Evaluating the Impacts of Anaerobic Digestate Applications on Soil Nutrients and Macrofauna in South Wales

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List of Nomenclature

GHG – Green House Gas

AD – Anaerobic Digestion

CM - Cow Manure

EBA - European Biogas Association

HD – High Digestate added site

MD – Medium Digestate added site

LD – Low Digestate added site

CF – Chemical Fertilizer added site

REF - Reference site

Abstract

The use of renewable energy is popular at the present as a solution to the increasing energy demand with population growth and to reduce climate change caused by greenhouse gas emissions. Biogas production through anaerobic digestion is key here. As a by-product of this anaerobic digestion, solid and liquid digestate are produced. Today, this digestate is used as a bio-fertilizer for sustainable agriculture. But there is a need for scientific studies on the effects of the use of this digestate on soil microorganisms and soil nutrients. Accordingly, in this study, the impacts of adding biogas digestate to the soil on soil nutrients and earthworms were studied. Eight lands controlled under two AD plants were selected for this study. Correspondingly, the percentage of C, H, N, O, S, C: N ration and the biomass of earthworms after adding digestate were calculated. The results of this study showed that adding digestate to the soil has negative and some positive impacts on C sequestration and N, and S percentages. However, it was not as much as expected. These results can be impacted by the soil characteristics, quality of the soil, cultivated crops, crop management systems, climate and weather changes, quality of the digestate, frequency of digestate addition and some other factors still not identified. Hence, this study should be further research in a more comprehensive manner to reach a complete conclusion. Also, the addition of digestate can have negative impacts on earthworms and shown a decrease of earthworm biomass with the increase the amount of digestate added. This results can be determined on many factors such as the pH of digestate, available nutrient amount in digestate, amount of heavy metal in digestate, pathogens, salinity, DO, and some other unknown factors. Therefore, further research need to be done regarding this negative impacts and what are the solutions for that.

1. Introduction

1.1. Renewable energy and Biogas

The global human population is growing expeditiously, hence global energy consumption is also upraising along with that. According to the United Nations, the global population was around 2.6 billion in 1950 and it expanded up to 6 billion in 1999 (UN, nd). From 1999 to October 2011, the human population on the earth reached up to 7 billion and within the next 30 years, this population is predicted to grow by another 2 billion people. Statistically, it is expected to reach its peak around in 2100 making up 11 billion of the human population (UN, nd). According to the Office for National Statistics (ONS, January 2021), the United Kingdom has reached 66.8 million population up to mid of 2019 (ONS, 2021). Compared to the year 2018, the population increased by 0.5% in mid-2019, which means an additional 361,000 people were added to the society (ONS, 2021). ONS 2018-based major national population forecasts show that the UK population will approach 69.6 million by mid-2029 and achieve 72 million by mid-2041 – rising of 4.2% and 7.8%, correspondingly, from mid-20191. The UK population is predicted to continue to increase (ONS, 2021).

As the human population increases tremendously, their needs also become more complex and the demand for energy has increased remarkably. While the advancement of technology is positive, its detrimental effect is to increase the demand for energy. At the moment, the primary source of energy supply is fossil fuels which fulfil 88% of the energy demand (Ionel and Cioabla, 2010). According to statistics, oil and its derivatives, natural gas, and coal are the most prevalent forms of fossil fuels utilised today (Ionel and Cioabla, 2010; Weiland, 2010). The combustion of fossil fuels releases greenhouse gases (GHG) into the atmosphere, most notably carbon dioxide (CO_2). The effects of GHG on global warming have led to considerable climate changes. GHG emissions must be controlled to less than half of the 1990 global emission levels in order to lessen the associated consequences of global warming and climate change (Weiland, 2010). The stability of the energy supply is also important, as a majority of the primary energy sources are centred within a few regions which are economically and politically corrupted. Therefore, environmentally friendly, economically attractive and technically well-grounded alternatives are being studied to generate energy around the world to overcome this crisis. By 2030, it is predicted that the structure of energy production will be based on the following principal sources: 75-85% combustion of conventional fuels, 10-20% nuclear power, 3-5% hydropower, and around 3% by renewable energy (Popescu and Mastorakis, 2010)

Solar, geothermal, wind, and biomass-related energy are the most prevalent types of renewable energy. Limit fossil fuel usage and reduce CO_2 emissions, organic structure, usage of mostly locally accessible resources, and ability to meet all demands while best serving and directly benefiting the community are the typical benefits of usage of renewable energy sources (Ionel and Cioabla, 2010; Lee *et al*, 2021; Popescu and Mastorakis, 2010; Scarlat *et al*, 2018).

During the past few decades, biomass-related energy production shows a significant improvement. The production of biogas under controlled microbial conditions plays a major role in the earth's carbon cycle. Around 590-880 million tonnes of methane are emitted into the atmosphere annually as a result of microbial activities such as decomposition (Kossmann *et al*, 2000). After identifying these biochemical processes, technologies have been developed to decompose biomass and extract the energy produced throughout that process (Bond and Templeton, 2011).

The term "biogas" is used to refer to the gas created by the anaerobic digestion (AD) of organic materials. Methane (CH_4) and Carbon dioxide (CO_2) are the major components of biogas, and it can be between 50–70% CH_4 and 30–50% CO_2 based on the substrate (Ionel and Cioabla, 2010; Kossmann *et al*, 2000; Lee *et al*, 2021; Sasse, 1988; Scarlat *et al*, 2018; Weiland, 2010; Yekta *et al*, 2019). Even though biogas contains a tiny amount of hydrogen sulphide (H_2S), CH_4 is the major component of energy production and contains around 21–24 MJ/m3 or 6 kWh/m3 calories (Bond and Templeton, 2011; Dimpl, 2010). Although biogas derived from renewable resources is currently an effective replacement for fossil fuels, numerous raw materials must be taken into account to fulfil the rising demand for bioenergy. The most prevalent organic substance on Earth, lignocellulose available in a variety of forms, amounts, and quality making it a viable raw material for the generation of biogas (Petersson *et al.*, 2007; Wyman, 2018).

The entire biogas production process can be logged into three main steps such as hydrolysis, acidification, and methanogenesis (Bond and Templeton, 2011; Ionel and Cioabla, 2010; Kossmann *et al.*, 2000, Tambone *et al.*, 2009; Yekta *et al.*, 2019). All these steps are activated by numerous complex and diverse microorganism species found in biogas, remarkably methanogens (Kossmann *et al.,* 2000). The organic matter is enzymolyzed externally by extracellular enzymes (cellulase, amylase, protease, and lipase) of microorganisms in the first stage (hydrolysis). Bacteria metabolized complex compounds (carbohydrates, proteins and lipids) into constituent units such as monosaccharides, amino acids and peptides (Bond and Templeton, 2011; Ionel and Cioabla, 2010; Kossmann *et al.,* 2000). The next step is acidification, where acid-producing bacteria produce acetic acid (CH_3COOH), hydrogen (H_2) and carbon dioxide (CO_2). For this process, they use bond oxygen in organic matter and as a result of that, it creates an anaerobic condition within the digester. These conditions help for the final step, methanogenesis (Bond and Templeton, 2011; Kossmann *et al.,* 2000). This process can be mesophilic (20–40 °C) or thermophilic (above 40 °C) (Bond and Templeton, 2011) and methanogenic archaebacteria utilized CH_3COOH, H_2, CO_2 and produce CH_4 and CO_2 (Kossmann *et al.,* 2000). This step is very sensitive, and it depends on several factors such as composition of the substrate (Arthurson, 2008), temperature (Hattori, 2008; Pycke *et al.,* 2011), pH (Kossmann. *et al.,* 2000), concentration of acetate (Hao *et al.,* 2011) and synergetic stress of acids and ammonium (Lü *et al*., 2013). In general, mesophilic conditions are more favourable for bacteria and archaebacteria and have a low digestion rate (Pycke *et al.*, 2011; Yu *et al*., 2014). Even though thermophilic conditions create less diversity, higher temperatures increase the capability of organic degradation (Fernández-Rodríguez *et al*., 2013) and increase biogas production (Siddique *et al*., 2014; Yu *et al*., 2014).

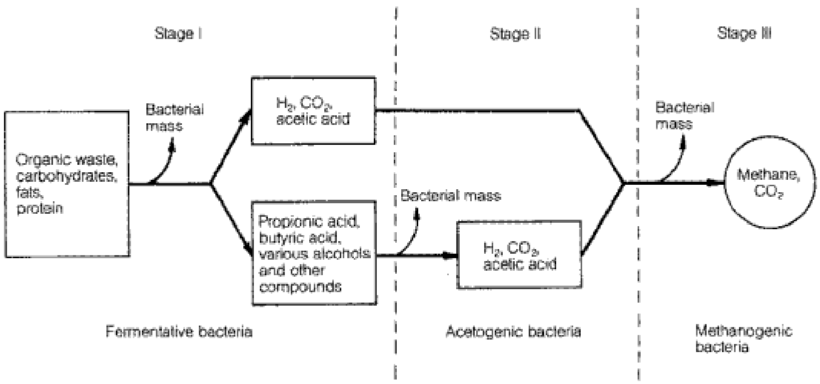


Figure1.1: Three major steps (hydrolysis, acidification, and methanogenesis) of biogas production and the main bacterial categories involved in each step (Eggeling, 1985).

Generally, the composition of biogas can be categorized as 50%–70% methane (CH_4), 25%–40% carbon dioxide (CO_2) and 2%–8% of water vapors and traces of O_2, N_2, NH_3, H_2S (Singh *et al*., 2020). According to the Singh *et al* (2020), if the percentage of CH_4 in biogas is 60%, the amount of energy that can be produced from it is about 6kWh per cubic meter (m^3) and when the CH_4 percentage intensifies up to 97% the outcome will be 9.67 $kWhm^{-3}$ (Singh *et al*., 2020). Also, a study by Sefeedpari *et al.*, (2012), has shown that biogas' energy production has reduced CO_2 emission by 414g per kilowatt-hour (Sefeedpari *et al*., 2012).

1.2. Anaerobic digestion row materials

The raw materials used for biogas production is vary from country to country. As shown in the figure, throughout Europe 63% of biogas is produced by agricultural products (European Biogas Association, 2020).

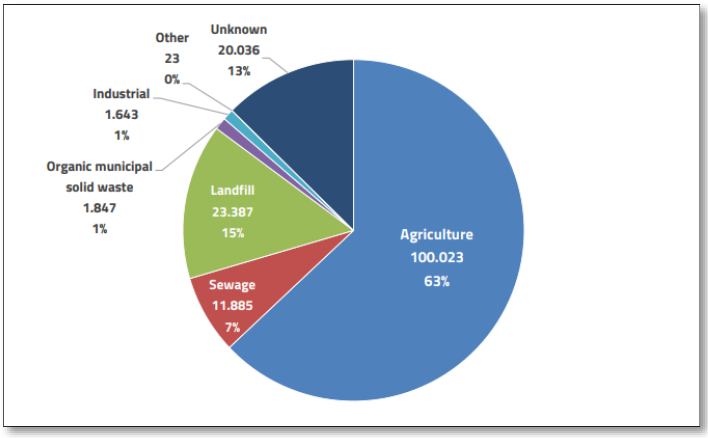


Figure1.2.1: Biogas production per plant in Europe (GWh). This chart illustrates the raw sources used for biogas production throughout Europe (European Biogas Association, 2020).

In Germany, approximately 92% of biogas is produced using livestock and high-energy agricultural crops (O'Keeffe and Thrän, 2019). Among those crops, the most prominent and widely used crop is Maize silage (*Zea mays L.*). This is nearly 70% of the total mass of energy crops used to produce biogas (O'Keeffe and Thrän, 2019). The next most noteworthy energy crops used are grasses and cereal (whole crops) plants (Daniel-Gromke *et al*., 2018). However, although GHG emissions are reduced by the production of biogas in this process, between 50% - 70% of GHG emissions occur during the entire process of growing these energy crops (Bachmaier *et al*., 2012; O'Keeffe and Thrän, 2019).

Additionally, sewage sludge is being used to produce biogas across Europe (7%) and particularly in the UK. This entire process can be categorised into two main steps, in the first step, the primary sludge is produced by using raw sewage and in the second step, secondary sludge is produced by biological treatment (Kor-Bicakci and Eskicioglu, 2019). This primary sludge is mixed with the secondary sludge and anaerobic digestion is done through several steps using bioreactors (Verlicchi and Zambello, 2015). The final sludge produced at the end of this anaerobic digestion process is called "Bio-solid" and can be used as a soil amendment (Verlicchi and Zambello, 2015).

Apart from the above-mentioned raw materials, cow manure (CM) is used throughout the world to produce biogas, especially in Europe the annual cow manure production reaches up to 1.2 billion tons and they use that for the anaerobic digestion (Li *et al*., 2021). There are several significant reasons that cow manure is used for anaerobic digestion. Firstly, the moisture content of CM is > 70% and with the advantage that CM can be used for direct thermochemical or biochemical digestion processes to produce energy (Font-Palma, 2019). Also, CM is a good source of nutrition and other trace elements especially phosphorus 7.89 g kg^{-1}, potassium 38.45 g kg^{-1} and nitrogen 2–8.1 g kg^{-1} (de Mendonça Costa *et al*., 2015).

Also, as illustrated in the figure another 1% of total biogas production is carried out by using municipal solid waste. A study done by Vrabie in 2021 points out that, the biomass of municipal solid waste is a combination of 78% biodegradable waste and 21.6% non-biodegradable waste. Of that bio-waste, 57.3% is organic waste and the rest contain Paper 14.1%, Wood 1.5%, Cotton and wool 3.7% and leather 1.8% (Vrabie, 2021).

1.3. The history of biogas production and present developments

When investigating the historical information about the production of biogas, there is no precise evidence that can be proved with certainty, but there are various opinions about it. Among them, there is speculation that biogas was used to heat water for bathing in Assyria as far back as the 10th century BC, and suggestions have been made that, ancient Chines empires was taken advantage of the anaerobic digestion process (Bond and Templeton, 2011; He., 2010; Kabeyi and Olanrewaju, 2022). However, documented history is suggested that this anaerobic digestion process and the use of its results began with the discovery of CH_4 by Italian physicist Alessandro Volta in the 18th century (Chasnyk *et al*., 2015; Kossmann *et al.*, 2000). The chemical structure of CH_4 was established by John Dalton (1766–1844) (Abbasi *et al*., 2012) and discussion of the concept of anaerobic digestion began with Jean-Louis Mouras's introduction of the septic tank system in around the 1870s (Grando *et al*., 2017). Later, Louis Pasteur (1822–1895) discovered that biogas could be used to produce heat and light, and in 1895, Donald Cameron used biogas for street lighting in Exeter, England (Bond and Templeton, 2011; Grando *et al*., 2017; Kossmann *et al.*, 2000). From here until 1921 there was a transitional period during which research was carried out on what resources could be used to generate CH_4. Those efforts succeeded in discovering the basic principles of the process, and in the 1840s the first unit digester was built in Otago, New Zealand (Grando *et al*., 2017). However, Brakel's review report (1980) mentioned that in 1859 a unit digester was built to heat the leper colony in Mantunga, Bombay, India (Brakel, 1980). Until the end of the 19th century, the main focus was on the production of energy using organic materials, but currently, India, China and Western Europe have introduced many innovations (Abbasi *et al*., 2012; Ji *et al.*, 2017). By the end of 1988, about 4.7 million domestic biogas digesters were reported throughout China (Ni and Nyns, 1996). Nevertheless, at the end of 2010, the number of domestic biogas digesters had grown to 38.5 million and the number of large and medium-sized biogas plants had grown up to 27,436 (Chen *et al*., 2012). Meanwhile, by 1999, India was also focusing extensively on anaerobic digestion, and by then the number of domestic biogas digesters exceeded 3 million (Bond and Templeton, 2011). During 1981-82, the government of India introduced a National Policy on Biogas Development (NPBD), which further expand domestic digesters throughout the country (Ministry of New and Renewable Energy, India, 2007). Also, by the year 2007, the government of India started providing subsidies for the construction of biogas digesters in the range of 30% to 100%. Accordingly, by 2019 India has become the 4th major producer of biogas in the world (Young, 2014).

