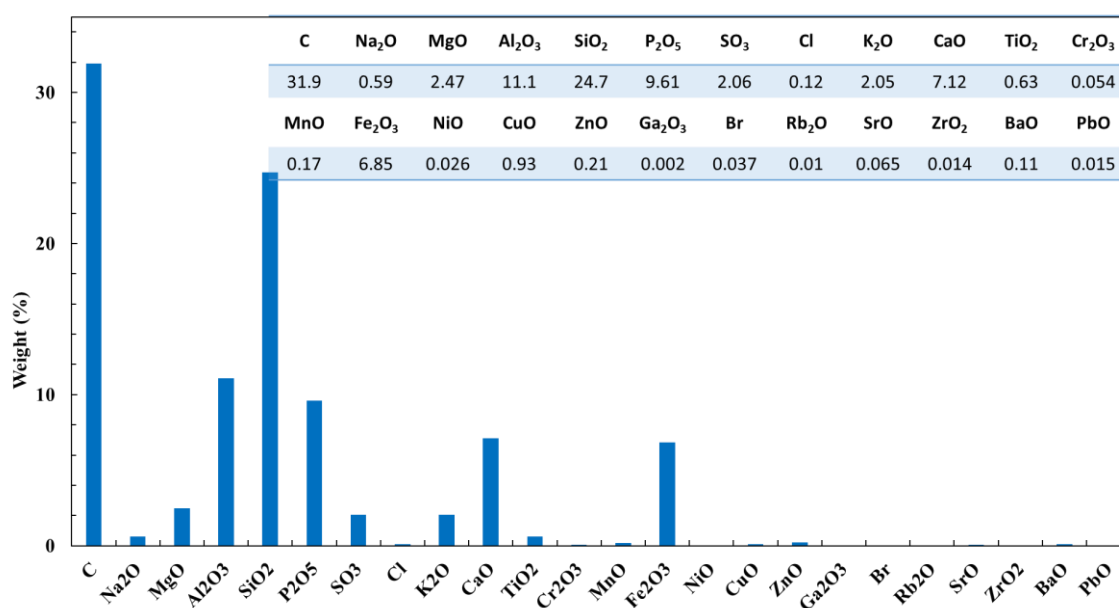


Figure 5.5. XRF results of char

In addition, the concentration of phosphates leached out of char were determined using ion chromatography and the percent of phosphates leached with respect to initial phosphate content in char was calculated to be 4.3% using Equation 5.1. Application of sludge residual char in agriculture for phosphorus recycling is valuable as P serves as a potential nutrient for soil. These results from XRF and leaching tests show that as high as 10% of P in char could be a useful resource for recovery and by improving the method for extraction, a higher yield of P could be recovered and recycled for agricultural purposes.

5.2.4 Characterisation of bioliquid

Bioliquid comprised of two phases: aqueous and oil. By visual appearance, the aqueous fraction was orange coloured and less viscous than the oil fraction that was dark brown coloured and sticky. The phases were allowed to separate by gravity in a test tube. It was observed that 60% of the liquid was the aqueous fraction while 40% was the oil phase. This greater proportion of water-soluble phase is the presence of water/ humidity in the dried sludge before pyrolysis that were not completely volatilised as the drying took place at around 50-60 °C. To confirm this, pyrolysis of sample dried at 105 °C were performed and the bioliquid obtained was 40% aqueous and 60% oil phase. Separation of phases takes place due to the differences in polarity and density of the obtained liquid products.

From the GC-MS results, the percentage composition of different class of compounds in both phases of liquid were identified as presented in Figure 5.6. It can be seen that in the oil phase, there is a greater percentage of phenolic compounds followed by hydrocarbons like alkanes, alkenes, ketones, cyclic compounds. Unlike the aqueous fraction where the total percentage of organics present was 6- 8%, out of which the percentages of the most abundant compounds differ with a higher percentage of hydrocarbons, followed by phenolic and nitrogen containing compounds. There was higher fraction of nitrogenic and furan/pyran molecules in aqueous phase than oil while some alcohols that were present in the oil phase were absent in the aqueous phase. The possible explanation for these results is that there is a difference in abundance for different compounds present in both fractions. It is necessary to also consider the chromatograms to better understand these differences.

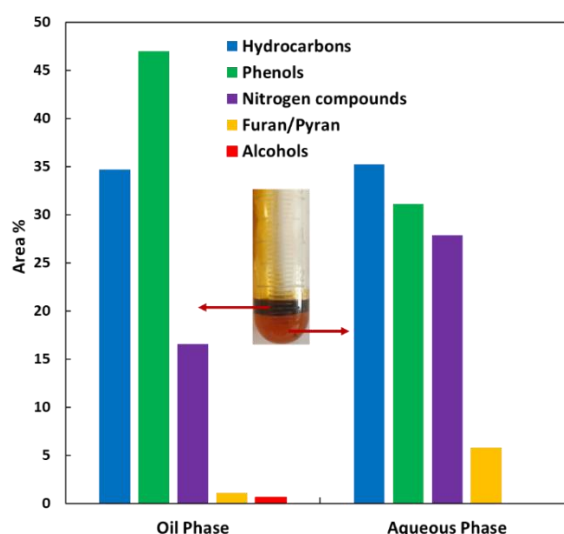


Figure 5.6. GC-MS results of oil and aqueous phase of bioliquid

In addition, some solubility tests using solvents like n-hexane, toluene, and diethyl ether were done to have better understanding of the class of organic molecules present in oil and aqueous phases. Table 5.2 gives the results denoted with + for being soluble and – for insoluble in a particular solvent. It can be seen that the oil phase is completely insoluble in water but slightly soluble in non- polar solvents with higher solubility in toluene. This could be because this fraction contains hydrocarbons, aromatics, phenolic and nitrogen compounds that are more towards the non-polar side. The aqueous phase on the other hand seems to comprise of a mixture of both polar and non-polar molecules with a greater fraction of polar ones due to their high solubility in water and insolubility in n-hexane and weak solubility in toluene and ether. The probable molecules could be carboxylic acids, amides, alcohols, and phenols.

