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Methane potential of oat husk waste streams in anaerobic digestion

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The production of oat grains creates side streams of oat husks that are mainly unsuitable for human consumption. Globally it reaches around 1500 million tons of agricultural waste from cereal production only. CH-Bioforce's process utilizes oat husk powder to extract polymers, producing hemicellulose permeate as a waste stream. In this study, methane is produced by anaerobic digestion using industrial inoculum and oat husk powder to determine oat husk powders methane potential. Oat husk powder and hemicellulose permeate are analysed for their chemical composition to estimate digestion properties and potential as a biobased material.

Methane production digestion setup included inoculum control, microcrystalline cellulose control and sample, with three replicate samples of each. Digestion is located in a shaker incubator (35 °C, 200 rpm, 30 days). Biogas produced in the digestion is lead to sodium hydroxide (5 M) to capture carbon dioxide, after which methane is collected.

Detected production of digestion control was approximately 300 ml of methane, and in double digestion methane production of digestion control was 210 ml of methane and oat husk powder sample by 100 ml of methane in 30 days. Other digestions did not indicate any biomethane produced. Compared to maximum potential of 1930 ml digestions did not exceed predicted methane production. These are not scientifically viable results, because only one control and one sample showed results between two digestions.

Oat husk powder is considered as a potential methane production substrate according to the chemical composition and literature references. Hemicellulose permeate was not considered as a possible methane production substrate due to high sodium concentrations.

Key words: anaerobic digestion, methane potential, agricultural waste stream, oat husk.

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Abbreviations

BMP	Biochemical methane potential
DC	Dry content
GC	Gas chromatography
HMDS	1,1,1,3,3,3-Hexamethyldisilazane
KKJP	oat husk powder (fin. kaurankuorijauhepöly)
KKJ-H-perm	oat husk hemicellulose permeate
TDC	Total dry content
TMCS	Chlorotrimethylsilane
VS	Volatile solids

1. Introduction

1.1. Oat husks utilization

Oats were produced 22,74 million metric tons in 2022 globally (Statista 2024) and 1,174 million tons in Finland (Luonnonvarakeskus 2022). Oat is a particularly important crop in Nordic countries because of quick flowering and maturing with long daylengths (Buerstmayr et al. 2007). The production of oat grains also creates side streams of oat husks that are mainly unsuitable for human consumption with approximately for every ton of cereal production comes 1,5 tons of agricultural waste. Globally, it exceeds 1500 million tons of agricultural waste from cereal production only. Oat husks are approximately 25-35 % of the seed weight (Redaelli and Berardo 2007). Most traditional way of utilizing husks is using it as feed because of the high fiber content, energy and crude protein (Yu et al. 2002). However, husks nutritional quality is relatively low in its natural state even for ruminants due to its low rate of degradation, digestibility and voluntary intake. (Yuan and Sun 2010; Sun et al. 2015.)

As the biomass waste from oat agriculture is vast, it should be utilized for materials and products that use virgin materials in production. By using side streams instead of virgin materials, the source of virgin materials can be saved or used elsewhere. A step towards a circular economy can be taken by utilizing the side streams for human consumption, products or as a source of energy, as the oat husks would otherwise be consumed by animals or left to rot. (Hansen and Plackett 2008.) Landfills have methane content of around 45-55 % and carbon dioxide (CO_2) content of 30-40 %, already. (Rasi et al. 2007.)

The carbon cycle of organic side streams is visualized in figure 1. Carbon dioxide from the atmosphere is converted into sugars and other organic materials that can be utilized in biomethane production. Biomethane can then be used as a fuel or an energy source, where burned carbon dioxide returns to the atmosphere. In contrary, fossil fuels bring more carbon from below the ground surface into the atmosphere, which is eventually in the form of carbon dioxide, causing various problems to nature and chemical balance.