1.3.1. Anaerobic digestion development in Europe

Apart from Asia, Europe is a world leader in the renewable energy sector. The majority of Europe's electricity comes from fossil fuels or nuclear power, while only a limited fraction is generated utilizing renewable energy sources. In the last decade, with the introduction of favourable policies encouraging the use of renewable energy by the governments of European countries, significant growth can be seen in the biogas sector. With a 2% growth rate between 2018 and 2019, the biogas industry has been expanding gradually over the past few decades (European Biogas Association, 2022). However, the most current statistics show that the EU continues to rely largely on fossil gas. The most recent figures demonstrate a consistent rise in natural gas utilisation since 2014. The amount of natural gas utilised in 2019 was 23 times that of biogas (European Biogas Association, 2022). To facilitate this demand as illustrated in figure 1.3.1.1, the number of biogas plants in Europe has grown dramatically.

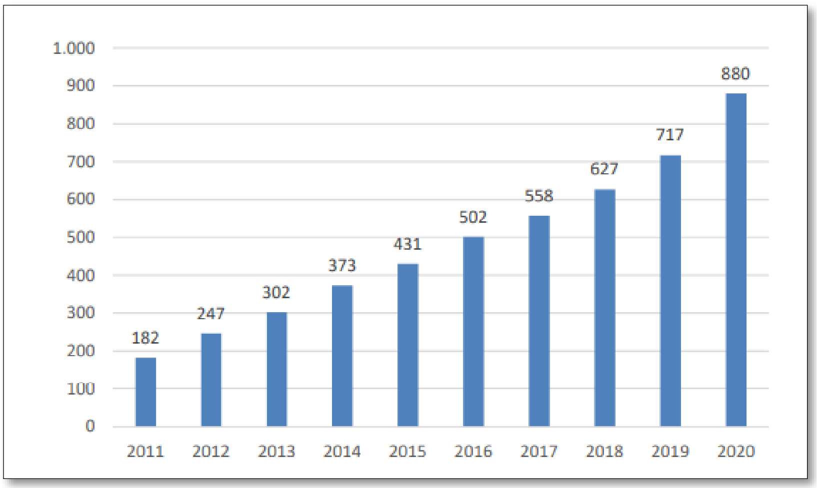


Figure 1.3.1.1: Development of number of Biogas plants in Europe from 2011 to 2020. (Bio-Energy Europe statistical report, 2022).

According to the Bio-Energy Europe report 2022, the total inland energy consumption of biogas increased dramatically from 1.376 ktoe in 2000 to 14.716 ktoe in 2020. Over the past ten years, landfill gas recovery has contributed continuously to the production of biogas. The statistic report of Bio-Energy Europe (2022) reveals that, in 2020, biogas accounted for 10.5% of the EU27's consumption of bioenergy and 4.5% of the EU27's total gross inland gas consumption. EBA (European Biogas Association) estimates that biogas will be able to replace 10% of the gas consumption for the EU27 by 2030 and up to 30% to 40% by 2050. These numbers show that biogas may be a dependable option for the low-carbon energy transition. However, it should be with the appropriate legislative framework.

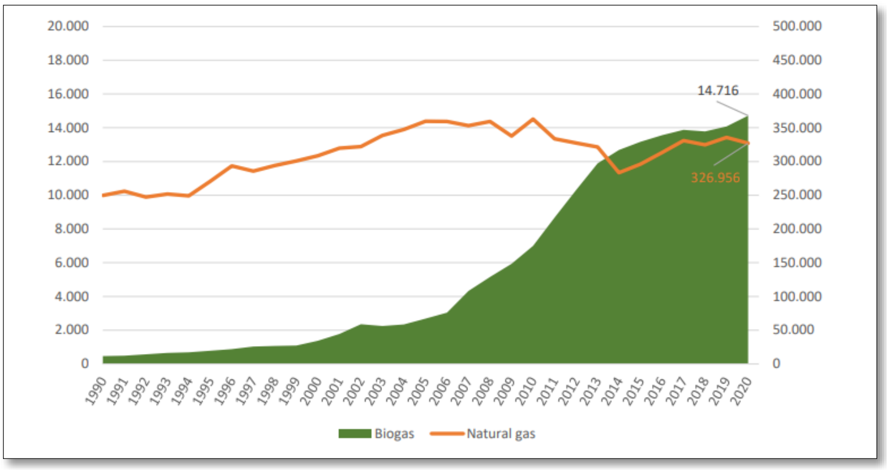
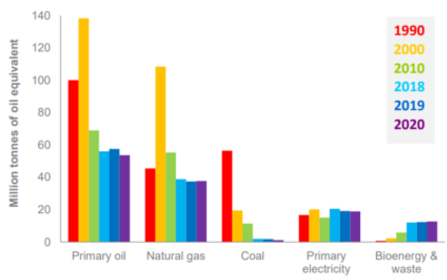


Figure 1.3.1.2: The development of biogas's gross inland energy consumption (left axis) and natural gas consumption (right axis) in EU27 (ktoe). (Bio-Energy Europe statistical report, 2022). This graph clearly shows that biogas energy consumption increased and in 2020 the inland consumption of biogas exceed natural gas consumption.

1.3.2. Anaerobic digestion development in the United Kingdom, Wales.

When it comes to the United Kingdom (UK), according to the UK's energy brief in 2021, still the main source of energy production is fossil fuel, followed by natural gases. According to the UK energy brief, the energy production in the fossil fuel sector has declined by 3.1% in 2020, compared to 2019 and at the same time, bioenergy production has upgraded by 0.3% (Department for Business, Energy & Industrial Strategy, 2021).



Million tonnes of oil equivalent

	1990	2000	2010	2018	2019	2020
Primary oil	100.1	138.3	69.0	56.0	57.4	53.6
Natural gas	45.5	108.4	55.3	38.8	37.4	37.7
Coal	56.4	19.6	11.4	1.9	1.8	1.2
Primary electricity	16.7	20.2	15.1	20.5	19.2	18.9
Bioenergy & waste	0.7	2.3	5.8	12.0	12.3	12.7
Total	**219.4**	**288.7**	**156.7**	**129.3**	**128.2**	**124.1**

Figure1.3.2.1: The UK primary energy production from 1990 to 2020 (Department for Business, Energy & Industrial Strategy, 2021).

According to the most recent statistics of the European Biogas Association (2022), there are 572 anaerobic digestion plants in operation or in construction. Among those plants, there are 77 waste-fed and 80 farm-fed biogas plants in existence, with a total capacity of 160 MWel. In addition to such, the UK water sector has 146 biogas plants based on wastewater treatment all around the nation that use 1.6 million tonnes of sludge (European Biogas Association, 2022). In addition, there are 75 landfill-based biogas plants spread across the UK with a capacity of 1402 MWel (European Biogas Association, 2022). Analysing this data, it is clear that the UK is very optimistic about renewable energy compared to other countries in the world.

Of the UK countries, Wales is one of the most capitalistic regions in its use of renewable energy. Wales is a large region in the United Kingdom and is an area rich in natural resources. Accordingly, the Wales government introduced new policies to reduce carbon dioxide emissions. Accordingly, for the convenience of implementing those policies, Wales was divided into 22 local authorities. The main aim of these policies is to generate electricity and heat for the whole of Wales using as much renewable energy as possible and to reduce carbon dioxide emissions. Accordingly, the first anaerobic digestion plant in Wales was established in 2012 at “Rainbarrow Farm”.

According to the Wales government (2018), the annual energy consumption is around 91 TWh and 16% of it is used for electricity generation and the rest of 76.1TWh is used for industrial usage, transport and heating (Wales Gov, 2018). The Energy Generation in Wales (2018) reveals that, compared to previous years, 2018 shows a slight decrease in renewable energy-related projects. That is, in the year 2016, the growth of related projects showed an increase of 21% and it decreased to 4% by 2018 as illustrated in figure 3 (Wales Gov, 2018).

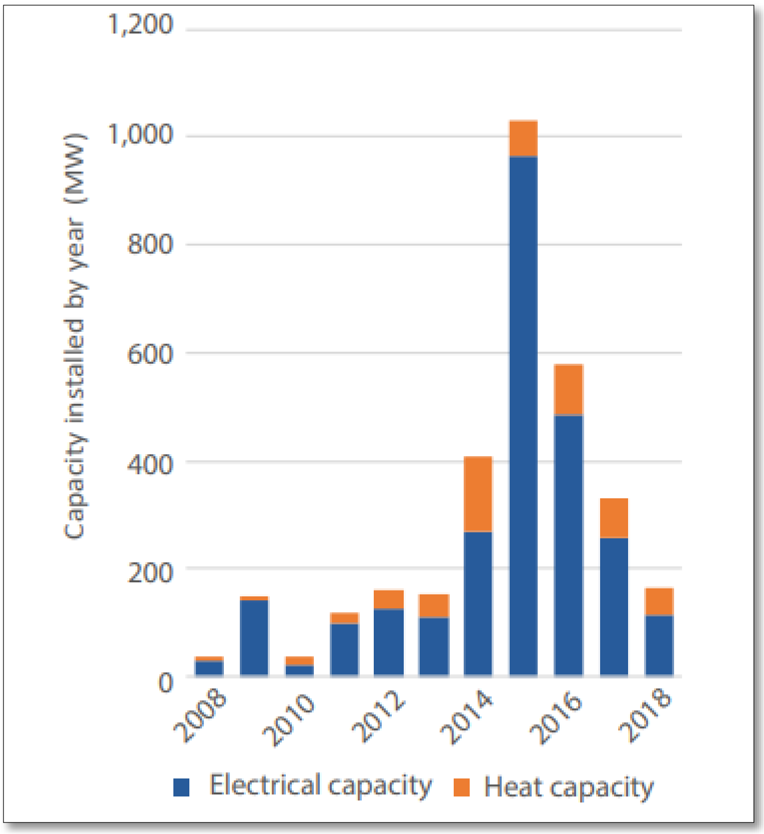


Figure 1.3.2.2: The renewable energy projects capacity installed by the year (Wales Gov, 2018).

Table 1.3.2.1: Wales renewable energy technologies and their production up to 2018 (Wales Gov, 2018).

Renewable Energy Technologies	Number of Projects	Electricity		Heat	
		Capacity (MW)	Estimated generation (GWh)	Capacity (MW)	Estimated generation (GWh)
Anaerobic digestion	**45**	**19**	**100**	**8**	**48**
Biomass heat	3,345	-	-	443	1,356
Biomass electricity and CHP	48	131	756	119	658
Energy from waste	1	30	125	-	-
Heat pump	4,928	-	-	56	108
Hydropower	364	182	389	-	-

Landfill gas	24	31	117	-	-
Offshore wind	3	726	2,200	-	-
Onshore wind	740	1,106	2,779	-	-
Sewage gas	6	9	34	11	68
Solar PV	54,560	978	925	-	-
Solar thermal	4,664	-	-	13	8
Total	**68,728**	**3,213**	**7,426**	**651**	**2,249**

As of 2018, there were 45 anaerobic digestion plants in operation across Wales, with a total capacity of approximately 27 MW. According to the government of Wales, as shown in figure 4, 19 MW of that was allocated for electricity and the remaining 8 MW for heating. A more remarkable thing is, that 16 local authorities out of 22 in Wales mentioned earlier have installed at least one AD plant within their area. The number of anaerobic digestion plants in Wales tripled between 2014 and 2016, and this growth declined to 4% due to deductions in tariff concessions in 2017.

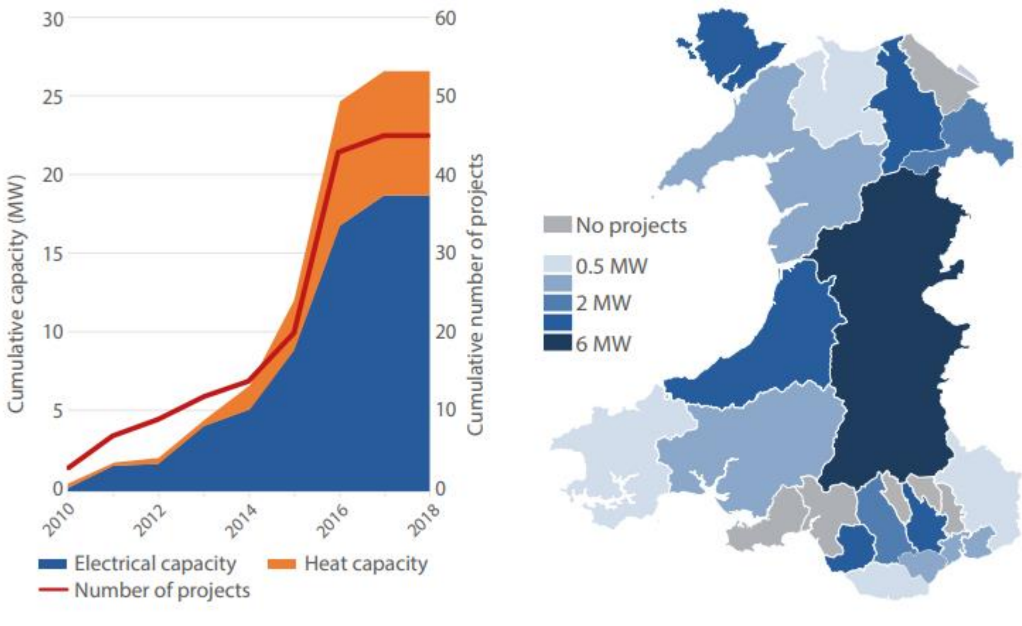


Figure 1.3.2.2: The left side graph illustrates the AD plant development in Wales since 2010 to 2018 and how electrical, heat capacity developed as a result of those projects. The map in right side shows the geographical distribution of those AD plants (Wales Gov, 2018).