Table 5.2. Results of solubility tests

Solvent	Oil phase	Aqueous phase
n-Hexane	+ - - - -	- - - - -
Toluene	+++ - -	+ - - - -
Diethyl ether	++ - - -	+ - - - -
Water	- - - - -	++++ -

+ soluble

- insoluble

To identify the functional groups, infrared spectroscopy was performed on dried sludge and on both oil and aqueous fractions of the liquid product, and the results obtained are given in Figure 5.7. The FTIR spectrum for dried sludge shows a very wide and strong absorption band around 3300 cm^{-1} , which can be assigned to the O–H and N–H groups bound to H, two small bands in the 2955 and 2920 cm^{-1} region due to aliphatic C–H stretching. Peaks at 1532 and 1241 cm^{-1} may also be due to secondary amides. The region between 1506 and 1328 cm^{-1} is due to the vibrations of the CH_2 and CH_3 groups. A strong band at 1064 cm^{-1} was also observed which may have been caused by stretching vibrations of the C–O and C–O–C groups. Below 700 cm^{-1} , skeletal vibrations occurred, often difficult to interpret. In all cases, two partially overlapping bands at 1720 and 1660 cm^{-1} were observed. The first was assigned to C=O of esters and acids, and the second to amides. Comparing the FTIR spectra, the dried sludge sample bears greater resemblance to the spectrum of the oil phase of pyrolysis liquid. The graph of the aqueous phase has the typical and most intense molecular hydrogen bonds O–H stretching vibration at 3300 cm^{-1} and this agrees with its aqueous nature on comparing it water spectra. Also, in this aqueous spectrum, water is being greatly absorbed as compared to the minor compounds present shown at band $1300\text{--}1500\text{ cm}^{-1}$. On the other hand, it does not have the typical bands

of aliphatic C stretching, confirming that this fraction contains fewer hydrocarbon compounds. In addition, the 1064 cm^{-1} band present in the dried sludge is related to oxygenated compounds such as alcohols, carboxylic acids, ethers, and esters, is totally absent or present only at very low intensity in pyrolysis oil phase. The relative intensity of the bands in the oil spectra indicates both an increase in the concentration of aliphatic carbon and a decrease in the concentration of oxygenated compounds.

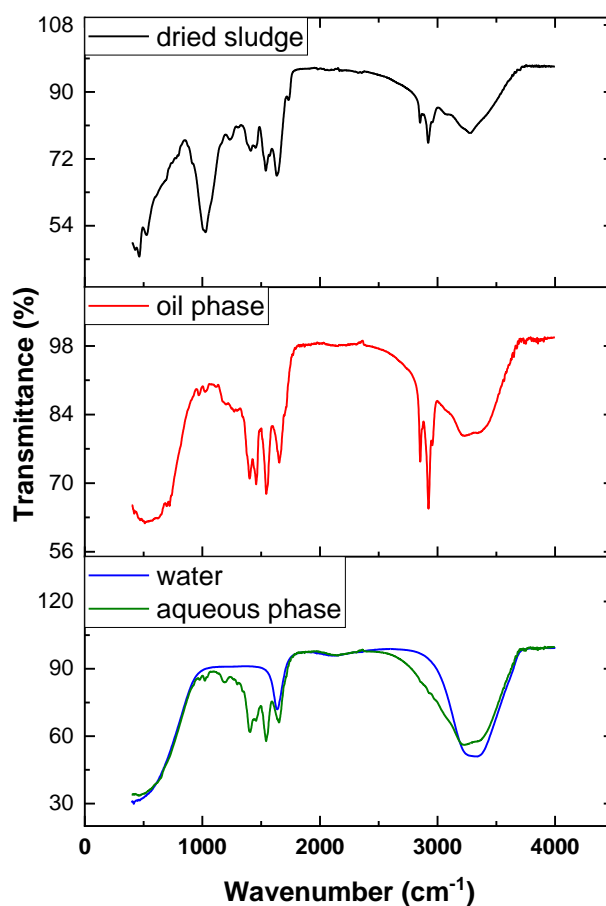


Figure 5.7. FTIR spectra of the dried sludge, liquid fractions and pure water obtained after pyrolysis at 500°C .

5.2.5 Characterisation of gaseous product

The composition of the gaseous fraction is analyzed by GC-TCD. The data were expressed in terms of yield (ml/min/g) of the gas in the mixture leaving the pyrolysis system. Figure 5.8 (a) show the gas analyses at 500°C pyrolysis while (b) are the pyrolysis results from 800°C .