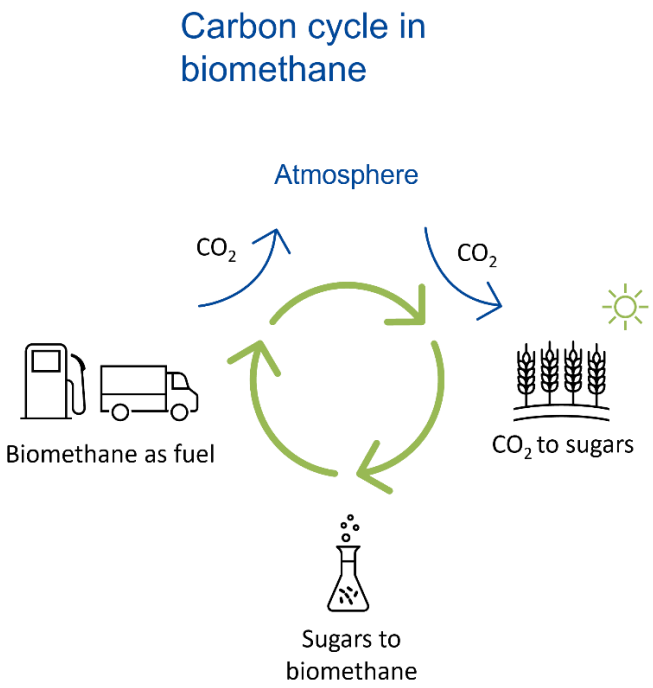

Figure 1. *Carbon cycle of organic side streams used in biomethane production.*

Because the source of material in this process is a side stream product, it could open new doors as resources are depleting all over the world. Hemicellulose-rich biomass has had increasing demand because of the possible applications in packaging films, foams, gels, paper additives, color components, as well as surfactants, flocculation aids, antimicrobial agents and coating components (Alekhina et al. 2014; Bouxin et al. 2010; Deutschmann and Dekker 2012; Edlund et al. 2010; Hansen and Plackett 2008; Laine et al. 2013, Mikkonen et al. 2015; Oliveira et al. 2017; Petzold-Welcke et al. 2014; Pohjanlehto et al. 2011; Reis et al. 2005). Similarly, lignin shows great potential for producing chemicals, biofuels and other materials because of the aromatic backbone (Rosado et al. 2021).

By using controlled fermentation, the production of methane and other greenhouse gases can be controlled and utilized further as biomethane in energy production without fossil fuel usage. As the carbon released from fossil fuels is the main factor in carbon dioxide emissions, it is important to favor non-fossil fuels and other sources of energy. Therefore, biobased products and applications must be invented and optimized as the current economy is increasingly driven by sustainable products and methods.

Various biomasses among oat husks are studied in literature when it comes to anaerobic digestion, such as land weeds, grass silage, sugar beet tops, leaves of fast-growing tree

species, paper mulberry, paddy straw, sugarcane bagasse, sugarcane trash, winter rye straw, oilseed rape straw, faba bean straw, wheat straw, corn straw, rice straw, manure from various animals and urban wastes. These feedstocks are considered as a good source for bioenergy conversion from side streams to energy, alternatively for biomethane or bioethanol. (Chanakya et al. 1999; Demirbas 2006; Lehtomäki et al. 2007; Møller et al. 2004; Petersson et al. 2007; Torres-Castillo et al. 1995; Zhang 1999.)

To avoid pushing the side streams to a wastewater treatment plant, it is hoped to have enough biomethane potential for hemicellulose permeate to be utilized in the fermentation process instead of loading the wastewater plant and saving energy as there is no need to purify or transport the side streams. An alternative to the fermentation process is coalification. However, it is considered less optimal, as it consumes energy.

1.2. Source of substrate

This study is made in cooperation with CH-Bioforce Oy. CH-Bioforce is a Finnish company that produces polymers – dissolving cellulose, polymeric hemicellulose and sulfur-free lignin – from different biomasses via Bioforsense technology. With their current fractionation technology, they can produce up to 98 % α-cellulose. Dissolving cellulose is currently wanted in textile industries. With the Bioforsense technology over 95 % hemicellulose can be produced. It can be utilized in take-away cups, cosmetics, and food. By fractionation, CH-Bioforce can produce sulfur-free lignin as a potential replacement for plastic. (CH-Bioforce 2023.)

Before fractionation, oat husks are mechanically processed, and the raw material oat husks powder (KKJP) is collected. During fractionation process oat husks hemicellulose permeate (KKJ-H-perm) is collected. The hemicellulose permeate is concentrated before use in this study as its concentration is low. The hemicellulose permeate has naturally a pH of 4.7 because of the acetic acid attached to hemicellulose.