1.4. Biogas Digestate

In addition to CH_4 production, the AD process produces a liquid or solid slurry which is rich in nutrients, generally known as "Digestate" (Bibby *et al*., 2010; Fermoso *et al*., 2018; Mukhuba *et al*., 2018). The quality of the digestate can be varied according to the micro-organisms, feedstock, operational conditions, digestate processing methods and the layout of the anaerobic digestion system (Koch *et al*., 2015; Opatokun *et al*., 2017). The moisture content is one of the major physical characteristics of digestate, which can be influenced by thermophilic or mesophilic conditions. According to Kim and Oh (2011), the dry anaerobic digestion process generates 30%-40% solid digestate, hence is no need the solid-liquid separation during the dry digestion process (Kim and Oh, 2011). Generally, the biogas digestate is rich in NPK (Nitrogen (N), Phosphorous (P), and Potassium (K)) and other macronutrients such as Calcium (Ca), Sulphur (S), Magnesium (Mg) and micronutrients including Chlorine (Cl), Manganese (Mn), Ferrous (Fe) etc. (Logan and Visvanathan., 2019; Zirkler *et al*., 2014). However, the organic and inorganic composition of the digestate depends on the feedstock, energy crops, management strategies and techniques (Möller and Müller, 2012). Follows table 1.4.1 illustrates the typical characteristic of the anaerobic digester and according to Logan and Visvanathan (2019), the liquid digestate has a high N percentage and solid digestate has a high P percentage (Logan and Visvanathan, 2019).

Table 1.4.1: Typical characteristics of anaerobic digestate (Keotiamchanh, 2018; Logan and Visvanathan, 2019; Peng and Pivato, 2017; Tampio *et al*., 2016).

Digestate type	pH	Total solids (TS) (%)	Volatile solids (VS) (%TS)	Total Kjeldahl nitrogen (%TS)	N–NH3 (g/L)	Carbon/ nitrogen	Chemical oxygen demand (g/gVS)
Whole	8.3	0.72–51.2	62.1	2.79–14	1.7–7.5	1.3–29.8	1.62
Solid	8.8	7.23–94.78	68.0–71.0	-	-	12.1–20.9	-
Liquid	8.34–8.80	2.0–19.20	66.4	-	3.84	2.7	-

Table 1.4.1 data indicates, that the digestive pH varies between 8-9, which makes the digestate slightly alkaline. This can be due to ammonium carbonate ($(NH_4)_2CO_3$) which is formed by ammonium (NH_4^+) and carbon dioxide (CO_2) (Arthurson, 2009), lack of volatile fatty acid throughout the process or accumulation of basic cations such as Ca^{2+} and K^+ (Logan and Visvanathan, 2019). Organic and inorganic nitrogen creates the ammonium concentration of the digestate, but inorganic nitrogen is more volatile rather than organic nitrogen (Maurer and Müller, 2012; Rotz, 2004). During thermophilic anaerobic digestion, NH_4^+ volatilization is inevitable. Hence there is an equilibrium between ammonia (NH_3) and NH_4^+ as follows, (Pantelopoulos *et al*., 2016)

$$NH_4^+{}_{(aq)} \leftrightarrow NH_{3\,(g)} + H^+$$

With higher manure temperature and pH, non-volatile NH_4^+ dissociates more readily to easily volatile NH_3, increasing the amount of NH_3 in the manure (Guštin and Marinšek-Logar, 2011). According to a study done by Sommer *et al* in 2006, NH_3 emissions are caused by the gradient in NH_3 concentration that develops between the manure and the ambient air. The vigorous aeration frequently used in current drying systems might increase nitrogen losses (Sommer *et al*., 2006). It has been demonstrated that acidification of animal manure reduces nitrogen losses throughout the storage and agricultural application stages of manure management (Fangueiro *et al*., 2015). Because the acidic reagents may keep the pH of manure at a low level, powerful acidifying chemicals like H_2SO_4 have been shown to be effective as NH_3 emission inhibitors (McCrory and Hobbs, 2001). Additionally, the greater ammonium concentration in the acidified solids may be due to the creation of ammonium sulphate ($(NH_4)_2SO_4$) granules following the interaction of H_2SO_4 with the NH_4 component of the solids (Mahimairaja *et al*., 1994). Studies have revealed that slurry undergoes additional physicochemical changes after being treated with strong acids, even if acidification as a slurry treatment reduces ammonia emissions (Hjorth *et al*., 2013). The elimination of the majority of the inorganic C should have caused the overall carbon content of S to significantly fall as a result of acidification. It is true that significant losses should happen during acidification. Slurry acidification transforms the C to H_2CO_3 and CO_2, with a conversion rate of 72–96% of bicarbonate/carbonate to CO_2 at pH 5–6 (Fangueiro *et al*., 2009). All life forms on the earth depend on phosphorous (P) and it is a fundamental element which needs by agricultural lands. As the hard P is a finite resource, currently typically supplied to agricultural areas through secondary P supplies. Around 7 million tonnes of P per year are supplied by the secondary P resources including animal slurries and manure as well as digestate from the generation of bioenergy, which is equivalent to 40% of the total mined P (Cordell *et al*., 2009). However, due to the mobility of potentially hazardous materials or elements as well as the availability of nutrients, using secondary P resources as a fertiliser on agricultural fields has encountered difficulties (Li *et al*., 2016; Pivato *et al*., 2016). Generally, microorganisms in the digester do not utilize the P and K (Logan and Visvanathan, 2019). Therefore it is really important to recover the P from the digester to use as a fertilizer. However, there are two major issues with the P recovery process. The first issue is to liquefy the insoluble P (Dai *et al*., 2015). The second challenge is to develop a system that is highly selective for phosphorus as the organic matter adds various nutrients to the run during this anaerobic digestion process (He *et al*., 2017). As a solution for this, some studies have proposed the hydrothermal method (Shen *et al*., 2016) and some other studies have proposed the crystallization as struvite ($MgNH_4PO_4$) (Fang *et al*., 2016). However, a study done by Fagbohungbe *et al* (2019), has indicated that it is possible to recover phosphorus by changing the pH value during the digestion process. According to their study, when the acidity of the solution increase, the phosphorus content will increase (Fagbohungbe *et al.*, 2019). Noteworthy, after all these processes the ammonia content in the digestate of its total nitrogen content is approximately 60% to 80% (Logan and Visvanathan, 2019). As well as, the carbon content of digestate plays a major role as it is a good energy source for microorganisms and impacts soil. Potassium (K) also an important nutrient for life growth in agricultural fields. In digestate K can be contained at around 0.001–2.52% (Barampouti *et al*., 2020) and presence of these basic cations (K^+, Ca^{2+}) the pH of digestate can be increase and ultimately it can cause carbonates (CO_3^{-2}) and phosphates (PO_4^{-3}) precipitation (Drosg *et al*., 2015).

For a more effective land application, digestate is presently separated on-site into two different fractions (solid-liquid). It strives to reduce its bulk and, thus, transportation costs, prevent the composition process of degrading reactions, and enhance its handling. The solid fraction of the digestate contains 40- 90% of P, 20-25% of N and 40-80% of dry matter. The liquid fraction, in fact, has lower levels of dry matter, phosphorus, and organic carbon but is greater in ammonium nitrogen (70-80%) and potassium.

Table 1.4.2: liquid and solid components of digestate's composition on a wet basis (Barampouti *et al*., 2020; Ledda *et al*., 2013; Tambone *et al*., 2017; Vaneeckhaute *et al.*, 2017).

	Liquid fraction	**Solid fraction**
pH	7.8–7.9	7.7–8.5
TS (%)	3.3–6.6	19.3–24.7
Total C (% DM)	2.64–3.15	9.0–10.1
Total N (% DM)	0.32–0.51	0.33–0.65
Total NH4 (% DM)	0.17–0.3	0.13–0.3
NH4 (% Total N)	40–80	26–49.4
Total P (% DM)	0.03–0.1	0.08–0.25
Total K (% DM)	0.29–0.52	0.25–0.48
Total Mg (% DM)	0.03–0.05	0.09–0.10
Total Ca (% DM)	0.04–0.06	0.16–0.19

However, there is a concerns about the digestate, because of the raw materials used. As discussed above majority of the AD plants used ow manure, sewage sludge, landfills, municipal waste etc. Hence the digestate generate from those materials can contain pathogens (Nag *et al*., 2021). Even though pasteurisation take place during the AD process complete inactivity of pathogens are unlikely (Nag *et al*., 2020). As a result of this World Health Organization point out the risk of direct application of digestate for ready-to-eat agricultural purposes.

1.5. Applications of Biogas Digestate

Due to its cost-effectiveness and rich composition in microelements and microorganisms, digestate has been promoted in many nations and areas as an alternative to chemical fertiliser for farmland (He *et al*., 2018). Especially in Europe, around 95% of AD digestate is used for agricultural purposes, mainly as an organic fertilizer (Verheijen et al., 2010). The digestate serves a variety of purposes that are good for the soil, plants, and crops. First of all, digestate is renowned for its fertilising properties that increase plant production since it contains vital minerals for plant growth. Second, their importance in promoting soil performance via nutrient cycling in the soil, carbon transformation, and preservation of soil structure cannot be understated (Przygocka-Cyna and Grzebisz, 2018). However, the stability and physicochemical characteristics of anaerobic digestate will determine how they should be used for soil application, since using unstable digestate may result in nitrogen (N) immobilisation and/or oxygen depletion owing to an unsustainable spike in soil microbial activity (Mortola *et al*., 2019). Throughout the world, during past decades multiple studies have been done to identify the impacts of digestate use as a bio-fertilizer. Horta and Carneiro (2021) has done a study on the applications of digestate as an organic amendment to vegetable crops. According to their study biogas digestate cause positive impacts on soil organic matter. Also it helps to increase the soil pH and exchangeable base cations (K^+, Ca^{2+}) (Horta and Carneiro, 2021). According to the Ronga *et al* (2020), the direct application of digestate does not helps to improve the crop yield as mineral fertilizer does. This was supported by the study done by Panuccio *et al* (2019). According to their study by raising the amount of phenols and flavonoids, digestate enhanced the quality of cucumbers (*Cucumis sativus*) cultivated on sandy-loam soil (Panuccio *et al*., 2019). However, the digestate can be used as a combination of bio char and it helps to improve the crop yield (Ronga *et al*., 2020). Furthermore, the addition of biochar helps to reduce the CO_2 emission after added to the soil (Mukherjee *et al*., 2016). According to Mukherjee et al (2016), there was a saturation effect in the C degradation process because the rate of CO_2 emission was not proportional to the quantity of C given to the systems. Biochar helps to retain the C within the system and Mukherjee *et al* (2016) show that the directly added digestate release more than 40% of CO_2 while biochar and digestate mixture released 3%. However, these process can be impacted by the climate conditions. According to a study done by Alburquerque *et al* (2012), the nitrogen availability of digestate reduces with time and cold climate conditions. When the temperature is reduced, the microbial activity rate decline and in long term, it reduces the fertiliser capacity of the digestate (Alburquerque *et al*., 2012). Ammonium is rapidly converted to nitrate when digestate is applied to the soil, hence the primary aspect for farmers is to prevent volatile ammonia loss during spreading, nitrate via leaching, and gaseous N_2O emissions (Möller and Müller, 2012). Even though there is optimistic usage, applying digestate to agricultural areas may have some pessimistic effects. In fact, if it is disposed of improperly, nutrients may flow off (Nkoa, 2014). The total ammonium nitrogen (TAN) content in digestate is elevated as a result of the anaerobic breakdown of protein-rich biomasses, which restricts its usage in regions that are considered particularly susceptible to nutrient contamination of ground and surface waters. Similarly, because it contributes to the eutrophication phenomena, phosphorus (P) concentration is a concern in digestate management (Catenacci *et al*., 2022). In order to effectively fertilise agricultural fields without endangering the environment, national laws in Europe specify maximum nitrogen (N) amounts that can be applied to the fields, a minimum amount of digestate holding capacity, and a mandatory spreading season. The potential nitrogen surplus declined as the gap between inputs and outputs closed between 2000 and 2015, according to the gross balance of nitrogen added to and withdrawn from agricultural land in the EU. From an average (EU-28) of 62.2 kg per hectare in the years 2000 to 2003 to an average (EU-28) of 51.1 kg per hectare in the years 2012 to 2015, the excess nitrogen applied to agricultural land decreased by around 18% (Directive 91/676/EEC; Europe Environment Agency, 2019).

However, there is a knowledge gap about how this biogas digest addition impact soil microclimate especially how it impacts biomass and soil elements such as Carbon (C), Nitrogen (N), Oxygen (O), Hydrogen (H) and Phosphorus (P). The main objective of this study is to evaluate the impacts on the soil of the addition of biogas digestate. Also, this study aims to find out the impacts of biogas digestate addition on soil characteristics and provide reliable insight for advanced sustainable management of large-scale digestate addition to agricultural lands while achieving the renewable energy demand.

In this study, four interrelated hypotheses will be evaluated. The first hypothesis is, the soil carbon content in biogas digestate added soil is different from background soils with implications for carbon cycling. Secondly, as a result of biogas digestate addition, the decomposition of organic materials can be affecting mineral nitrogen and phosphorus in the soil. Thirdly, Earthworm biomass and density are higher as a result of hypotheses one and two. Evidence generated in this study will help to understand the soil biotic conditions of soil after the addition of biogas digestate, but also provide insight into the putative effects of greenhouse gases' impact on climate change can be reduced. Nevertheless, we try to compare the biogas digestate added to soil and artificial fertilizer added to soil, to get a better understanding what is the best solution for safe and productive agriculture farming.

2. Study Area

The study was conducted mainly based on two AD biogas plants, GP Biotec and Bryn Power Ltd within South Wales, United Kingdom.