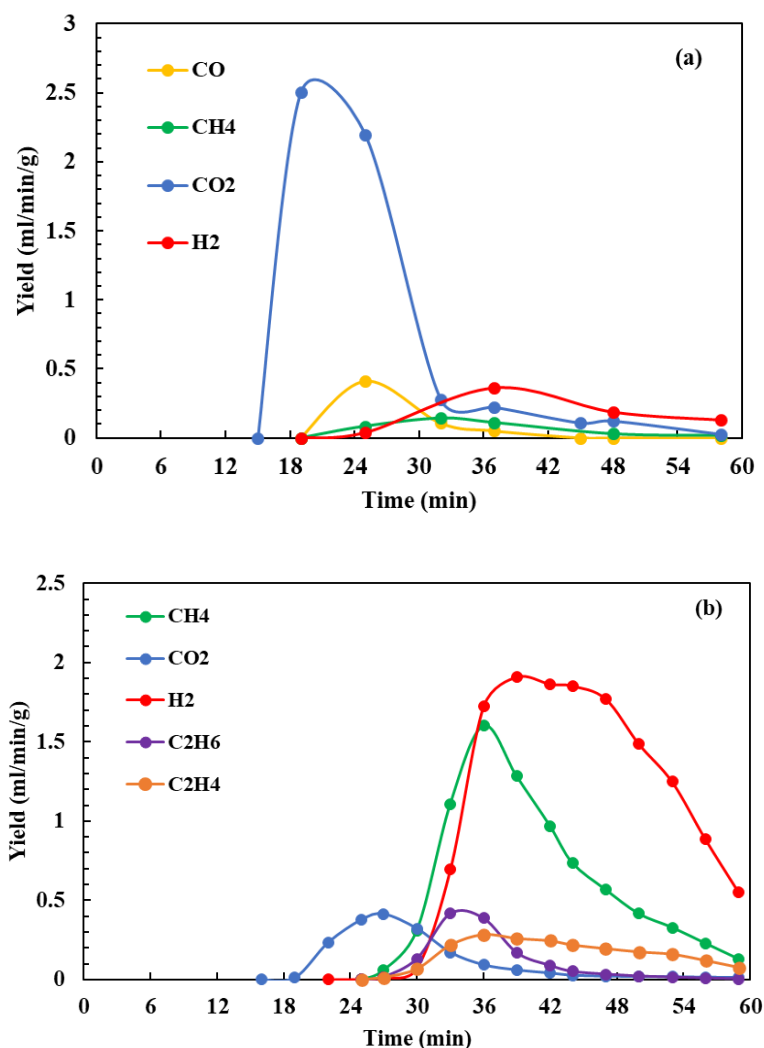


Figure 5.8. Yield of gases from pyrolysis at (a) 500 °C and (b) 800 °C

The main gases evolved at 500 °C were CO₂, CO, CH₄, H₂ while at 800 °C, C₂H₄ and C₂H₆ were additionally measured but CO was not detected. The primary gaseous product at lower temperature of 500 °C was CO₂ with a maximum yield of about 2.0- 2.5ml/min/g within the first 30 mins. CO was also seen to have an increasing trend. Other gases had flow rates between 0-0.5 ml/min/g during 1 h residence time. Whereas, at 800 °C, hydrocarbons were released after 30 mins with an almost constant flow rate in case C₂H₄. The flow rates of CH₄, and H₂ evolved showed an increasing trend with a more or less constant rate of release of H₂ gas between 36- 48 mins. For both pyrolysis, CO₂ was the first gas to be released.

5.3 Conclusions

Pyrolysis of a stabilised filter pressed sludge from a municipal wastewater treatment plant was studied using a fixed bed reactor system. The investigation of the pyrolysis product yields was done at 500 °C and 800 °C. At 500 °C, there is higher yield of char and liquid products while at 800 °C, the yields of all products were quite comparable between 31.8- 34.9%. However, the gas yield at 800 °C was twice that at 500 °C. Among the gases measured, CO₂ was more prominent at 500 °C whereas CH₄, and H₂ had higher flow rates at 800 °C. Additionally hydrocarbons like C₂H₄ and C₂H₆ were also evolved at 800 °C. It is clear that the temperature affected the product distribution of pyrolysis. Since biochar and bioliquid were the products of interest in this study, further characterization was performed only on the products of pyrolysis at 500 °C which gave higher yields of solid and liquid products. Higher heating value were estimated using the proximate parameters obtained from thermogravimetry analysis. These results showed that the dried sludge and biochar had the potential to be used as a solid fuel. According to values from X-ray fluorescence test, 10% phosphorus on basis of dry matter was found in the char. Carrying out leaching test using acetic acid solution, 4.3% of phosphates were extracted and determined using Dionex from char. Thus, the char from this stabilized sludge is suitable either for energy recovery or to be exploited for agricultural purposes. The utilization of biochar as soil amendment can result in a twice benefit. The relevant content of phosphorus which can be released into the soil candidates the biochar as an alternative to synthetic fertilizers. Moreover, the fixation of carbon into an inert solid carbon phase can be seen as a way to sequester carbon. This last approach it is very interesting since in the specific case studied for each kg of sludge 0.423kg and 0.333 kg of biochar are produced at 500°C and 800°C, respectively. That corresponds at 0.135 kg and 0.106 kg of carbon and 0.495 kg and 0.389kg of CO₂ fixed as biochar at 500°C and 800°C, respectively. In order to make negative the carbon balance of the process the energy necessary for pyrolysis should come from renewable sources. Further studies will be necessary to evaluate the sustainability of the process. Concerning the use as soil amendment additional investigations will be necessary to assess how the presence of some contaminants in the sludge will affect the suitability of the biochar in agriculture.