In this study, the KKJP and concentrated KKJ-H-perm are analysed to determine potential for fermentation and other alternatives. KKJ-H-perm is expected to contain some of the same polymers as the oat husks. The oat husks are considered to be potential carbon source to substitute fossil-based carbon sources in biofuels, enzymes, organic acids, for

example. (Demirel et al. 2018). In the process of fractionation arabinoxylans are removed, and KKJ-H-perm could be utilized in the fermentation process. As the KKJ-H-perm is expected to contain fermentable polysaccharides, it could be used in other applications as well. Therefore, they can be evaluated as potential alternatives to pure carbon sources for the production of value-added products. The oats used in this study are first used for food production, so the grain itself is already removed from the husks. This removes the ethical problem of using consumable food for energy or materials as the edible part is not used.

1.3. Oat husk composition in literature

Oat husks contain large concentrations of cellulose, hemicellulose and lignin, that can be utilized in different polymer applications (Chopda et al. 2020; Oliveira et al 2017; Valdebenito et al. 2017) and anaerobic digestion uses mainly cellulose and hemicellulose to produce methane. (Kusch et al. 2011.) In a comprehensive study of Knudsen (1997) the contents of oat husk meal were analysed, and the percentages in dry content of total sugars were 1.4 %, of which monosaccharides had percentages of 0.4 %, sucrose 0.7 %, raffinose 0.2 % and stachyose 0.1 % and 2.0 % of glucose. Polysaccharides, other than cellulose and hemicelluloses, percentages were as follows: starch 21.3 %, fructan 0.2 % and insoluble non-cellulosic polysaccharides 29.5 %. (Knudsen 1997) Oat carbohydrate and lignin consistency are collected in table 1.

Table 1. *Oat carbohydrate and lignin consistency modified from the paper of Knudsen (1997). Table shows concentrations of important substrates such as starch, glucose, hemicelluloses and unwanted compounds in digestion such as lignin and dietary fibre.*

	Oat Hulled (Mean) (g kg^{-1})	Standard deviation (Oat Hulled)	Oat Hulless (Mean) (g kg^{-1})	Standard deviation (Oat Hulless)	Oat hull meal (g kg^{-1})
Monosaccharides	2	1	not measured		4
Sucrose	11	2	not measured		7
Raffinose	3	1	not measured		2
Stachyose	2	1	not measured		1
Total sugars	17	4	not measured		14
Starch	468	25	557	38	213
Fructan	3	2	not measured		2
β-glucan	28	3	41	8	14
Soluble non-cellulosic polysaccharides	40	13	54	7	13
Rhamnose	0	0	0	0	0
Arabinose	3	1	3	1	2
Xylose	2	3	2	1	0
Mannose	2	1	1	1	1
Galactose	2	1	2	0	0
Glucose	28	5	45	7	8
Uronic acids	3	4	2	1	1
Insoluble non-cellulosic polysaccharides	110	9	49	10	295
Rhamnose	0	0	0	0	0
Arabinose	15	0	10	1	26
Xylose	78	8	21	7	212
Mannose	1	0	2	1	1
Galactose	5	0	2	0	9
Glucose	5	2	11	2	12
Uronic acids	7	0	3	1	35
Cellulose	82	5	14	6	196
Total non-starch polysaccharides	232	10	116	19	505
Klason lignin	66	9	32	6	148
Dietary fibre	298	19	148	23	653

Cellulose is one of the main sources of carbon and energy to the methanophilic microbes. In total oat husks contain approximately 40 % cellulose (Oliveira et al. 2017; Valdebenito et al. 2017). According to Neitzel et al. (2023) 66 % of oat husks cellulose content is holocellulose and 30 % is α-cellulose. In another study, 45 % of an oat husks cellulose content is α-cellulose (Skiba et al. 2020), so the concentration of cellulose varies depending on used methods and cultivar.

In total hemicellulose concentration is approximately 30 % (Oliveira et al. 2017; Valdebenito et al. 2017). Hemicellulose concentrations of four species of Nordic oat husks are shown in the study of Schmitz et al. (2020). They found concentrations of hemicelluloses to average as follows: xylose 24 %, arabinose 3.4 %, galactose 1.3 %, mannose 0.1 %, galacturonic acid 0.5 % and glucuronic acid 0.7 % (Schmitz et al. 2020). In another study, xylose was found to have a concentration of 0.0 %, arabinose 2.8 %, β-glucan 1.4 %, galactose 0.9 %, mannose 0.1 % and uronic acids 3.6 % (Knudsen 1997).