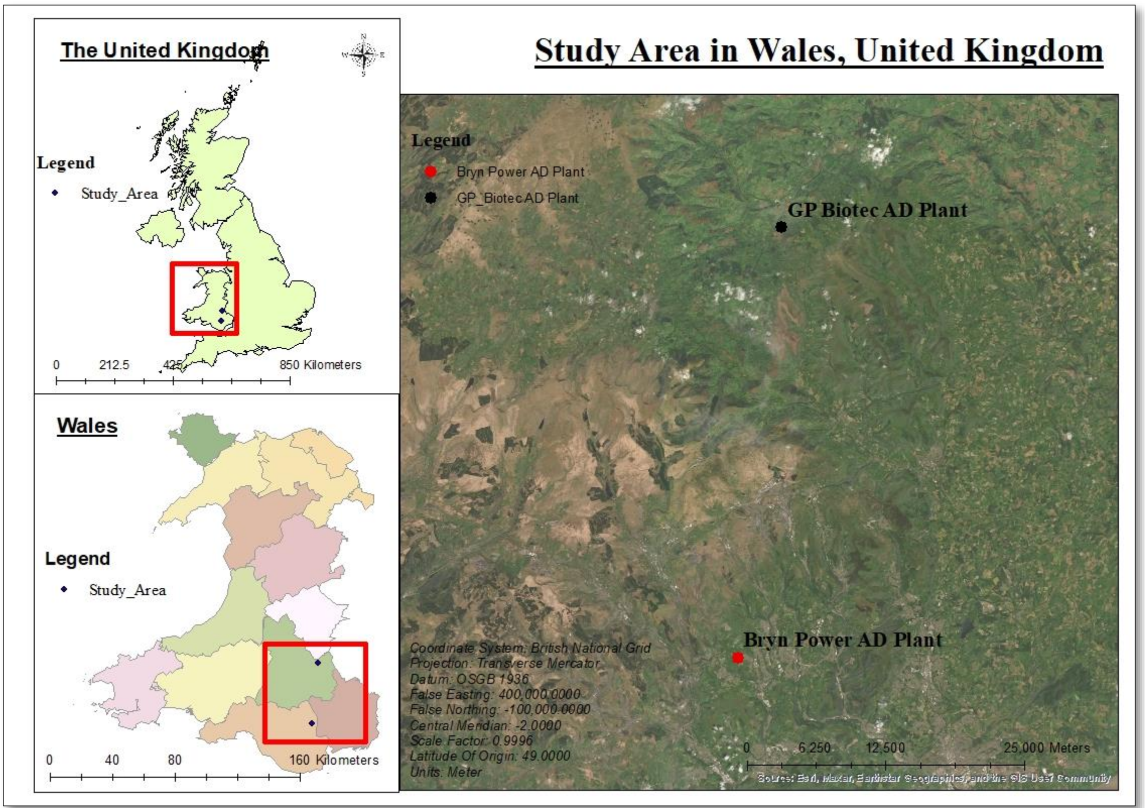


Figure 2.1: Map of the study area.

2.1. GP Biotec

The first anaerobic digester (AD) plant was GP Biotec, which is located in Powys, LD3 0DL as shown in figure. This AD plant operates since 2014 and uses a wet, mesophilic (35-42ºC), two-stage process to produce biogas. This helps to improve the mixing of row materials, biological stability and control over digestion conditions with less energy consumption. Food waste, energy crops and biodegradable waste from abattoirs are the main raw materials used in this AD plant (GP Biotec, 2021). The digestion process in this AD plant can be categorized in to four parts as pre-treatment & loading, primary digestion, secondary digestion, pasteurisation. The pre-treatment process comprises the maceration of solid feedstock, which are subsequently placed into the main digester with the liquid wastes after being combined with a tiny amount of digestate. In a liquid and gas-tight, insulated concrete tank with a flexible double membrane roof and a heating system, the primary digestion phase is conducted. Between 35 and 42 ^{o}C, the process happens without oxygen. For the necessary retention time, the substrate stays in the primary digester. The primary digestion stage is when the majority of the biogas generated by the process is collected. The substrate is piped to the secondary digester, where the residual biogas is collected after it has naturally settled at the bottom of the digester tank after being digested. Following the second round of digestion, the digestate descends to the bottom of the tank once more. It is then pasteurised in two steel tanks using a batch pump. In order to kill pathogens, 10m3 volumes of digestate are heated to 70^{o}C for 60 minutes.

Figure2.1.1: Primary digester tank

Figure 2.1.2: Secondary digester

Depending on the crop variety, the digestate is often delivered to the appropriate fields through umbilical pipes, where it is coupled to a tractor-mounted dribbling bar system that may distribute a 3m direct injection or a 12m, 18m, or 24m spreading band. The tractors have specialised technology that enables precise mapping, recording, and application rates. According to the GP Biotec they apply biogas digestate 25 m3 or tons per ha, and their crop available nitrogen 72 kg/ha, total phosphate 53 kg/ha, total potash kg/ha. Furthermore as per there records the annual income after adding digestate 92£/ha**.

2.2. Bryn Power Ltd

The second AD plant was Bryn Power Ltd, which is located in Hengoed, CF82 8FY. This is a large group of a company involved in recycling, aggregating, renewable energy and farming. Since 2016, the AD power plant has been operating and currently, 24,000 tonnes of food waste and cattle slurry turn into electricity and bio-fertiliser using a thermophilic process. The Bryn power AD plant converts the biogas into electricity by burning it in two engines that drive two generators, creating 1 MW of clean electricity that is used locally and the balance that is sent into the national grid. Additionally, the engines generate heat that is utilised to operate the pasteurizer and keep the digester warm.

About 650 Holstein Friesian cows are managed by the Bryn group as a milking herd. In order to produce the high-energy silage that is used to feed cows, the Bryn group manages more than 350 hectares of pasture and grassland. The digestate bio fertilizer created by the anaerobic digestion process at Bryn Power is applied to those fields three or four times a year to fertilise them.

Figure2.2.1: Primary and secondary digesters of Bryn group power plant

Figure 2.2.2: Secondary digester of Bryn group power plant

3. **Method**

First, five sites were selected within the GP Biotec agricultural lands to collect samples. Those sites were categorised as follows,

The first site was an Oats (*Avena sativa*) grown field as shown in the appendix figure. This site was categorized as a high digestate added site (HD) because annually 25 tonnes per hectare on 2 separate occasions a total of 50 tonnes per hectare was fertilized by biogas digestate. The soil within this field was quite compact and very dry. However, this could be caused by the heat wave condition during the sample collection time period (June 2022).

The second site was categorised as a medium digestate added site (MD). This land is used to grow grass for sheep and cattle to graze on. Once every two years, the digestate has been added to this site. The soil on this site was not much dry and it was quite red in colour.

As the third soil sample collection field, a grass field with low digestate addition was selected. This site was categorised as a Low digestate added site (LD). This site has been used for maintaining livestock and the soil in this site is not much hard and it was quite dark brown in colour.

The fourth field was a Wheat (*Triticum*) grown field and only chemical fertilisers are added for this cultivation. Hence this site was categorised as CF. For this site annually they added 34.5% ammonium nitrate 3 times total 450 kg per ha.

Finally, all these sites were compared with a natural grassland where no fertiliser or digestate was applied. This site was categorised as the reference site (REF).

In Bryn group AD plant, three sites were selected for sampling. All these three sites were used for silage. The first site was categorised as a high digestate added site (HD), because annually 4 times they added liquid digestate for this site. Second site was categorised as medium digestate added site (MD) as it has been fertilised by digestate annually one time. Third site was categorised as low digestate added site (LD), as they added digestate to this field a year after a year.

From each site, 5 soil samples were collected as shown in figure for analysis and kept in an ice cooler. Soil sample size was 15,625 cm^3 (25cm x 25cm x 25cm). Using these samples Total Organic Carbon (TOC), and Soil Fertility by analysing Nitrogen (N) and Phosphorus (P), Density of Earthworms, Biomass, Soil Moisture Content, and Biota were analysed.

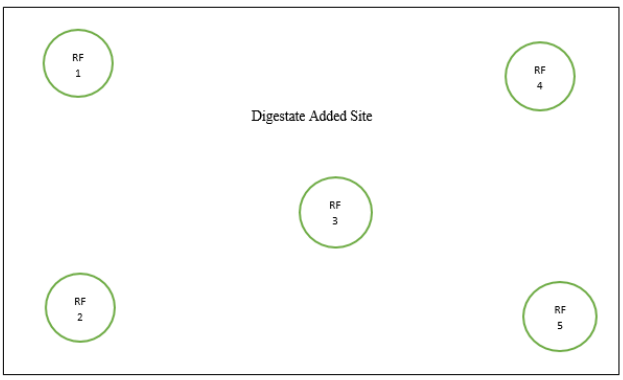


Figure 3.1: five soil samples were collected from each site as shown in this figure.

3.1. Element Analysis

Three soil samples from each site were used for element analysis. First, all soil samples were sieved using the #4 (4, 75 mm) sieve and then ground to get homogeneous fine soil samples. If the soil samples are wet, then it needs to oven dry over one night before do the sample analysis. Then five fine soil samples were placed in 50mL falcon tubes and finally sample analysis procedure was started. To analyse the samples, the UNICUBE element analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany) was used. This is designed to analyse the Carbone (C), Hydrogen (H), Nitrogen (N), Sulphur (S) and Oxygen (O) content in the solid and liquid sample, ranging from 0.1 mg chemical substance to 15 mg of liquid fuel to 1 g of inhomogeneous soil (Elementar Analysensysteme GmbH, 2022). It uses TPD technology which utilises gas separation to detect the elements. First, desktop PC was turned on and started the software by selecting the suitable mode. Then reference test was run as follows.

Table 3.1.1: Reference test running sample chart.

Column Number	**Sample**	**Contain**
1-3	Empty samples	Nothing in these three columns
3-6	Blank samples	Only the sample containers which made by Titanium (Ti).
6-9	RunIn samples	5 mg Sulfonamides samples
9-12	Standard samples	5 mg Sulfonamides samples

Then soil samples were weighted into Ti containers. The weight must be very accurate, hence Microbalance for Pipette Calibration MYA 5Y.P (RADWAG UK Ltd, Macclesfield, United Kingdom) was used. To get accurate weight first an empty Ti container was kept in balance and zero the balance. Then 2mg of soil was placed in to the Ti container. Then the container was sealed to avoid fine soil come out from the container during the combustion. After finishing the sealing, again the sample was weighted and entered that data into the software. Like these all five soil samples were weighted. Then these samples were kept in the UNICUBE element analyser as follows.

Table 3.1.2: Test sample running chart.

Column Number	Sample site	Contain
12-15	Site 01	5mg reference site soil
15-18	Site 02	5mg fertilised soil
18-21	Site 03	5mg low digestate added soil
21-24	Site 04	5mg medium digestate added soil
24-27	Site 05	5mg high digestate added soil

After the input of all soil samples inside the columns as illustrated above, the UNICUBE element analyser was run through all these samples one by one and burned them using Helium (He) and initiate the results after one day.

To analysis the Oxygen, rapid OXY cube machine (Elementar Analysensysteme GmbH, Langenselbold, Germany) was used. But the process of sample preparation was same as the above. The only difference was instead of sulfonamides standard sample, benzoic acid was used. All weighted samples were kept inside the machine as mentioned in table 1.3 and then run the software. It was taken one day to process all samples and generate the results.

Table 3.1.3: Test sample running chart for rapid OXY cube machine.

Column Number	Sample site	Contain
1-3	Empty samples	Nothing in these three columns
3-6	Blank samples	Only the sample containers which made by Titanium (Ti).
6-9	RunIn samples	5 mg Sulfonamides samples
9-12	Standard samples	5 mg Sulfonamides samples
12-15	Site 01	5mg reference site soil
15-18	Site 02	5mg fertilised soil
18-21	Site 03	5mg low digestate added soil
21-24	Site 04	5mg medium digestate added soil
24-27	Site 05	5mg high digestate added soil

Both UNICUBE element analyser and rapid OXY cube machines were produced how much C, H, N, O and S percentages included within the tested soil samples as graphs and tables.

3.2. Soil Biota and Earthworm Analysis

3.2.1. Earthworm Analysis

To calculate the biomass of earthworms, a 25cm x 25cm x 25cm size block was dug up from the ground as mentioned above. All the earthworms in the sod were then put into labelled containers and a small amount of 100% ethanol (C_2H_6O) was added to preserve the earthworms. In this way, earthworms were collected from 25 locations in 5 sites as five locations from one site. Then these earthworms were classified as follows and the biomass was measured using an SG-402 scale (Fisher Scientific, Leicestershire, England).

First, those earthworms were categorized into two groups, juveniles and adult earthworms based on their age and then the adults were further classified into four ecotypes as mentioned in the table.

Table 3.2.2.1: Earthworm categorisation according to age and ecotype.

Ecotype	characteristics	
Anecic earthworms	▪ Fresh litter feeder ▪ Soil dweller ▪ Digs deep, vertical, unbranching burrows. ▪ have tails that are lighter and are darker in colour at the end of head (red or brown). ▪ Large size. (ESB, 2022)	 Figure 1: Anecic earthworm (*Apporectodea longa*) (ESB,2022).

Endogenic earthworms	▪ Soil feeder ▪ Mineral soil dweller (0-50 cm) ▪ No skin pigmentation ▪ Lighter in colour from grey to pale pink. ▪ Small to medium size (ESB,2022).	 Figure 2: Endogenic earthworm (*Allolobophora chlorotica*) (ESB,2022).
Epigeic earthworms	▪ Pigmented skin ▪ Frequently blood red or reddy-brown and no stripes. ▪ Small in size (ESB,2022) .	 Figure 3: Epigeic earthworm (*Lumbricus castaneus)* (ESB,2022).
Compost earthworms	▪ Blood red in colour and have a stripy body.	 Figure 4: Compost earthworm (*Dendrobaena veneta*) (ESB,2022).

4. Results

4.1. Earthworm Data Analysis

In June 2022, soil samples were collected from both sites as mentioned in the methodology. During the sample collection earthworm, analysis was also done according to the above mentioned procedure. During the soil sample collection, all the earthworms within that 25cm x 25cm x 25cm size soil block was collected and separated according to the age and ecotype (Anecic earthworms, Endogenic earthworm, Epigeic earthworm and Compost earthworm). However, in this classification, the juvenile earthworms did not classify according to the ecotype as they are not morphologically matured.

During the soil sample collection in chemical fertilizer (CF) added soil and high digestate (HD) added soil couldn't find any earthworms. The maximum weight of earthworms recorded in the LD site which an outlier (2.27) as shown in the figure. The highest number of earthworms were found in the reference site (REF) where no digestate or chemical fertiliser. The second higher number of earthworms were present in the low digestate added soil and finally, the lowest number of earthworms were present in medium digestate added to the soil (please find the table in the appendix).

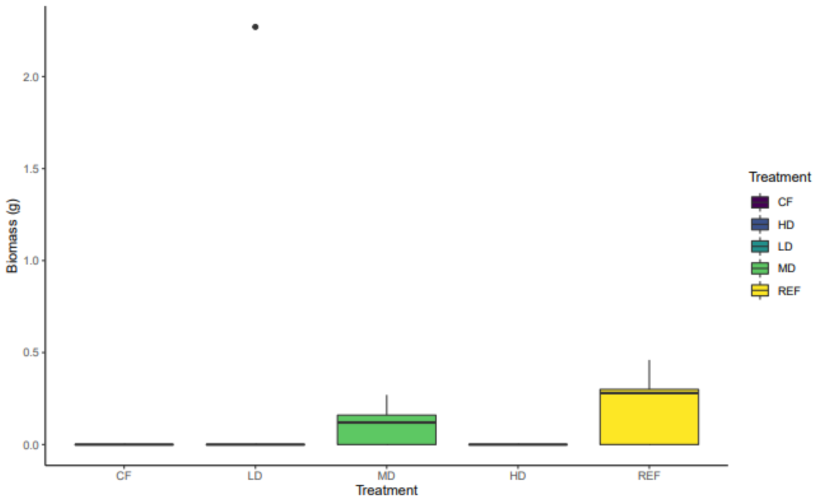


Figure 4.1.1: The earthworm's biomass variation according to the treatment within GP Biotec sites. There was no significant changes among the treatments (*chi-squared* = 7.8258, *p-value* = 0.09817, *M* = 0.1544).