The bioliquid was divided into two fractions by gravity phase separation: viscous, less dense oil phase and less viscous, dense aqueous phase. Oil fraction appeared to consist of larger proportion of phenolic compounds while aqueous phase constituted largely of hydrocarbons as

per GC-MS results. Also, the solubility tests proved that the aqueous phase was mainly water soluble compounds like low molecular weight hydrocarbons as it was almost soluble in water though some of its components were insoluble in water but slightly soluble in non-polar solvents like toluene and diethyl ether. This water soluble phase was a result of the humidity and crystallization water present in the sludge sample before pyrolysis. The oil phase showed a mixed solubility behavior in non-polar solvents and was completely insoluble in water. The FTIR spectra of dried sludge was more like that of the oil phase but with an increase in the intensity of aliphatic carbon whereas the aqueous phase resembled higher humidity with the broad peak at 3300 cm^{-1} . Considering the above results, it can be concluded that the sludge which was to be disposed in landfills, could be pyrolyzed and the char can be utilized either as solid fuel or as nutrient supplement in agriculture. While the oil fraction of the bioliquid can be further investigated for their ability as feedstock for biofuel production. Thus, pyrolysis looks like a feasible solution for this sludge from an environmental aspect.

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Chapter 6: Pre-treatment and hydrolysis of brewer's spent grains (BSG)

6 Pre-treatment and hydrolysis of brewer's spent grains (BSG)

Europe is the world's second largest producer of beer after China with more than 8490 breweries producing about 400million hectolitres of beer as per reports in 2016 [1]. One of the main by product obtained during beer production in the brewing industry is brewer's spent grains (BSG). It is the most abundant agro-industrial waste created and accounts to over 3.4 million tons of BSG generated yearly in Europe [2]. BSG is the insoluble part of the malted barley grain that is separated during the mashing process before fermentation of the soluble liquid wort. About 30% of the initial malted barley end up as BSG at the end of brewing [3]. European legislation 'New Waste Framework Directive- 2008/09' was introduced for the management of food wastes like BSG and to define methods for their reuse and recycle [3]. BSG is rich in cellulose, hemicellulose, lignin, and proteins and are generally used as animal feed [4]. The composition of BSG including its high fibre content makes them a suitable raw material for biotechnological processes, enzyme production, renewable energy, production of bread, ethanol, activated carbon among others [5]. For effectively valorising BSG, it is necessary to destroy its lignocellulosic structure. Pre-treating BSG can help to facilitate this objective by making the organic molecules readily available. Thus, pre-treatment strategies like chemical, physical, thermal methods are important for improving the valorisation process of BSG [6]. The effect of several pre-treatments like microwave [7], thermal [8], acid hydrolysis [9] and alkali hydrolysis [10] have been studied in the past.

The aim of the work is to try to valorise BSG similar to valorisation of sludge firstly by pre-treating them and then by examining suitability of the liquid for obtaining molecules as feedstock and solid for fuel properties after pre-treatments. This chapter is part of a brief and recent research period at Micro, Nano and Bioprocess Engineering department of Wroclaw University of Science & Technology, Poland and is still an ongoing work.

6.1 Materials and Methods

6.1.1 Materials

BSG were obtained as a by-product from the brewery located at the premises of the Wrocław University of Science and Technology (Poland). The grains were dried at 50 °C for 48 h and ground and then stored at room temperature in a dry place for further experiments.

6.1.2 Pre-treatments

Different BSG pre-treatments were done with water, acid, or alkali as the medium. For acid medium, 0.5N hydrochloric acid (HCl) and 0.5N sulphuric acid (H₂SO₄) were used while for alkaline ones, 0.5N sodium hydroxide (NaOH) and 0.5N potassium hydroxide (KOH) were utilised. One set of pre-treatments were done by adding 5g of BSG in 100 ml of aqueous medium in a 230 ml Erlenmeyer flask and shaking in a water bath shaker (Elpin+ type 357) at 100 rpm for 1 h at room temperature. Ultrasonication was performed in a sonicator bath (Elmasonic S30H) with a maximum power of 280 W maintaining ambient temperature by water circulation for reaction time of 1 h. Low temperature thermal treatment was also done in Erlenmeyer flasks at 70 °C in water bath shaker for 1 h. Microwave pre-treatment was done in a plastic bottle using a modified commercial microwave reactor (Panasonic A554W) operating at 70% of total power for reaction time of 4 min. After the experiments, samples were separated into solid and liquid fractions by vacuum filtration. Solid was dried at 50 °C in an oven for 24 h and stored while liquid was stored at 4 °C until further analyses.

6.1.3 Sample analyses

On the liquid part, total sugars are measured using refractometer (Francuski refraktometr 0-32 °BRIX Browamator, Poland). Results are given in Brix degrees, 1 °Bx is equal to 1 g of sucrose in 100 g of solution. Measuring the concentrations of total reducing sugars was done using 3,5-Dinitrosalicylic acid (DNS) by Miller's test [11]. The absorbances values were measured at 550 nm using UV-1800 Spectrophotometer (Shimadzu, Kyoto, Japan) and concentration was determined according to the standard calibration curve. Total polyphenols were done by Folin–Ciocalteu method and concentration of polyphenols is calculated as a gallic acid equivalent at 765 nm [12]. Total protein measurements were performed by Lowry method using Lowry and

Folin reagents at 750 nm in UV-1800 Spectrophotometer (Shimadzu, Kyoto, Japan). Calibration curve was prepared using albumin as a reference protein [13].