Lignin is a phenolic polymer, that works as a structural support in higher plants between cellulose, hemicellulose and pectin (Kärkönen and Koutaniemi 2010). It is located especially in the husks of oat kernel (Karmanov et al. 2023). Lignin concentration should remain low in value, because it has been studied to lower bioactivity of the fermentation by binding to polysaccharides (Dai et al. 2020). In the fermentation process the lignin degrades to polyphenols, that are severe inhibitors in the process (Guo et al. 2021). According to Knudsen (1997) the concentration of Klason lignin is 148 g/kg.

Phenolic compounds have inhibitory effects to the methane fermentation. Ferulic acid, for example, binds lignin and hemicelluloses with ester bonds (Schmitz et al. 2020). This affects methane production, because the hemicellulose cannot be used by the microbes once attached to lignin. Phenolic compounds concentrations, p-hydroxybenzaldehyde, vanillin and ferulic acid, are on average 0.01 %, 4.75 % and 1.97 % (Schmitz et al. 2020). However, the concentrations are highly affected by climate and weather conditions. (Welch et al. 1983.)

Highest mineral concentrations in oat husks are on average calcium (995 µg/g), potassium (4 300 µg/g), magnesium (873 µg/g) and phosphorus (1 815 µg/g). These mineral concentrations too, as phenolic compounds, are affected by climate and weather conditions (Welch et al. 1983). These minerals are also considered as valuable nutrients

for agriculture. The extractive concentrations of oat husks in cold water are 14 %, in hot water 17 %, in 1 % sodium hydroxide (NaOH) 42 % and in ethanol-toluene 2 %. (Neitzel et al. 2023.)

After hydrolysis the oat husks contained approximately 25 g/L of xylose, 7 g/L of glucose, 5 g/L of arabinose, 2 g/L of phenols, 3 g/L of acetic acid and 0.48 g/L of furfural (Demirel et al 2018; Soleimani and Tabil 2014) which differs slightly from oat husk consistency before hydrolysis. This is something that should be looked further into, as during the anaerobic digestion slight hydrolysis happens and changes the composition of substrates.

1.4. Biogas and biomethane in anaerobic digestion

Biogas is a flammable gas, that is generated in anaerobic digestion by biomass fermentation. It consists mainly of over 50 % methane (CH_4) and under 50 % CO_2 as well as under 5 % of other gases water (H_2O), hydrogen sulfide (H_2S), hydrogen gas (H_2), nitrogen gas (N_2), carbon monoxide (CO) and oxygen gas (O_2). After the purification and separation process of CH_4 and CO_2 the methane gas can be used as biomethane (Lecharlier et al. 2022).

In the study of Wu et al. (2010) biogas production from oat straw with swine manure was found to be higher than with bare manure, corn stalks or wheat straws on the seventh day of digestion. Experimental potential of methane production in oat husks was reported to be 242N CH_2/kg depending on both potential of the substrate and total fermentation time (Kusch et al. 2011). Common methane production graph is shown in figure 2.