According to figure 4.1.1 illustrates that the abundance of earthworms higher in REF site compared to other sites. But it had slightly lower biomass compared to LD site (LD = max biomass, 2.27 g). This abundance and biomass variation might be a result of digestate quality and available organic material content. However, to identify this more replications need to proceed.

Finally, when analysing all the data, the reference site (REF) had the highest number of earthworms of all other treatment sites and the lowest number of earthworms present in the MD site and HD, CF sites did not have any earthworms.

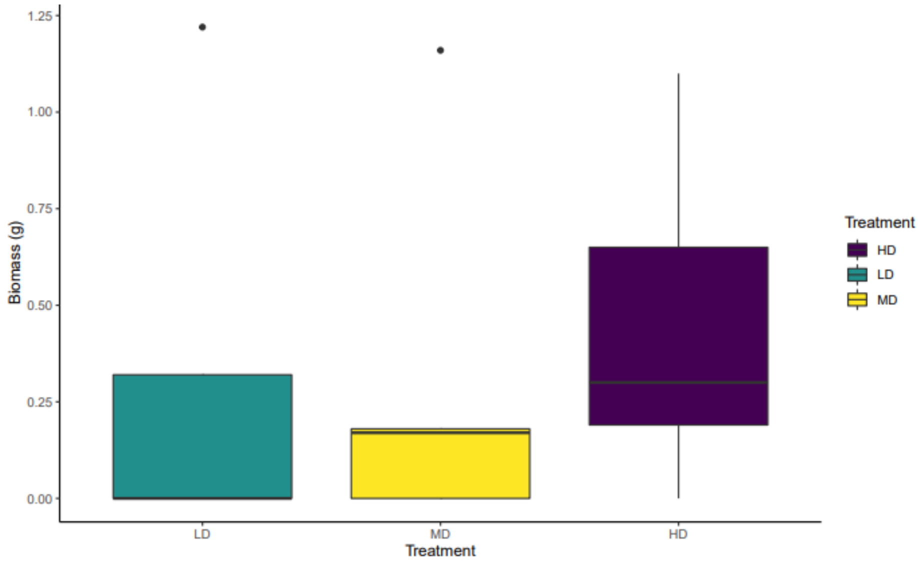


Figure 4.1.2: The earthworm's biomass variation according to the treatment within Bryn Power sites. (*chi-squared* = 2.1014, *p-value* = 0.3497, Anova *W* = 0.88009, *p-value* = 0.08788)

In Bryn power sites, samples were collected from three sites according to the digestate addition. They were LD, MD and HD. Interestingly, all those three sites contained earthworms of different proportions. As shown in figure 4.1.2, the highest biomass of earthworms was recorded in the HD site and then LD and MD. The mean biomass within these 3 sites were 0.3527 and maximum biomass was 2.24g. However, between those three sites could not find any significant difference ($p < 0.05$).

In Bryn group sites both adult and juvenile earthworms were present. More significantly all the adult earthworms present in the sites were Epigeic earthworms. But the adult earthworm percentage < juvenile earthworms (Adult= 3, juvenile = 12). This is an interesting point for further investigation. Finally, when comparing both GP Biotec and

Bryn power sites, there is one common result. That is always the adult earthworm percentage < juvenile earthworm percentage.

4.2. Element Analysis

4.2.1. Carbon Analysis Results

According to the procedure, soil samples were collected from all REF, LD, MD, HD and CF sites and the elemental analysis. First, the Carbon (C) content in the soil was analysed and figure 4.2.1 illustrates the results of the analysis.

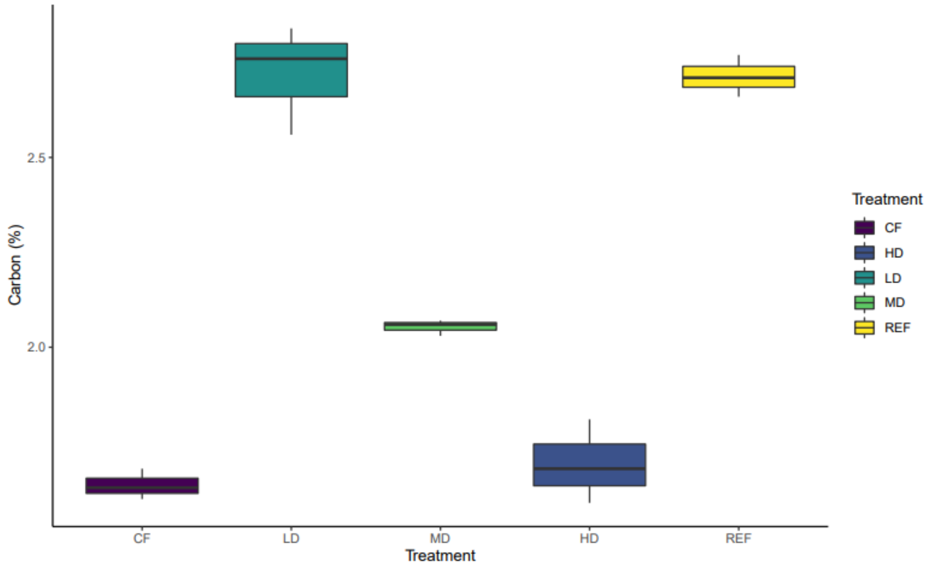


Figure 4.2.1.1: The percentage of C in soil after adding the biogas digestate. According to the analysis there are significant variations between sites (M = 0.8421, F = 1.0202, $Pr(>F)$ = 0.4424). The C content variation in sites with the treatments, HD = CF, REF = LD and LD > CF, MD > CF, REF > CF, LD > HD, MD > HD, REF> HD, MD < LD, and REF > MD.

There are some significant variations of C content between the sites according to the TukeyHSD test (see figure 4.2.1.1). When comparing the REF – LD and HD – CF, there was no significant variation between those sites (p > 0.05, (REF = LD, p = 0.9999793) and (HD-CF, p = 0.9263761)). However, there are significant variations between REF and MD, HD and CF. The highest differences were observed between LD-CF (diff = 1.083333333, p = 0.0000002) and REF-CF (differ = 1.076666667, p = 0.0000003). Apart from that, still there were significant variations between MD-CF (*p* = 0.0011885), LD-HD (*p* = 0.0000004), MD-HD (*p* = 0.0035413), REF-HD (*p* = 0.0000004), MD-LD (*p* = 0.0000226) and REF-MD (*p* = 0.0000247).

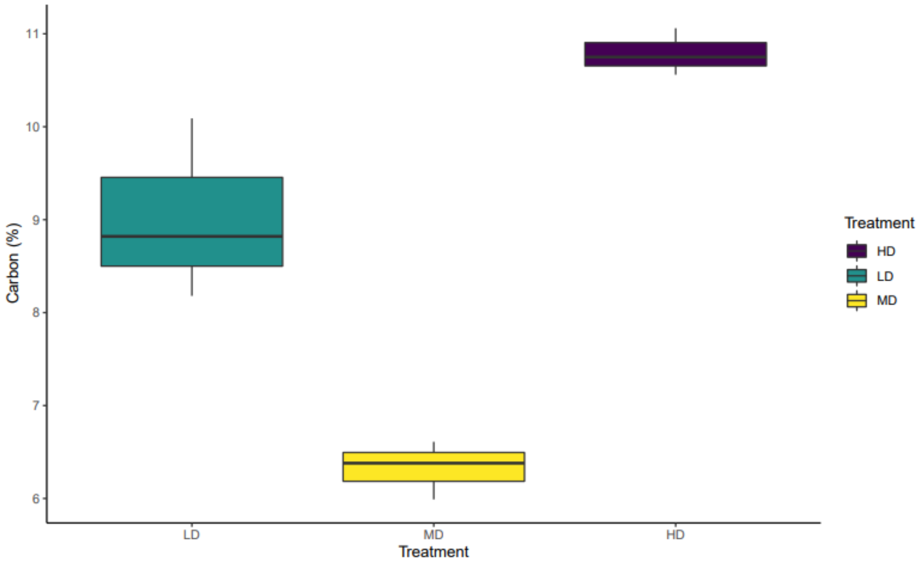


Figure 4.2.1.2: The percentage of C in Bryn Power sites, after adding biogas digestate. According to the analysis there are significant variations between sites (F = 1.3111, $Pr(>F)$ = 0.337, M = 15.163). The C content variation in sites with the treatments, LD < HD, MD < HD and MD < LD.

In Bryn group sites the highest C percentage was observed in the HD site and the lowest C content was recorded in the MD site (see figure 4.2.1.2). There were significant differences between LD-HD (*p* = 0.0263568), MD-HD (*p* = 0.0010146) and MD-LD (*p* = 0.032588). These results are quite opposite to the GP Biotec site results. However, these results are also consistent with the first hypothesis.

4.2.2: Nitrogen percentage in Soil.

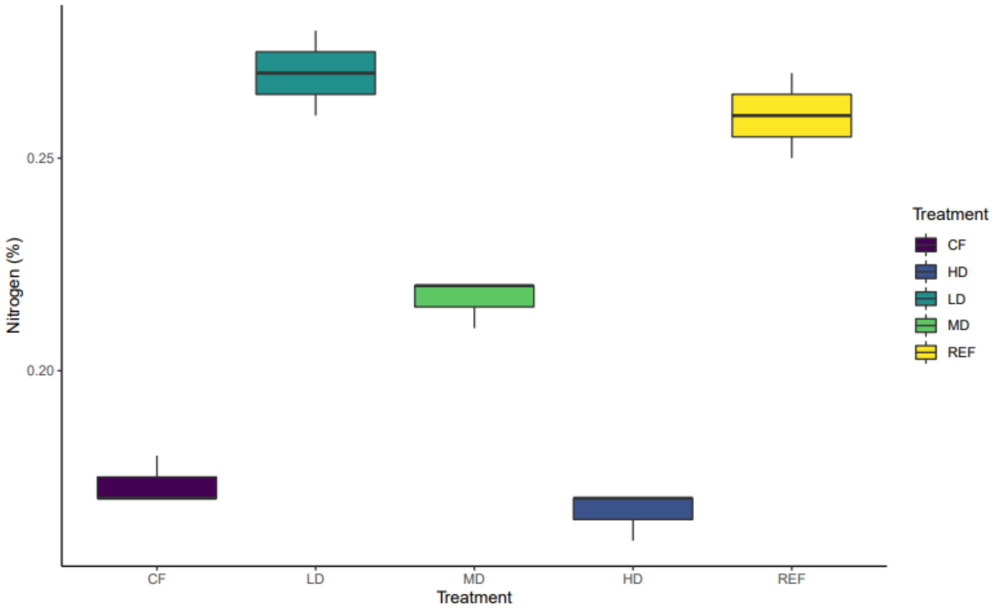


Figure 4.2.2.1: The percentage of N in GP Biotec sites, after adding biogas digestate. According to the analysis there are significant variations between sites (F= 0.3, $Pr\ (>F)$ = 0.8714, M = 0.006823). The C content variation in sites with the treatments, HD = CF, HD = CF, LD > CF, MD> CF, REF > CF, LD > HD, MD > HD, REF > HD, MD < LD and REF > MD.

There are some significant variations of N content between the sites according to the TukeyHSD test (see figure 4.2.2.1). When comparing the REF – LD and HD – CF, there was no significant variation between those sites (p > 0.05, (REF = LD, p = 0.9999793) and (HD-CF, p = 0.8250378)). However, there were significant differences between REF and MD, HD and CF. The highest differences were observed between LD-HD (diff = 0.103333333, p = 0.0000001) and REF-HD (differ = 0.093333333, p = 0.0000003). Apart from that, still there were significant variations between MD-CF (p = 0.0003322), LD-CF (p = 0.0000002), MD-HD (p = 0.0000991), REF-HD (p = 0.0000003), MD-LD (p = 0.0000565) and REF-MD (p = 0.0003322).

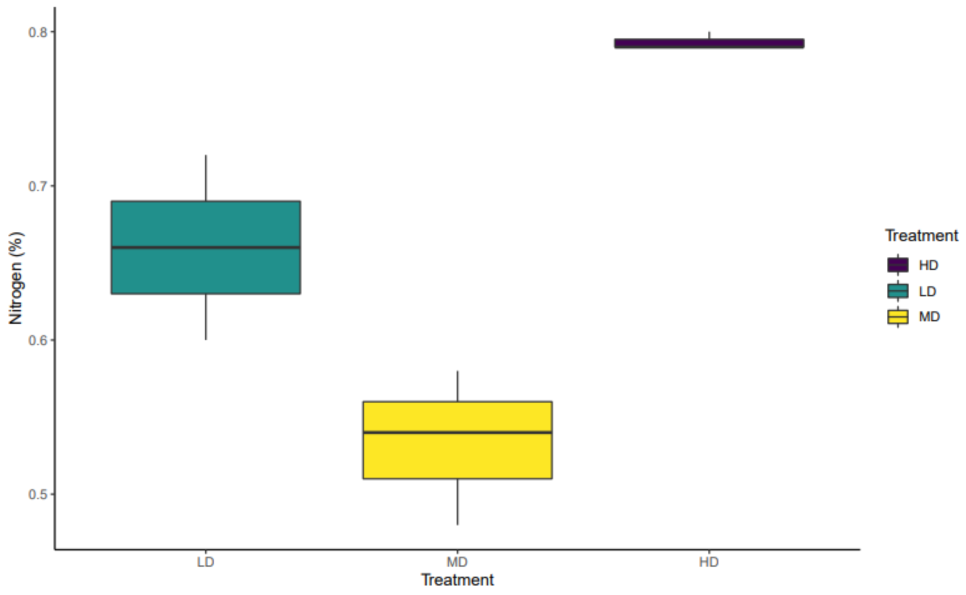


Figure 4.2.2.2: The percentage of N in Bryn Power sites, after adding biogas digestate. According to the analysis there are significant variations between sites (*F*= 1.5846, *Pr (>F)* = 0.2802, *M* = 0.05071). The N content variation in sites with the treatments, LD < HD, MD < HD and MD < LD.

There are significant variations of N content between the sites according to the TukeyHSD test (see figure 4.2.2.2). In Bryn group sites the highest N percentage was observed in the HD site and the lowest N content was recorded in the MD site (see figure 4.2.2.2). There were significant differences between LD-HD (p = 0.0263568), MD-HD (p = 0.0010146) and MD-LD (p = 0.0325884). These results are quite opposite to the GP Biotec site results. However, these results are also consistent with the first hypothesis.

4.2.3: Hydrogen percentage in Soil.