6.2 Results

6.2.1 Effect of pre-treatments on reducing sugar solubilisation and °Brix

Figure 6.1 shows the results obtained from DNS-reducing sugars and refractometer °Brix measurements on the liquid fraction after pre-treatments. Degrees Brix or °Brix is a measure of the total soluble solids (TSS) present in the sample and is mostly used in food processing industries. TSS is mainly made up of sugars but also includes other compounds like organic acids, fats, minerals, alcohols, or flavonoids. Sugar content is highest in comparison to other soluble solids. So °Brix is taken as a measure of total sugar contents and does not differentiate between the different sugars. From the graphs it was seen that in general, for all pre-treatments there is a higher concentration of reducing sugars that is being released into the liquid phase in acid medium followed by water and was lowest in alkaline solutions. During acid pre-treatment conditions, there is degradation of polysaccharides to monomers and subsequent breakdown of simple sugars to 5-hydroxymethylfurfural (HMF), levulinic acid or formic acid [8]. °Brix was higher in alkali medium on the contrary than in acidic conditions. This could be because alkaline pH is good for solubilisation of not only sugars but also other molecules from pre-treated BSG but did not favour the release of reducing sugars. An alkaline medium is responsible for the inter-crystalline swelling of cellulose by the breakup hydrogen bonding of adjacent glucose molecules to produce material of low bulk density. Also, hydrolysis of hemicellulose takes place. For shaking and ultrasonication pre-treatments, the concentrations of reducing sugars and °Brix were quite comparable irrespective of the aqueous medium used. °Brix measured during all the four pre-treatments for all samples were 1.4- 5.0. Combining BSG with water gave nearly the same values for both reducing sugars and °Brix except for microwave pre-treatment which could be due to the smaller reaction time utilised here as compared to other pre-treatments. When the aqueous medium used was acid and combining it with low thermal pre-treatment released the highest concentration of reducing sugars among others. In case of NaOH medium, it can be seen that microwave and thermal pre-treatment significantly reduced the concentration of reducing sugars with respect to stirring and

ultrasonication. This trend observed is similar to that in literature where no significant amount of monomers were solubilised when a dilute alkali was used in compared to acid additions [8].

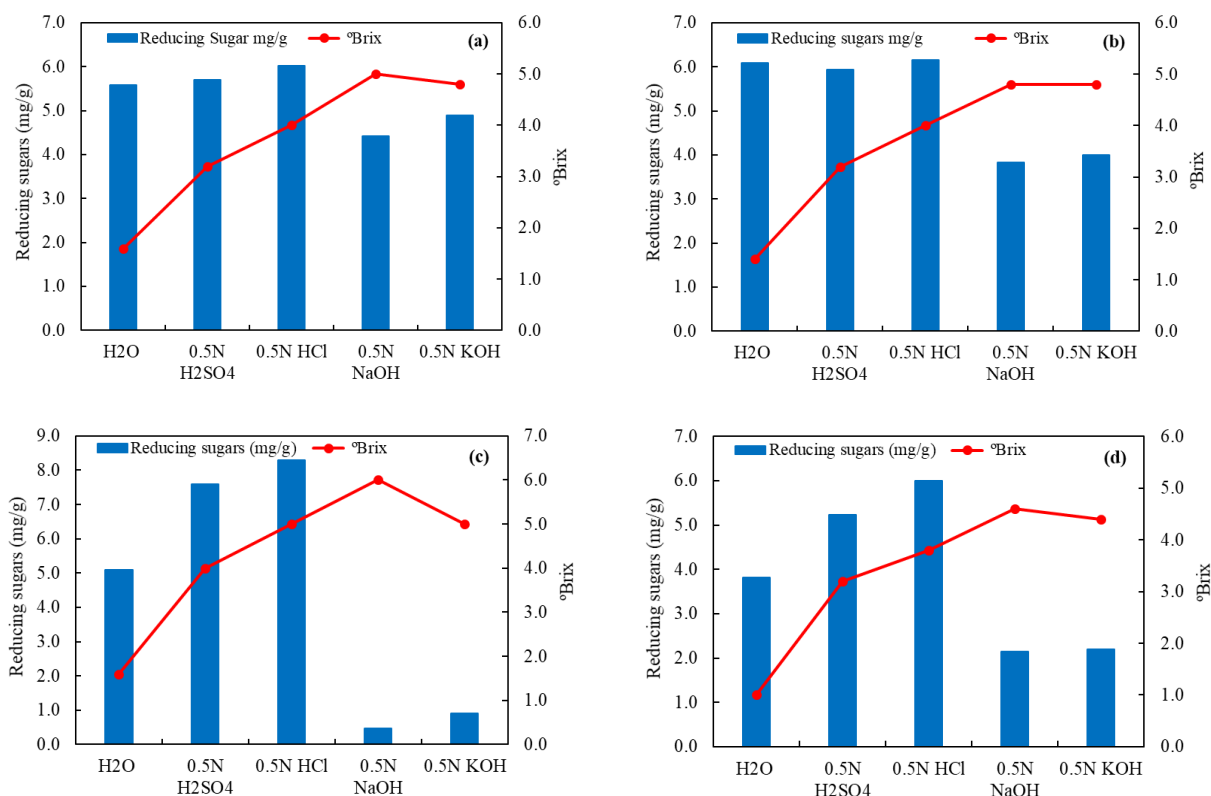


Figure 6.1. Graphs showing concentration of reducing sugars and °Brix after (a) shaking, (b) ultrasonication, (c) low temperature thermal, and (d) microwave pre-treatments in different medium