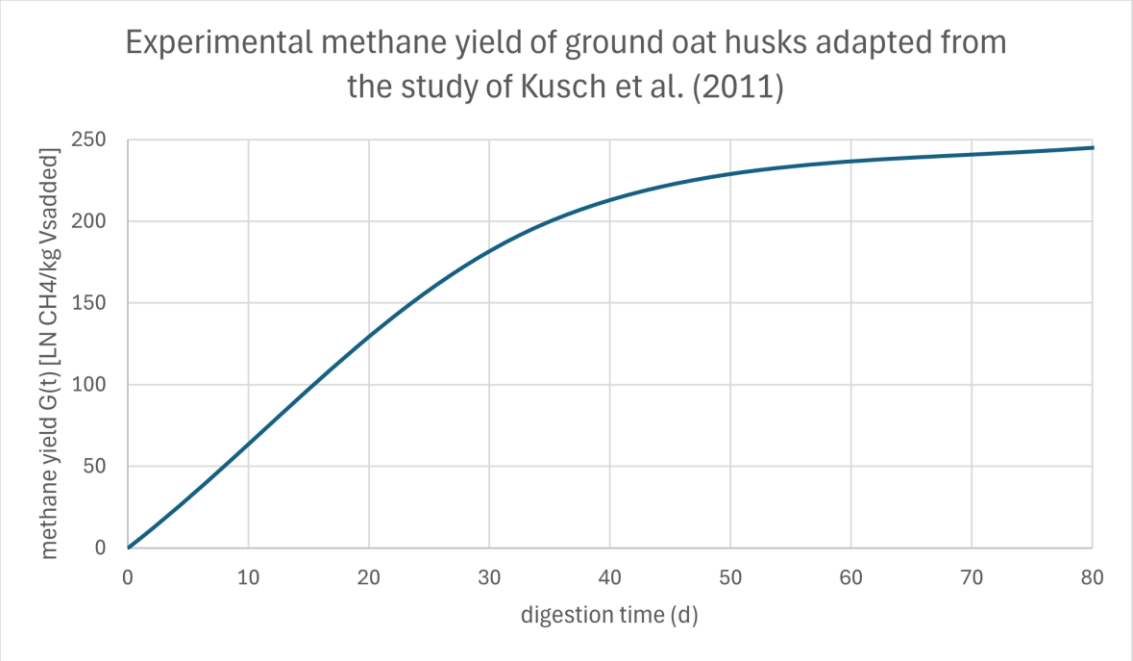

Figure 2. *Experimental methane production of ground oat husks by Hohenheim biogas yield test adapted from the study of Kusch et al. (2011).*

Study of Kusch et al. (2011) shows that oat has great success in methane production in digestors. Therefore, such performance is expected from the hemicellulose permeate as well. It was also detected that ground oat husks increased methane production efficiency in 30-day cultivations because of the higher surface area compared to untreated oat husks (Kusch et al. 2011). Taking this into account the KKJ-H-perm has high efficiency expectation due to lack of structure and consequent free polymers in the permeate.

In methane fermentation the properties of different methanogenic pathways were analyzed. Depending on the organism they can use different methanogenic pathways: hydrogenotrophic, aceticlastic and methylotrophic (Kurth et al. 2020). The key pathways are hydrogenotrophic and aceticlastic pathways in methane production. Cellulose and hemicellulose degradation in hydrolysis are the most important reaction in methane production as it is the source of energy for micro-organisms. (Guo et al. 2021.)

In hydrogenotrophic pathway of methane production the methanogens use hydrogen as electron donors and reduce carbon dioxide to methane through various reactions shown in figure 3. Aceticlastic pathway transforms acetate to methane and carbon dioxide via numerous reactions in the cell seen in figure 3. Methyl group is used in the methanogenic pathway to be reduced to methane (Costa and Leigh 2014; Kurth et al. 2020).

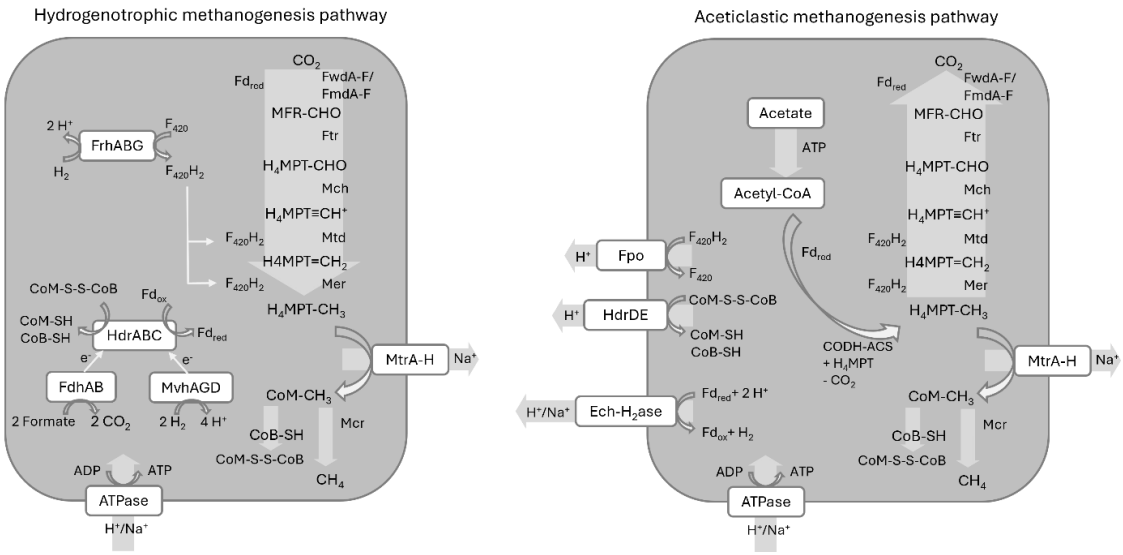

Figure 3. *Hydrogenotrophic and aceticlastic methanogenesis pathways adapted from the paper of Kurth et al. (2020). Hydrogenotrophic pathway uses hydrogen to reduce carbon dioxide to methane. Aceticlastic pathway uses methyl carbon of acetate to reduce to methane.*