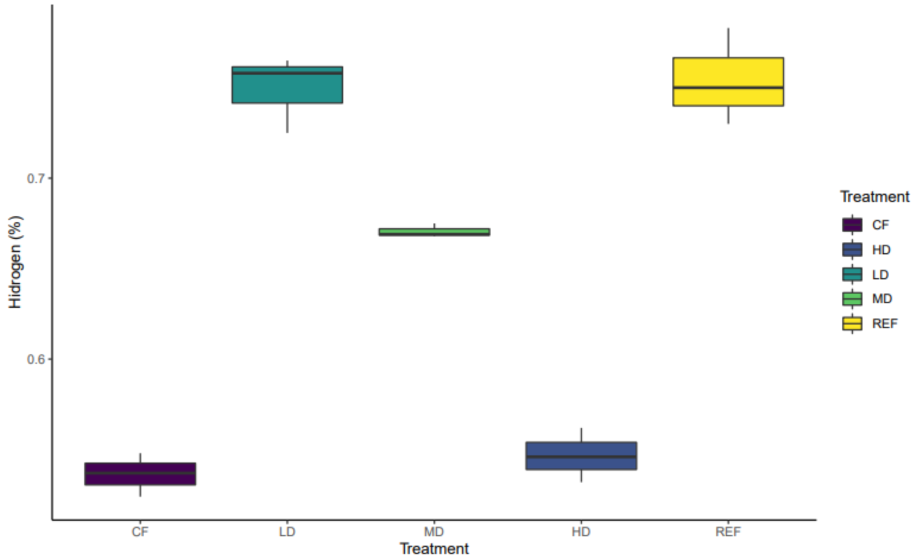


Figure 4.2.3.1: The percentage of H in GP Biotec sites, after adding biogas digestate. According to the analysis there are significant variations between sites (*F*= 2.0181, *Pr (>F)* = 0.1677, *M* = 0.03358). The H content variation in sites with the treatments, HD = CF, REF= LD, LD > CF, MD> CF, REF > CF, LD > HD, MD > HD, REF > HD, MD < LD and REF > MD.

There are some significant variations of H content between the sites according to the TukeyHSD test (see figure 4.2.3.1). When comparing the REF – LD and HD – CF, there was no significant variation between those sites (*p* > 0.05, (REF = LD, *p* = 0.9963505) and (HD-CF, *p* = 0.9476994)). However, there were significant differences between REF and MD, HD and CF. The highest differences were observed between REF-CF (diff = 0.21800000, *p* = 0.0000003) and LD-CF (differ = 0.21300000, *p* = 0.0000003). Apart from that, still there were significant variations between MD-CF (*p* = 0.0000231), LD-HD (*p* = 0.0000005), MD-HD (*p* = 0.0000472), REF-HD (*p* = 0.0000004), MD-LD (*p* = 0.0019922) and REF-MD (*p* = 0.0012466).

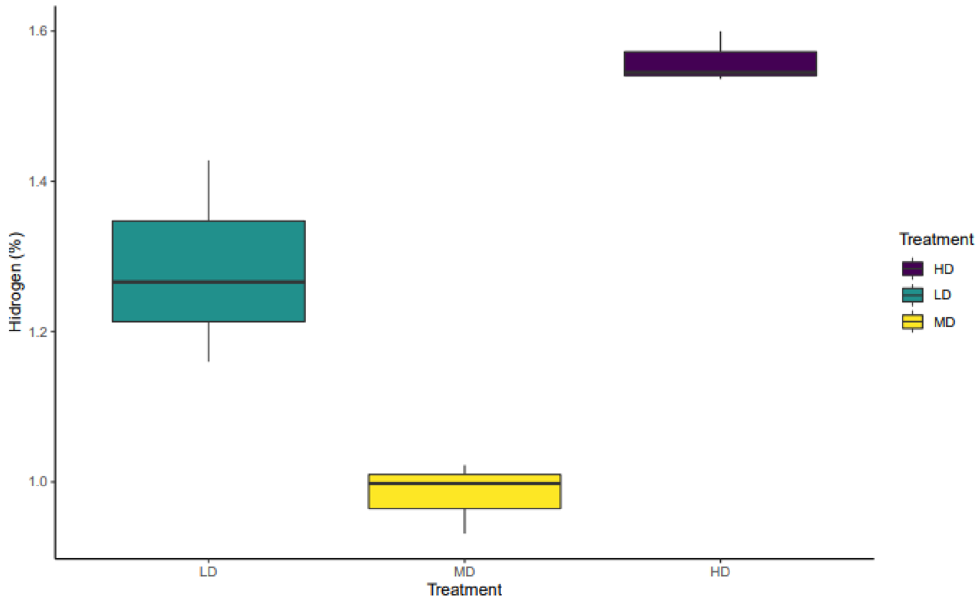


Figure 4.2.3.2: The percentage of H in Bryn Power sites, after adding biogas digestate. According to the analysis there are significant variations between sites (*F*= 1.6025, *Pr (>F)* = 0.2769, *M* = 0.24957). The H content variation in sites with the treatments, LD < HD, MD < HD and MD < LD.

There are significant variations of H content between the sites according to the TukeyHSD test (see figure 4.2.3.2). In Bryn group sites the highest H percentage was observed in the HD site and the lowest N content was recorded in the MD site (see figure 4.2.3.2). There were significant differences between LD-HD (*p* = 0.0172099), MD-HD (*p* = 0.0004025) and MD-LD (*p* = 0.0115481). These results are quite opposite to the GP Biotec site results.

4.2.4: Sulphur percentage in Soil.

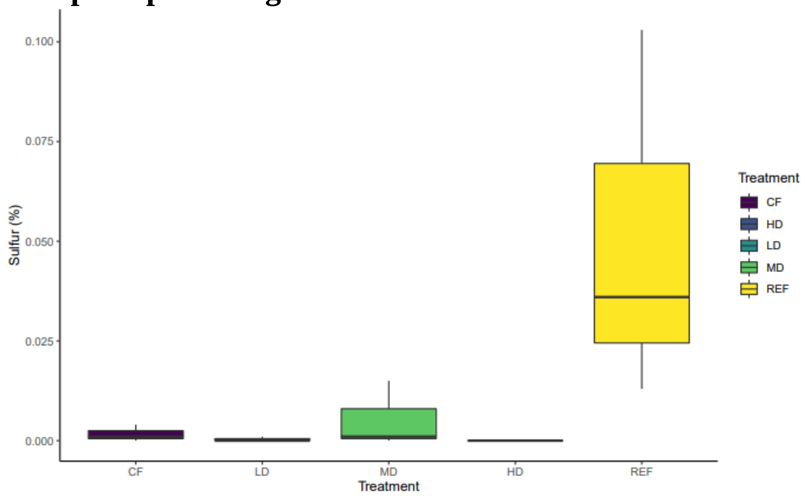


Figure 4.2.4.1: The percentage of S in soil after adding the biogas digestate. According to the analysis there are few significant variations between sites (M = 0.001336, F = 2.0181, $Pr(>F)$ = 0.1677). The S content variation in sites with the treatments, HD = CF, MD = CF, MD = LD, REF = LD, REF = MD and LD > CF, REF > CF, LD > HD, MD > HD and REF > HD.

There are few significant variations of S content between the sites according to the TukeyHSD test (see figure 4.2.4.1). When comparing the HD-CF, MD-CF, MD-LD, REF-LD and REF-MD, there were no significant variation between those sites ($p > 0.05$, (HD-CF, p = 0.1014182) (MD-CF, p = 0.8157484) (MD-LD, p = 0.1118777), (REF-LD , p = 0.9999403) and (REF-MD, p = 0.0929508)). However, there are significant variations between REF and MD, HD and CF. The highest differences were observed between LD-HD (diff = 0.267177686, p = 0.0004129) and REF-HD (differ = 0.272062788, p = 0.0003558). Apart from that, still there were significant variations between LD-CF (p = 0.0213340), REF-CF (p = 0.0176890), MD-HD (p = 0.0193117), and REF-HD (p = 0.0003558).

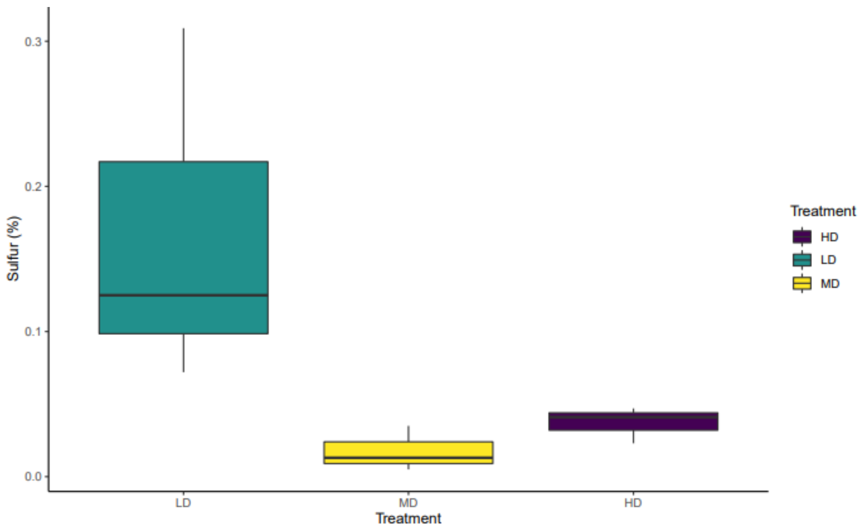


Figure 4.2.4.2: The percentage of S in Bryn Power sites, after adding biogas digestate. According to the analysis there was no significant variations between the sites. The S content variation in sites with the treatments, LD= HD, MD = HD and MD > LD.

As per the results of the Shapiro-Wilk normality test, the S content in the soil does not show a significant change with the treatments (*p* = 0.6558). However, there was a significant variations of S content between the MD-LD (*p* = 0.0342770).

4.2.5: Oxygen percentage in Soil.

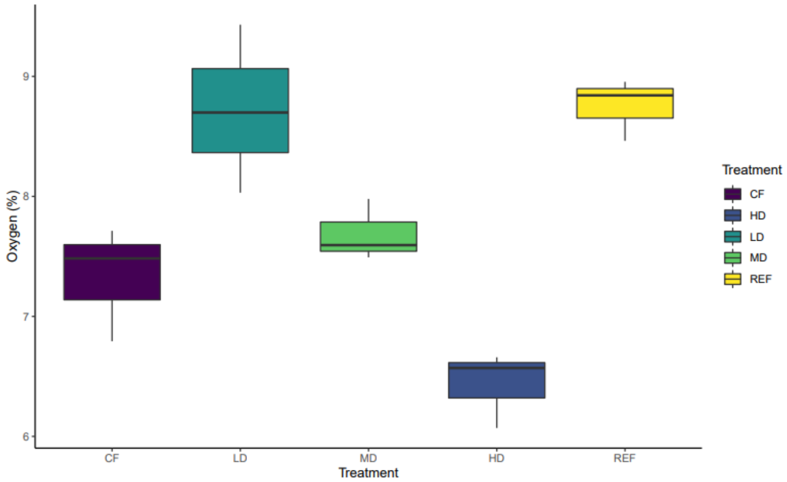


Figure 4.2.5.1: The percentage of O in GP Biotec sites, after adding biogas digestate. According to the analysis there are significant variations between sites (*F*= 2.0181, *Pr (>F)* = 0.1677, *M* = 0.03886). The O content variation in sites with the treatments, HD = CF, REF= LD, REF = MD, MD = CF, MD = LD and LD > CF,REF > CF, LD > HD, MD > HD, REF > HD.

There are some significant variations of O content between the sites according to the TukeyHSD test (see figure 4.2.5.1). When comparing the HD- CF, REF-LD, REF-MD, MD-CF, MD-LD, there were no significant variations observed between those sites (*p* > 0.05, (HD- CF, *p* = 0.1014182), (MD-CF, *p* = 0.8157484), (MD-LD, *p* = 0.1118777), (REF-LD, *p* = 0.9999403) and (REF-MD, *p* = 0.0929508)). However, there were significant differences were observed between REF-CF (diff = 0.21800000, *p* = 0.0000003) and LD-CF (*p* = 0.0213340), REF-CF (*p* = 0.0176890), LD-HD (*p* = 0.0004129), MD-HD (*p* = 0.0193117) and REF-HD (*p* = 0.0003558).

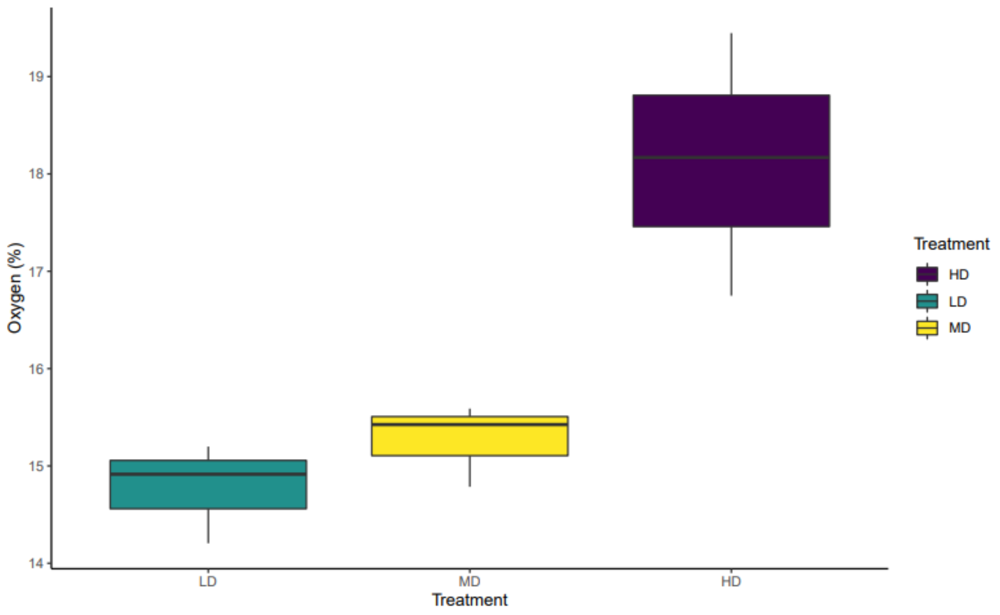


Figure 4.2.5.2: The percentage of O in Bryn Power sites, after adding biogas digestate. According to the analysis, there were no significant variations between sites (p = 0.8255).

As per the results of the Shapiro-Wilk normality test, the O content in the soil does not show a significant change with the treatments (M = 0.031596, p = 0.8255). However, there were significant changes according to the TukeyHSD test. According to that results, there were significant between LD-HD (p = 0.0063100) and MD-HD (p = 0.0144847). But between MD and LD sites there was no significant changes in O content (p = 0.7183801) (see figure 4.2.5.2).

4.2.6: C/N percentage in Soil.