6.2.2 Effect of pre-treatments on proteins and polyphenols solubilisation

Figure 6.2 shows the concentrations of proteins and polyphenols measured on the liquid fraction after all pre-treatments. It can be observed that the highest solubilisation of proteins and polyphenols were in alkaline medium and 0.5N KOH appears to be the better alkali than NaOH here. Higher solubilisation these organics were reported by previous works at operating conditions of 120 °C, 90 min and alkaline environment [14,15]. The concentrations of proteins and polyphenols are quite comparable for all different pre-treatments in water and acidic media. For shaking and ultrasonication pre-treatments, the contents of proteins and polyphenols solubilised were quite similar irrespective of the aqueous medium used. This trend was also observed in case of reducing sugars and °Brix which could suggest that sonicating at the

conditions used in this study did not significantly improve the values in terms of hydrolysis. From these preliminary results, low temperature thermal and microwave pre-treatment in alkaline mediums seems to be the most feasible option among others. However, further analyses for instance determination of ferulic acid in the solubilised liquid is necessary to have a better understanding of the results obtained.

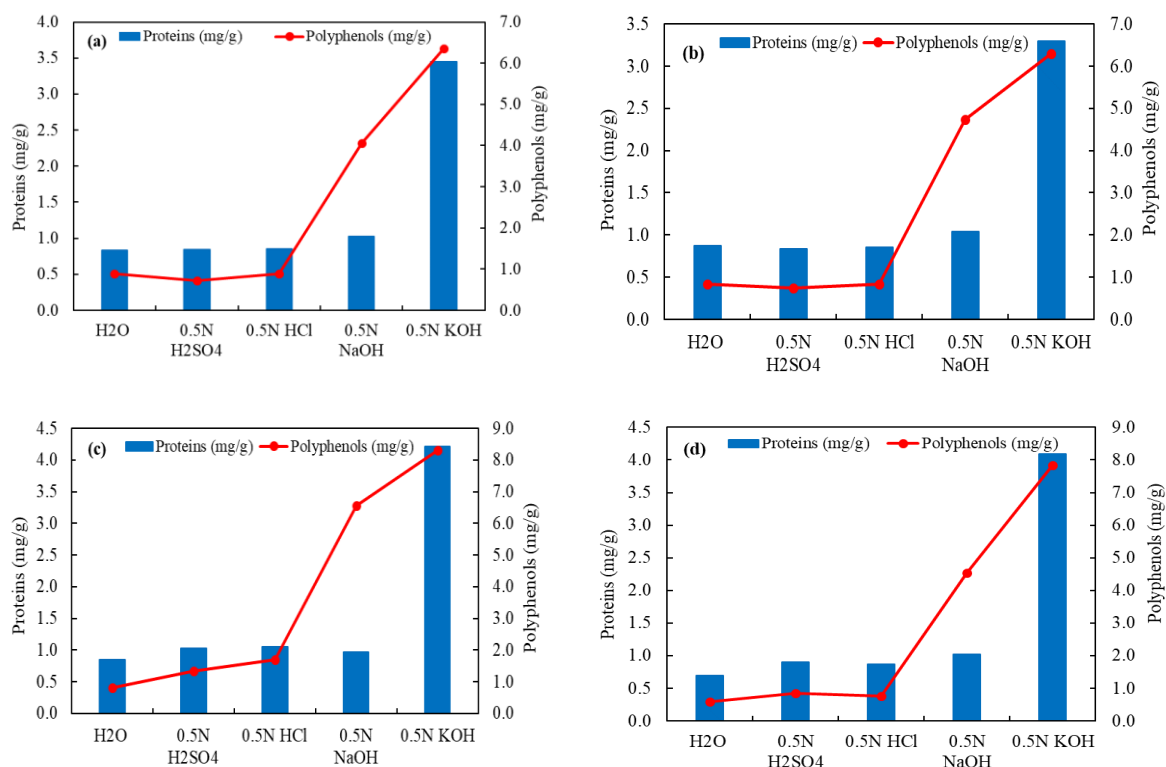


Figure 6.2. Graphs showing concentration of proteins and polyphenols after (a) shaking, (b) ultrasonication, (c) low temperature thermal, and (d) microwave pre-treatments in different medium

6.3 Conclusions

BSG is pre-treated by shaking, ultrasonication, low thermal treatment at 70 °C, and microwave treatments using different media like water, acids (0.5N HCl and 0.5N H₂SO₄) and alkalis (0.5N NaOH and 0.5N KOH). The effect of pre-treatments was investigated by the extent of solubilisation of molecules into aqueous phase after pre-treatments. Highest release of reducing sugars for all the different pre-treatments was found to be in water and acidic conditions while °Brix which could be used to estimate total sugar contents was enhanced in alkaline medium. Also, the concentrations of proteins and polyphenols solubilised were highest in alkaline

medium. These results could provide preliminary knowledge for valorisation of pre-treated BSG liquid and additional analyses on liquid and estimation of bioenergy on solid product is in progress.