1.5. Biogas down-stream processes

Biogas has a complex composition, which requires removal of impurities, such as N_2, O_2, H_2, H_2S and NH_3. Unremoved, these can cause problems in materials by corrosion, toxicity or reduction of heating value. (Yang et al. 2014.) Typically biogas is 50 % of methane and 40 % of carbon dioxide. (Sun et al. 2015; Yang et al. 2014.)

There are several methods on how methane is extracted from the other gases such as carbon dioxide and hydrogen sulphide in biogas. Most commonly used technologies for biogas cleaning and upgrading are water scrubbing and chemical scrubbing. Other methods to mention are pressure swing adsorption (PSA), membrane separation, cryogenic separation process, hydrogenation process and biological technologies. (Adnan et al. 2019; Angelidaki et al. 2018; Sun et al. 2015) Most of all available methods extract at least 95 volumetric percentages of methane from biogas (Sun et al. 2015). It is important to clean methane, because it could cause an explosive hazard with too much oxygen or oxidation to carbon dioxide (Lau et al. 2011; Rasi et al. 2007).

Water scrubbing is based on the solubility of carbon dioxide and methane, of which carbon dioxide solubilises more to water than methane. Compressed biogas is inserted to an absorption rain column, where carbon dioxide solubilises to the water and methane

floats up with 97 % purity (Adnan et al. 2019) and 90 % efficiency (Sun et al. 2015). (Angelidaki et al. 2018.) Chemical scrubbing is based on the reactivity of carbon dioxide and methane, of which carbon dioxide is more reactive than methane, for example sodium hydroxide bubbling (Anan et al. 2019).

These processes are simple and give high methane purity with minimal loss, but also require resources; physical method requires lots of water and energy, chemical process requires energy and chemicals (Anan et al. 2019. However, all available processes give high efficiency rates from 85 up to 98 volumetric percentages, which makes the upgrading highly profitable (Sun et al. 2015).

1.6. Anaerobic digestion to produce biomethane in literature

Methane can be produced in "solid fermentation" or "wet fermentation" with total solids of under 15 w-% (Kusch et al. 2011). "Wet fermentations" of methane in small scale can be conducted in capped bottles in temperature-controlled incubator. The production of methane must be anaerobic, so the used flask's air is displaced with nitrogen for a certain time depending on the volume of air in the flask (Guo et al. 2021).

Additives in methane production are anything what increases the production of methane. They can be biological such as plants, plant residues, manure or microbial cultures, or additives can be inorganic such as metal cations, heavy metals or iron or nickel salts. (Kusch et al. 2011.) Thermochemical pre-treatment includes high temperature treatment. pH alterations or other chemicals can be added. Ultrasonic pretreatment can be difficult to use in high volumetric cultivations. (Yadvika et al. 2004) Steam pressure disruption fractions down fibers and releases soluble organic components after steaming it in high temperatures and depressurizing (Liu et al. 2002).

Methane production in oat husk digestors reaches 90 % of methane production of oat husks after 49 days and 61 % after 26 days. (Kusch et al., 2011) Therefore, it is more profitable to have longer digestions going in industrial scale methane fermentation. In an experimental laboratory setup shorter time is sufficient, and more valuable due to time consumption and possible extrapolation from early-stage methane production (Møller et al. 2004).

The methane from anaerobic digestion is mainly from cellulose and hemicellulose. Lignin has to be enzymatically digested for the microbes to have access to holocellulose in order to produce methane. (Tong et al. 1990.) To avoid resistance for the methane producing reactions, often pre-treatments are added to the process. Some pre-treatment technologies are known such as additive, thermochemical and ultrasonic pre-treatment as well as stream pressure disruption. (Liu et al. 2002; Mosier 2005; Yadvika et al. 2004.)