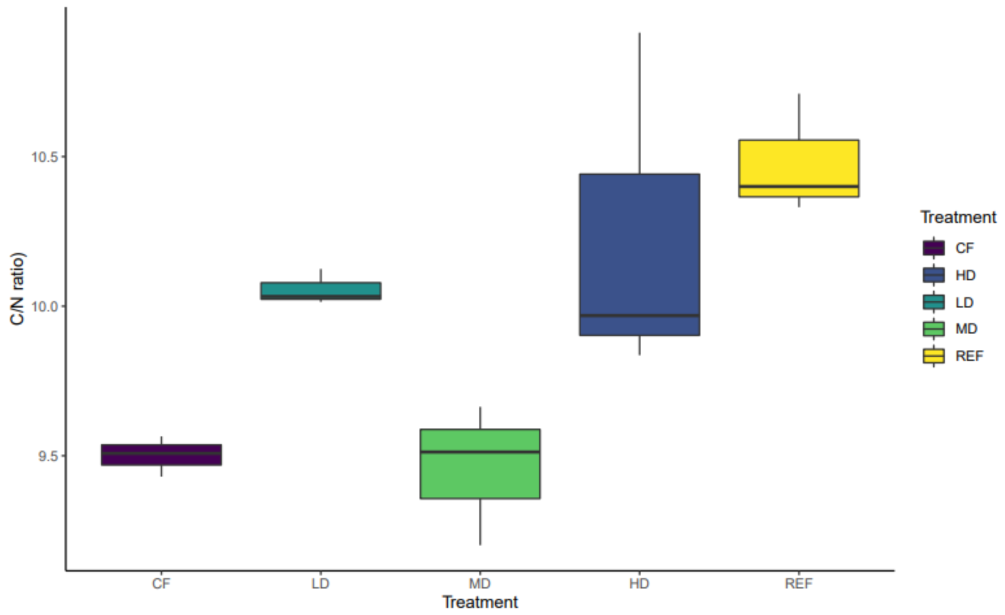


Figure 4.2.6.1: The percentage of C: N ratio in soil (GP Biotec) after adding the biogas digestate. According to the analysis there is no significant variations between sites (*M* = 0.005119, *F* = 2.0181, *Pr(>F)* = 0.1677). The C: N ratio variation in sites with the treatments, HD = CF, LD = CF, MD= CF, LD= HD, MD = HD, REF= HD, MD = LD, REF= LD, and REF > MD, REF > CF.

According to the results of the Shapiro-Wilk normality test, the C content in the soil does not show a significant change with the treatments (*M* = 0.005119, *p* = 0.211). Similarly, the TukeyHSD test (see figure 4.2.1.1) also did not show any considerable significant difference between sites (p > 0.05). According to the analysis, HD = CF (*p* = 0.0690495), LD = CF (*p* = 0.2005433), MD= CF (*p* = 0.9996442), LD= HD (*p* = 0.9489866), MD = HD (*p* = 0.0513446), REF= HD (*p* = 0.8449600), MD = LD (*p* = 0.1519747) and REF= LD (*p* = 0.4632245).

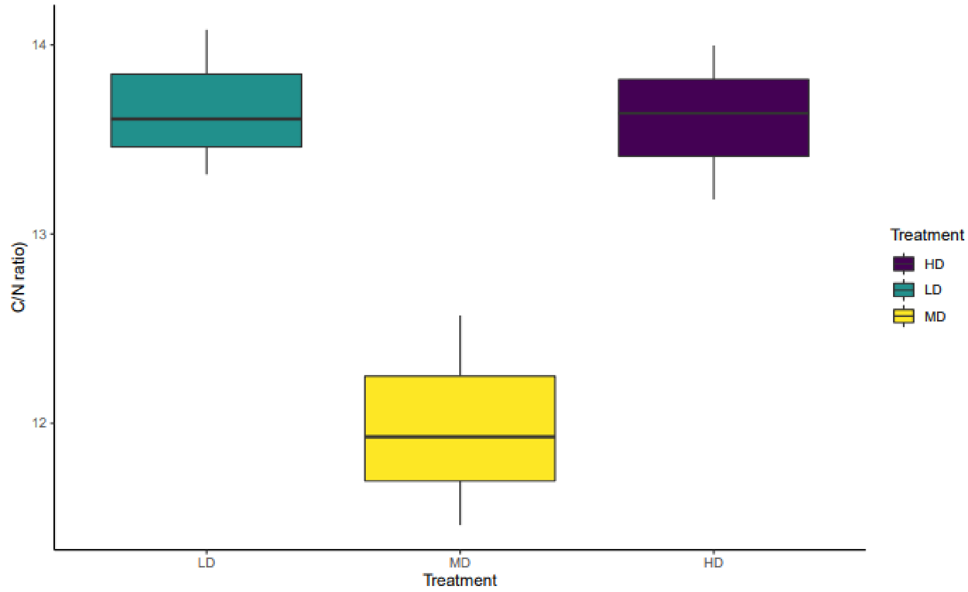


Figure 4.2.5.2: The percentage of C: N ratio in Bryn Power sites, after adding biogas digestate. According to the analysis there are some significant variations between sites (F= 1.6025, Pr $(>F)$ = 0.2769, M = 0.01442). The C: N ratio variation in sites with the treatments, LD = HD, MD < HD and MD < LD.

There are some important significant differences in C: N ratio between the sites according to the TukeyHSD test (see figure 4.2.5.2). There were significant differences between MD-HD (p = 0.0113992) and MD-LD (p = 0.0096549).

5. Discussion

5.1. Soil Carbon Content

It is well acknowledged that the dynamics of soil and atmospheric carbon (C) interact extensively and that terrestrial ecosystems can operate as a partial CO_2 buffer. Given that the amount of carbon stored in soil and plants is around three times greater than that in the atmosphere, (Schlesinger, 1995) any increase in soil carbon sequestration could considerably rise the atmospheric CO_2 and the accompanying global warming. The natural carbon cycle is an important part of the ecosystem and the carbon bonded with organic matter in the soil is called as "organic carbon" (Abbas *et al*., 2020). According to the carbon analysis results of GP Biotec, the high amount of digestate added site (HD) had a lower amount of carbon in soil compared to reference site (REF) and low digestate added sites (LD & MD). These results are supported by the studies done by (Barłóg *et al.*, 2020; Béghin-Tanneau *et al*., 2019; Caracciolo *et al*., 2015; Fontaine *et al.*, 2004; Möller *et al.*, 2011; Möller and Müller, 2012; Möller, 2015).Low C content in the soil can be caused by the low C content in the digestate compared to the feedstock (Möller and Müller, 2012).

According to Marcato *et al* (2009), the carbon oxidation rate in digestate is higher than in the undigested slurry. This is further explain by Möller (2015) and the reason for this is when the majority of the C in the digester coverts into biogas, the remaining C eventually increase its oxidation relatively. Furthermore, Möller (2015) concluded that most of the carbon-rich substances such as raw proteins, majority of volatile fatty acids (>90%), hemicellulose (>80 %) and cellulose (>50 %) are partially digested during the anaerobic digestion process. As a result, the amount of C content in the digestate can be reduced. As well as, the biogas digestate has a lower amount of lipids, amide, and polysaccharide content (nearly 15% absolute) rather than other fertilizers (Möller, 2015). Also the richness of thermostable compounds in digestate such as aromatic lignin shows an increase from 32% to 625% (Gómez *et al*., 2005). Because of these reasons, long aliphatic chains are formed in this system, but due to the absence of micro-organisms which can digest those chains within the anaerobic conditions, those aliphatics remain without degradation and substrates with easily degradable C decompose rapidly (Gómez *et al*., 2011; Marcato *et al*., 2009; Möller, 2015; Tambone *et al*., 2013;). Furthermore, this further differs from liquid digestate to solid digestate. As shown in table 1.4.2 solid digestate (9.0–10.1) has the highest amount of C compared to liquid digestate (2.64–3.15). GP Biotec uses liquid digestate for their croplands and this one also can be a reason for reduce the C content in the soil.

However, in Bryn power sites, the amount of C in soil has shown the opposite results. HD has recorded the highest C percentage compared to LD and MD. This can be a short-term impact of biogas digestate addition. Because the Bryn group has added digestate to the HD site two days before the sample collection and this high C percentage can be a result of that. However, when comparing the MD site and LD site, it shows similar results to GP Biotec.

Even though both sites has shown slightly different results, both sites were able to prove the first hypothesis of this study. Hence, it can conclude as the biogas digestate addition to the soil can caused an impact on the soil. Furthermore, the digestate addition can reduce the C content in the soil, but this can be different in short and long term. Similar conclusions have been made by Fontaine *et al.*, (2004), however, this should be further investigated.

5.2. Soil Nitrogen and Phosphorus Content

The nutrient that is particularly vulnerable to changes that might increase the risk of inefficient losses is nitrogen. GP Biotec highest N percentage (0.270%) was recorded in the LD site and the REF site recorded a slightly reduced amount (0.260%) of N compared to the LD site. Compared to the REF site, the lowest amount of N was recorded in the HD site (0.167%). When closely monitoring the results there is a pattern and it seems that when biogas digestate addition increases the amount of N in soil has been reduced. However, in Bryn power group sites the highest N percentage was recorded in the HD site (0.793%) and the second highest N percentage was recorded in the LD site (0.660%) while the lowest percentage was reported in the MD site (0.533%). These results quite deviate from GP Biotec results as the HD site had a high amount of N. But the MD and LD sites show a similar trend to GP Biotec. This exception can be a result of the digestate addition time. Because as mentioned before the Bryn group HD site was fertilized with digestate very recently. According to Eickenscheidt *et al* (2014) and Abubaker *et al* (2015), digestate helps to increase the soil nutrition level including nitrogen as a short time effect. However, this can depend on the soil conditions and digestate quality.

Even though digestate improves the soil nutrients for a short time period, the results of this study indicate that high digestate addition does not improve the soil nitrogen content as expected. This can be caused by nitrogen volatilisation, denitrification or immobilization. Several studies have shown that the main reason for N loss is nitrogen volatilisation. This can be caused during thermophilic anaerobic digestion. According to Pantelopoulos *et al* (2016) and Möller and Müller (2012) during the digestion process is an equilibrium between ammonia (NH_3) and NH_4^+,

$$NH_4^+{}_{(aq)} \leftrightarrow NH3_{(g)} + H^+$$

NH_3 is more volatile than NH_4^+ and when NH_4^+ concentration increases pH is increased. (Guštin and Marinšek-Logar, 2011). Not only that but also carbonates also led to increasing the pH of digestate due to the following equilibriums (Christensen *et al.*, 2013; Möller and Müller,2012).

$$CO_2 \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-},$$

$$CH_3COOH \leftrightarrow CH_3\ COO^-$$

When these carbonates were formed, there is a high potential to combine those with NH_4^+ and form ammonium carbonate as follows. This also leads to reduce the N content in the digestate (Arthurson, 2009; Webb and Hawkes, 1985)

$$NH_4^+ + CO_3^{2-} \leftrightarrow (NH4)_2CO_3$$

Furthermore, these carbonates can be bonded with basic cations (e.g. Ca^{2+}, K^+) and make a precipitate. These reactions also help to increase the pH level of the digestate (Drosg *et al.*, 2015). According to GP Biotec, recently their biogas digestate has recorded a high amount of K^+ (GP Biotec, 2022). When basic cations concentration increases the electric charge of the solution becomes more negative and reduces the H^+ ions. When H^+ ions reduce, the pH of the digestate increase and it leads to volatile N.

Moreover, the N loss can be impacted by climate conditions. According to Clemens *et al* (2006), during the summer biogas digestate emitted more NH_3 and during the winter it is twice lower than in summer. This can be a good reason for the N reduction in this study. Because the sample collection was done on June 23rd 2022 and according to the UK Meteorological Office, during June- July 2022, the UK experienced unexpected heat waves and in Wales maximum temperature was recorded as 370C (Meteorological Office, 2022). This high temperature also can reduce the N content in the soil after applying the digestate.

Apart from the above-mentioned facts, both N and C can be limited by the "priming effect". The priming effect can be simply defined as the 'decomposition of old soil carbon accelerated by the addition of fresh soil carbon. Mason-Jones *et al* (2018) try to explain this and according to their findings, the addition of a high amount of new organic material into the soil helps to increase the priming effect compared to the low organic material addition (Mason-Jones *et al*., 2018). When the soil priming effect increase it can negatively impact N mineralization and C mobilization (Möller *et al*., 2011). This can reduce the N and C availability in soil.

The lack of carbon in digestate has been investigated by several studies and the majority of them suggested that applying biogas digestate combined with carbon-rich amendments such as bio-char will help to stabilize the C and N as well (Berglund *et al*., 2004; Holatko *et al*., 2021; Steinbeiss *et al*., 2009; Woolf *et al*., 2010). Biochar is an organic product which can be produced using wood, sewage or manure at high temperatures without air (Lehmann and Joseph, 2015). A study done by Hewage (2016) explains that single addition of biochar into biogas digestate at a low rate helps to increase biomass production. Furthermore, biochar helps to increase the surface area of digestate by its pours structure and supports holding more nutrients (Hewage, 2016; Kanthle *et al*., 2016; Laird *et al*., 2010). Furthermore, a study done by Cardelli *et al* (2018) found that biochar addition with biogas digestate cause short and long-term impacts on soil. In short term, it reduces the microbial activity in the soil, but in long term, it enriches the C sink in the soil and reduces the CO2 emission. This helps to reduce climate change due to GHS including CO2 (Cardelli *et al*., 2018). However, these results depend on the digestate and biochar characteristics, quality and the cultivated crop type (Artiola *et al*., 2012).

In this study, we were unable to measure the P percentage in soil. However, there are so many studies related to this. Several experiments have stated that microbial activities during anaerobic digestion do not impact the P availability in digestate (Bachmann *et al.,* 2011; Loria and Sawyer, 2005). However, the pH level of digestate can impact the P availability more than any other factor (Möller and Müller, 2012). When pH increases the possibility of formation of phosphate increases as follows equation (Nelson *et al.,* 2003).

$$HPO_4^{2-} \rightarrow PO_4^{3-}$$

Simultaneously, these PO43− can be bonded with Ca2+, Mg2+ and make precipitates in the soil as follows,

$$Ca^{2+} + PO_4^{3-} \rightarrow Ca_3\,(PO_4)_2$$

$$Mg^{2+} + PO_4^{3-} \rightarrow Mg_3\,(PO_4)_2$$

As shown in figure H content in reference and LD soil is higher than MD and HD. The lowest H percentage can be seen on the HD site. In fact, H^+ and the pH have an inverse relationship; when H^+ is low, the pH is high. Therefore, pH will be higher in HD compared to REF, LD and MD in GP Biotec, hence the C, N and P content can be lower in the HD site. Nevertheless, in Bryn sites HD had the highest H percentage as shown in the figure and it will increase the acidity of the soil. As a result of this, HD can have higher C, N and P content compared to LD and MD in Bryn sites as shown in figure1, 2 and 3. However, these results prove the second hypothesis of this study, which is the decomposition of organic materials can be affecting mineral nitrogen and phosphorus in the soil. Even though the results prove the hypothesis, this should be further investigated. Because all these factors can be impacted by the climate, type of cultivation, digestate quality, soil condition, soil type and some other unknown factors.