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Conclusions and future scope

Conclusions

During the three years of PhD research, different experimental approaches for the valorisation of wastewater sludge were investigated.

Unlike specific kind of biomasses (e.g. a particular variety of a lignocellulosic plant) the waste sludge is a complex mixture of different types of organic matter and inorganic materials. Therefore, the treatments studied aimed to explore processes which would be able to handle the challenging issue related to this kind of biomass.

First, chemical (alkali addition), thermal (low temperature), physical (ultrasonication) and a combination of these pre-treatments were performed on sludge. The treatments resulted in an increase in the concentration of dissolved organics. Among individual methods, the alkali addition treatment was the most significant. Upon comparing all pre-treatments and all tested parameters, the combined alkali–heating pre-treatment was found to be the most effective. It was seen that alkali-coupled treatments could be further explored to produce an aqueous liquor with organic constituents which can undergo subsequent biotechnological steps to produce value-added chemicals/materials. For all the treatments, the higher heating values (HHV) were approximately 10–11.8 MJ/kg and did not greatly vary with comparison to the untreated sample which shows that, after resource recovery, the solid part of sludge can be further utilized for its energy properties. The results of this part have been published (Reshma Babu, Gustavo Capannelli and Antonio Comite, 2021. Effect of Different Pretreatments on Sludge Solubilization and Estimation of Bioenergy Potential. *Processes*, 9(8): 1382).

Also, pre-treatments like the preliminary screening of hydrothermal process conditions for enhanced solubilisation of waste activated sludge was carried out using small batch reactions. The effect of reaction temperature, pH and time was studied individually. All the parameters had a significant influence on sludge solubilisation and the ideal values selected for big batch reactions were temperature between 150- 200 °C, alkaline pH and reaction time of 1 h. Based on the results of the preliminary experimental runs, further hydrothermal reactions on a larger scale were carried out at 180 °C, pH 3, 6 and 12 for 1 h of reaction. The effect of acidic medium was also studied in addition to optimised alkaline environment. There was enhanced reduction of solids of about 50-60% and increased solubilisation of organics of 57-67% after the treatments with the most efficient treatment being in alkaline medium. The effect of different

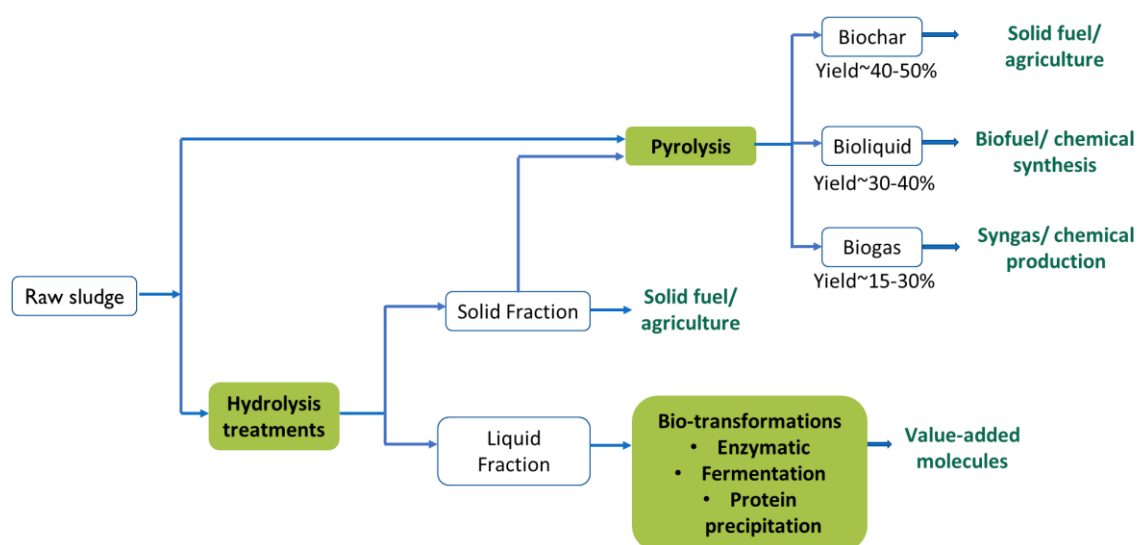
hydrothermal treatments on sludge solubilisation was evaluated which is the first step for a biorefinery exploiting sludge as a source.

Further, valorisation of solid and liquid fractions of hydrothermally treated sludge samples were explored. On the solid fraction, pyrolysis experiments at 500 °C were done. Char yield increased after all hydrothermal treatments and was about 47- 52% making them the highest fraction of pyrolysis products obtained. On the char, HHV values were estimated using the proximate parameters obtained from TGA and compared with sludge samples before pyrolysis (i.e., only after hydrothermal treatments). It was found that HHV of char (7- 14 MJ/kg) was lower than the non-pyrolyzed sample (10- 14 MJ/kg) but still sufficient to be utilized for energy studies. SEM elemental analysis showed that pyrolyzed sample had greater concentration of phosphorus than non-pyrolyzed sludge. Thus, the char from the hydrothermally treated sludge is suitable either for their bioenergy potential or to be used for agricultural purposes in organic fertilizers. On the liquid fraction, different tests were carried out with an aim to valorize it. Biotechnological transformations using commercial enzymes resulted in the decomposition of larger molecules into smaller molecular weight ones. Also, with the use of specific HPLC columns and cellulase enzyme, it was possible to obtain simple sugar like glucose which could be subjected to further separation. With GC-FID, production of bioethanol after fermentation test with yeast was confirmed and FTIR used for qualitative estimation of proteins that were precipitated using HCl acid. All the results on the liquid suggest a possibility to further explore this fraction for extraction, separation of a class of organic molecules or raw materials for fine chemicals production. However, the separation of the variety of molecules presents in the hydrolysate and in the broth after the enzymatic conversion is an issue that needs to be addressed.