5.3. C: N Ratio

The C: N ratio is an important characteristic of soil which helps to increase soil microbial activities. Increased bioavailability of C due to a high C: N ratio may encourage the growth of heterotrophic organisms like fungus and heterotrophic bacteria (Shi and Norton, 2000). According to the results of this study in GP Biotec the highest C: N ratio was recorded in the REF site and the second highest ratio was recorded in the HD site. In Bryn sites also the HD site had the highest C: N ratio. Similarly, in both sites, the MD site had the lowest C: N ratio.

This C: N ratio directly impact by the N and C availability in soil. As discussed above there was a significant N and C loss within both sites after the addition of the digestate. High NH_4+ concentration can increase the pH and alkaline the soil. Because of this high pH the C: N ratio can be reduced (Möller and Müller, 2012). However, studies related to this area are limited and the reasons for C: N ratio variations need to be further investigated.

5.3. Sulfur Content

Sulfur (S) is a significant necessary nutrient, and the plant available S form is Sulfate (SO_4^{2-}) (Scherer, 2001). S shortage is a developing concern in many areas. The results of

GP Biotec showed that the percentage of S was higher in the REF site compared to LD, MD, HD and CF sites. Even though there are no significant changes according to the statistical analysis of results, figure 1 has shown that comparative loos of S in the HD site is more than in others. In Bryn power sites the S percentage was higher in the LD site, compared to HD and MD. Furthermore, the S percentage in the HD site has shown a significant decline compared to LD and MD. Although there are some slight changes, as a whole it seems that the digestate addition leads to reducing the S availability in soil. This can be a pessimistic result of anaerobic digestion. Because during the AD process the redox level can be increased, and as a result of that, the S in digestate can be converted into sulfide (H2S). This can leads to reducing the S in soil (Abatzoglou and Boivin, 2009; Straka *et al*., 2007).

The pH is also an important factor related to the S content. The pH is also an important factor related to the S content. According to Möller and Müller (2012), when pH increase, sulfur can bond with C and precipitate. Furthermore, high pH can induce the formation of volatile H_2S. As per the findings of Eriksen *et al*., (1995), 50% of the S availability in soil, can be reduced as a result of this H_2S volatilization. Although the S content in soil can reduce for those reasons, still more research is needed to identify how digestate impacts the S content in the soil.

5.4. Oxygen and Hydrogen Content

According to the results of GP Biotec, the O percentage in soil has been reduced with the digestate addition. Because it has shown that, REF and LD sites have a high amount of O, compared to HD. However, In Bryn power sites, the O percentage in soil has increased with the digestate addition. This variation can be caused by the quality of digestate, application method, soil characteristics and etc. However, still cannot conclude these results as further research is needed. Also, could not find any related studies to this. Hence more research needs to be done about the digestate addition and O content variation in soil.

When considering the H content in the soil, after adding the digestate, it also showed a somewhat similar pattern to the O content variation. In fact, in GP Biotec, REF and LD sites had a high amount of H, compared to HD. However, In Bryn power sites, the H percentage in soil has increased with the digestate addition. However, still cannot conclude these results as further research is needed. Because this difference can be caused by the quality of digestate, application method, soil characteristics and etc. As well as, could not find any similar studies which focus on H content variation with digestate addition. Hence more research needs to be done about the digestate addition and H content variation in soil.

5.4. Earthworm Analysis

Earthworms are ecological engineers that modify ecological processes and attributes through their functional functions in the soil profile. They modify the soil which is suitable for crop growing (Bertrand *et al*., 2015), increase the water holding capacity by increasing the infiltration (Bouché and Al-Addan, 1997) and are responsible for increasing the crop yield (Van Groenigen *et al.,* 2014). Through bioturbation activities like their digging and feeding behaviour, they change the soil's characteristics (Blouin *et al.,* 2013). They are therefore often used as bio-indicators in environmental evaluations and soil monitoring programs (Huber *et al*., 2008). Hence, understanding the relationship between earthworms and digestate applications is important. According to the results of GP Biotec sites the highest biomass of earthworms was recorded in the Low digestate added site and the lowest biomass was recorded in HD and CF added sites. Comparatively the REF site and the LD site had higher earthworm biomass as shown in the figure. However, the abundance of earthworms is higher in the REF site compared to LD. Furthermore, when considering the age classification, all earthworms present in the GP Biotec were juvenile earthworms and could not find any adults. In order to the results of GP Biotec, it seems that low digestate concentrations help to improve the earthworm community and a high amount of digestate can reduce the earthworm density and biomass. A most recent study done by Natalio *et al* (2021) has shown similar results to GP Biotec. This study has shown that biogas digestate causes negative impacts on earthworms. Especially after the addition of digestate into soil adult earthworm mortality rate was higher compared to juveniles. As well as, a high concentration of digestate reduces the earthworm biomass and abundance rather than a low concentration of digestate (Natalio *et al*., 2021). Koblenz *et al* (2015) also tried to find out how digestate impacts earthworms and found that, as short-term impact biomass and abundance of earthworms can be reduced as a result of digestate addition (Koblenz *et al*., 2015). However, still the reasons for this is not clearly defined by studies. But this earthworm biomass and abundance reduction can be a result of biogas digestate toxicity (Natalio *et al.*, 2021). Becaues, as mentioned before digestate can contain pathogens, heavy metals and other toxic substances. These substances can cause negative impact on earthworms. Reinecke *et al* (2001) discovered that while Pb contamination had no influence on the efficiency at which earthworms produced cocoons, it had a considerable impact on the viability of those cocoons. Furthermore, Salminen and Haimi (2001) found that Copper (Cu) negatively impacts the size of earthworms. Nevertheless, these heavy metals can cause damage to the DNA and cell structure of earthworms (Wang *et al*., 1994; Yongcan *et al*., 1996).

Nevertheless, the salinity of the digestate (KCl, CaCl2) can negatively impact on earthworms (Edwards and Lofty, 1972; Koblenz *et al*., 2015; Natalio *et al.*, 2021). The earthworm epidermis is selective and sensitive to salinity (Raiesi *et al*., 2020). As previously discussed digestate is a nutrient-rich component and it has a high concentration of K^{+}, Ca^{2+} and Na^{+} which increase the salinity. Hence after the addition of the digestate, the soil (hypertonic) salinity can be increased. Meanwhile, the ion concentration of the body fluid can be lower (hypotonic). As a result of this osmotic pressure creates and water can come out from the epidermis. This mechanism can dehydrate the earthworms and can reduce biomass of earthworms (Laverack, 1960). The pH of soil after adding digestate can be impact on earthworm community. The results of this study it has shown that the digestate is more alkaline. Even though earthworms are able to tolerate some alkalinity, they are unable to tolerate high alkalinities (Di Carlo *et al.*, 2020). Hence with the high digestate addition, the earthworm biomass can be reduced. Moreover, Natalio *et al* (2021) found that water content also impact on the biomass and abundance of earthworms. According to their findings, with high amount of water, pores in the soil can be saturated with water. As a result of that, the amount of O2 in soil can be reduced and can be leads towards anaerobic conditions. Hence this O2 depletion also can be a reason for earthworm reduction (Natalio *et al*., 2021).

Apart from all those factors above, the absence of adult earthworms within GP Biotec sites can be a result of the morphology of earthworms. Due to the cylindrical body of earthworms get large surface area (Ezeokoli *et al*., 2021). This is an adaptation for respiration. However, this can be a reason for their absent. Because, when the surface area increases the impact of heavy metals, high pH, salinity and some other unrevealed factors can be impacted easily. Therefore adults can be impacted by digestate rather than juveniles. Even though digestate addition negatively impacts on earthworm population still it shows more positive results compared to chemical fertilizer addition. Earthworm biomass variation in GP Biotec significantly showed that the biomass of earthworms was higher compared to chemical fertilizer addition. According to Koblenz *et al* (2015), digestate directly supplies fresh organic foods for earthworms while chemical fertilizers indirectly supply organic matter at a slow rate. Hence it can be conclude as the biogas digestate addition to soil is more favourable for earthworm population compared to chemical fertilizer addition (Cuendet and Ducommun, 1990; Timmerman *et al*., 2006; Whalen *et al*., 1998).

Compared to GP Biotec, Bryn power sites have shown some exceptions in earthworm abundance and biomass. As shown in the figure, the highest abundance of biomass was recorded in the high digestate added site and the second highest biomass was recorded in the LD site. Although GP Biotec shows some negative impacts on earthworms, the Bryn sites show a positive impact on earthworms. A study done by Sizmur *et al* (2017), confirms that with the biogas digestate addition the biomass of earthworms increases. Furthermore, this effect can be increased if the digestate is added with straw (Sizmur *et al.*, 2017). This argument was further supported by Timmerman *et al* (2006) Long-term application of digestate helps to increase the biomass of earthworms (Timmerman *et al*., 2006). A most recent study done by Moinard *et al* (2021) discovered that, even though there are some negative short-term impacts of digestate applications, the long-term addition of digestate helps to increase the biomass of earthworms by 150%. This positive impact on earthworms could be due to the high availability of food sources. Because digestate is an organic nutrient-rich substance (D'Hose *et al*., 2018).

By contrast to GP Biotec, within the all three sites of Bryn power epigeic adult earthworms were recorded. Although epigeic adults were recorded still the abundance is low. Generally, epigeic earthworms are found in environments with plenty of organic debris. They feed on excrement, rotting plant roots, and leaf litter while living at or near the soil surface (Earthworm Society of Britain, nd). As a result of this, this species may have good tolerance for salinity and pH with compared to other earthworm species (Moinard *et al*., 2021). However, this results should be further research to find out what are the actual reasons for this.

Although there are positive and negative impacts of digestate on earthworm communities it should be further investigated. Because the earthworm population can be impacted by several factors including quality of the organic matter (Sizmur *et al*., 2017), C stability of soil (Thomsen *et al.*,2013), Physico-chemical properties of digestate (Moinard *et al*., 2021), land use pattern, cultivated crop type (Curry, 2004), rainfall pattern (Julka and Paliwal, 2005) and some other variable still could not define. However, as a whole, the results of this study partially fulfil the third hypothesis. Because GP Biotec results have shown earthworm biomass and density reduction after digestate addition, while Bryn power sites show earthworm biomass and density increment with digestate addition. Hence, more research needs to be done to come up with a conclusion.

6. Conclusions

In order to encourage sustainable farming practices and renewable energy production, the effects of applying biogas digestate into the soil and on the related living creatures need to be thoroughly evaluated. The results of this case study revealed novel insights into the impacts of anaerobic digestate on soil microclimate and earthworms. In this study, two different AD plants and their agricultural lands were used as study area. According to the results of both sites, the first hypothesis was fulfilled. Because both sites had shown C content variations after adding digestate. However, GP Biotec results indicate that, digestate addition and soil C content has a pessimistic relationship. But Bryn power sites had shown an optimistic relationship between digestate and soil C. This can be an outcome of multiple factors including soil characteristics, anaerobic digestion process, soil priming effect, pH, and digestate quality. However this should be further investigate.

The N content in both sites had shown similar trend with C. One of the main reason for that could be the pH. Other than that, presence of basic cations, N volatilization and precipitation and some other unidentified factors could be impact on N content in soil after adding digestate. More significantly, both C and N content results can be impact on C: N ratio in soil after adding digestate. In fact, the results of this study showed that C: N ratio increments with digestate addition. Moreover, the results of this study had shown that after adding the digestate the S content in soil can be reduced. Finally, the digestate addition can negatively impact on earthworm population. However, there may be short–term and long-term positive and negative impacts of digestate addition. Hence, further research is needed to identify what are the reasons for C, N, and S reduction with digestate. Also, need to do more research on how soil characteristics, crop management, crop type, climate conditions, digestate quality, pH, earthworm species, digestate application time period and frequency impact on the results of this study. Investigations on the effects of various solid and liquid digestate on earthworm ecology, including species, behaviour, and functions, are still needed in a variety of soils. Furthermore, the heavy metal content in digestate is an important factor which can be impact on earthworm populations. Hence all these parameters need to further study with more replications. Even though the C, N, S and other element (H and O) capacities are less than expected, still cannot abandon the biogas addition. Because it always shows better results compared to mineral fertilizer addition as shown in the results. Finally, a better knowledge of the digestate addition and soil microclimate and biota changes can be made via a set-up controlled experiment.

7. References

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8. Appendix

Table 8.1: Total biomass of Earthworms and their classification, GP Biotec AD plant.

Date	Sample Collection site	Soil characteristics	Earthworm Classification	Earthworm Count	Total Biomass (g)
23/06/2022	Reference Soil	Around 50 degrees sloppy land with grasses.	Juveniles	8	1.04
	High Digest Applied Soil	Compacted soil. Oats field.	None	0	0
	Medium Digest Applied Soil	Red colour soil. Grasses were grown for maintain the livestock	Juveniles	4	0.55
	Low Digest Applied Soil	Dark colour soil. Grasses were grown to maintain the livestock	Juveniles	6	2.27
		Dry, Compacted,	None	0	0

	Fertiliser Applied Soil	Hard soil. Wheat was cultivated within the area			

Table 8.2: Total biomass of Earthworms and their classification, Bryn power AD plant.

Date	Sample Collection site	Soil characteristics	Earthworm Classification	Earthworm Count	Total Biomass (g)
24/08/2022	High Digest Applied Soil	Dark colour soil, not much compacted. Use for livestock feeding	Epigeic earthworm	1	**2.24**
			Juveniles	7	
	Medium Digest Applied Soil	Compacted soil with small rocks. Use for livestock feeding	Epigeic earthworm	1	**1.51**
			Juveniles	2	
	Low Digest Applied Soil	Compacted soil .Use for livestock feeding	Epigeic earthworm	1	**1.54**
			Juveniles	2	

Table 8.3: Elemental composition of GP Biotec soil samples.

Sample	Average Elemental Composition (%)					CHNSO proportion of total mass (%)
	Carbon	Hydrogen	Nitrogen	Sulphur	Oxygen	
Reference Soil	2.713	0.754	0.260	0.051	8.753	**12.532**
Fertilised Soil	1.637	0.536	0.173	0.002	7.330	**9.678**
Low Digestate Added Soil	2.720	0.749	0.270	0.000	8.720	**12.460**
Medium Digestate Added Soil	2.053	0.671	0.217	0.005	7.688	**10.634**
High Digestate Added Soil	1.693	0.547	0.167	0.000	6.433	**8.840**

Table 8.4: Elemental composition of Bryn group AD plant soil samples.

Sample	Elemental Composition (% by mass)					CHNSO proportion of total mass (%)
	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	
Low digestate added soil	9.030	1.285	14.773	0.660	0.169	**25.917**
Medium digestate added soil	6.327	0.984	15.267	0.533	0.018	**23.128**
High digestate added soil	10.790	1.560	18.122	0.793	0.037	**31.302**

Figure 8.1: Solid digestate (GP Biotec).

Figure 8.2: Applying liquid digestate to farm land (GP Biotec).

Figure 8.3: Solid digestate storage area in GP Biotec.

Figure 8.4: After applying liquid digestate (GP Biotec).