Additionally, pyrolysis of a stabilised filter pressed sludge was studied at 500 °C and 800 °C. At 500 °C, there is higher yield of char and liquid products while at 800 °C, the yields of all products were quite comparable between 31.8- 34.9%. Among the gases measured, CO₂ was more prominent at 500 °C whereas CH₄, and H₂ had higher flow rates at 800 °C. Higher heating value was estimated using the proximate parameters obtained from thermogravimetry analysis. The results showed that the dried sludge and biochar had the potential to be used as a solid fuel. According to values from X-ray fluorescence test, 10% phosphorus on basis of dry matter was found in the char. Carrying out leaching test using acetic acid solution, 4.3% of phosphates were extracted from char. Thus, the char from this stabilized sludge is suitable either for energy recovery or to be exploited for agricultural purposes. The utilization of biochar as soil

amendment can result in a twice benefit. The relevant content of phosphorus which can be released into the soil candidates the biochar as an alternative to synthetic fertilizers. However, additional investigations will be necessary to assess how the presence of some contaminants in the sludge will affect the suitability of the biochar in agriculture. Moreover, for each kg of sludge, about 0.495 kg and 0.389kg of CO₂ is fixed as biochar at 500°C and 800°C respectively which can be seen to sequester carbon. Further studies will be necessary to evaluate the sustainability of the process. The bioliquid of pyrolysis consisted of oil phase with higher concentration of organics whereas the aqueous phase resembled higher humidity of sludge. It can be concluded that the sludge which was to be disposed in landfills, could be pyrolyzed and the char can be utilized either as solid fuel or as nutrient supplement in agriculture. While the oil fraction of the bioliquid can be further investigated for their ability as feedstock for biofuel production. Thus, pyrolysis looks like a feasible solution for this sludge from an environmental aspect.

The figure given below summarizes the main results obtained (Chapters 2-5) in terms of valorisation of WWTP sludge which requires additional testing and further studies to check for the feasibility of the final products stage.



Summary of results- valorisation of sludge

Finally, pre-treatment and hydrolysis studies on brewer's spent grains (BSG) was done. BSG was pre-treated by shaking, ultrasonication, low thermal treatment at 70 °C, and microwave treatments using different media like water, acids, and alkalis. The effect of pre-treatments was investigated by the extent of solubilisation of molecules into aqueous phase after pre-treatments. This work was part of the project to valorise BSG similar to that of waste activated

sludge firstly by pre-treating them and then by examining suitability of the liquid for obtaining molecules as feedstock and solid for fuel properties after pre-treatments. The results obtained provided preliminary knowledge for valorisation of pre-treated BSG liquid and additional analyses on liquid and estimation of bioenergy on solid product is in progress.

Future scope

The PhD work has put some basis for future research activities in the field of valorisation of sludge. In particular, the valorisation of the liquid part after hydrolysis treatments is to be better explored (Chapter 4). Biotechnological transformations using enzymes on different pre-treated samples is to be better studied by optimising the operating variables and also to well understand the complex mixture of organics at the end of the transformations. Also, to separate different classes of compounds by using improved separation and extraction techniques and to perform quantitative analyses is expected. Since one of the problems faced in this explorative phase of the research was due to the availability of small amounts of treated liquid that was not adequate for screening tests with different membrane processes as well as ultrafiltration, nanofiltration and reverse osmosis. So, the preparation of larger quantities of treated liquid is underway. This will also aid the improvement of fermentation and protein precipitation tests that will be carried out to give better quantitative results. And in general, to quantify the class of organics produced after either hydrolysis or bio-transformations, the liquid samples will be subjected to GC-MS and HPLC with more suited columns.

The utilisation of biochar for energy recovery, as soil amendment and fixation of carbon in biochar for carbon sequestration (Chapter 5) deserves to be further investigated in terms of nutrient availability and release. Tests to assess the influence of the presence of some contaminants in the sludge and how they will affect the suitability of the biochar in agriculture needs to be done. Also, methodology to use renewable energy for pyrolysis process to have a negative carbon balance of the process is important to be studied and to check for their sustainability. Furthermore, on the bioliquid produced, efficient separation of the oil and aqueous phases. One method would be to increase the quantity of bioliquid produced by pyrolyzing more quantities of sludge so they can be subjected to proper separation techniques. Also, the eventual amount of water in the oil phase can be estimated by Karl Fischer method,

the determination of total organics in both phases and the ability to use oil fraction as feedstock for biofuel production will be carried out.

Chapter 6 deals with pre-treatment of BSG which is part of ongoing work in collaboration of Wroclaw University of Science & Technology, Poland. Additional measurements to check the suitability of the liquid for obtaining molecules as feedstock and solid for fuel properties after pre-treatments on BSG is in progress like thermogravimetric and FTIR analyses on solid and detection of ferulic acid, total organic carbon contents on liquid part.

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