Hydrolysis, acidic fermentation and methanogenesis occur in the digestion process. In hydrolysis, polysaccharides, proteins and lipids are hydrolyzed with enzymes, and products are mainly simple sugars, amino acid, glycerol and fatty acids. In acidic fermentation, volatile fatty acids produced in hydrolysis are converted to alcohols, lactate, formate, carbon dioxide and hydrogen. Methanogenesis is the stage where in anaerobic fermentation the methanogen microbes produce methane. (Czatzkowska et al. 2020)

During fermentation, some inhibitors are produced. Most severe ones are ammonia, sulfides, ions of light metals, heavy metals and organic compounds. Ammonia is produced during digestion of proteins or urea, that contain large amounts of nitrogen. Sulfides are reduced from sulfates, and in methanogenesis its inhibitory effect is by adding iron salt to the solution that precipitates the sulfides. Ions of light metals have inhibitory effect as their concentration is too high, and cause dehydration to the cells, lower the activity and inhibit methane production. Heavy metals accumulate to the microbes and start to inhibit methane production as they bind to protein groups of the cells. However, as oats are food grade, it is expected to contain minimally any heavy metals. (Czatzkowska et al. 2020.) Organic compounds include antibiotics, ethylene, acetylene, aliphatic compounds and phenols. Inoculum's mineral composition is also determined in this study.

As soluble sugars are produced in hydrolysis, furan and lignin derivatives are produced as byproducts. Main derivatives are furfural from furan derivatives and phenols and polyphenols from lignin derivatives. Furfural, phenols and polyphenols have been shown to slow the methane production but in certain circumstances produced more methane than without. (Barakat et al. 2012.)

Another inhibitory compound is lignin. Lignin inhibits methane production by slowing down the digestion process, as they bind to polysaccharides blocking their digestion.

(Barakat et al. 2012.) It is quite difficult to prevent lignin's inhibitory properties, but some pretreatments can lower the concentration of lignin, for example enzymatically.

These kinds of KKJP and KKJ-H-perm fermentations utilized in methane production has not been largely studied, so specific expectations lack trust. However, it is expected that the permeate functions the same or even better in the fermentation than raw oat husks, as the biomass is already in small pieces. The acidity of the permeate may be an obstacle to tackle, as it was often considered as too acidic for methane production in organisms.

Biomethane production in other studies from biomasses has been around 35-60% of produced biogas in timespan of 25-60 days (Wu et al. 2010; Dai et al. 2020b). This study has different raw material, so similar results are expected, but it is possible to undercut or exceed the expectations.

1.7. Research layout

The KKJP and KKJ-H-perm samples are characterized with chemical analysis by determining the main carbohydrate content (cellulose, starch and hemicelluloses) by fractioning the polysaccharides via hydrolysis and methanolysis. After fractionation the results are measured with gas chromatography (GC). Determining the placement of gas chromatography measurements, the molecules in sample can be solved. It is to be notified that hydrolysis may occur in anaerobic digestion, which affects the carbohydrate content (Demirel et al. 2018; Soleimani and Tabil 2014). However, it is outside the scope of this study and will be treated as having no effect.

Lignin concentrations of KKJP can be determined with Klason lignin methodology. Fractioned insoluble dietary fiber is added to cold sulfuric acid (H_2SO_4), stirred and incubated. After 2 hours the mixture is boiled for 2 hours and washed after filtration. Lignin will contain ash in this point of studies, which must be corrected after ash analysis. (Bunzel et al. 2011.) KKJ-H-perm is expected not to contain any lignin, so further analysis of lignin is not conducted.

Extractives can be determined gravimetrically by diluting sample to acetone and measuring it with GC. To measure ash concentration of KKJP and KKJ-H-perm, the samples must be dried to under 15 % of moisture to be burned in 525 °C (Schmitz et al.

2020). The ash content, inorganic residue, can be then analyzed via Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES). The chemical analyses mentioned in this chapter should cover over 90 % of the sample consistency. Extractives concentration determines how much fermentable sugars are in the substrate (Demirel et al. 2018).

The industrial methane production inoculum used in the study is unknown. Methane fermentation is suggested to be conducted at near atmospheric pressure (Lecharlier et al. 2022). Temperature in the fermentation is approximately 35 °C, as it provides more stable temperature control than thermophilic fermentation (Hilkiah Igoni et al. 2008). The pH has been higher in other studies than in the hemicellulose permeate (pH 4.7), up to pH 7, and some sources claim that sufficient alkalinity must be present in the fermentation. (Ezieke et al. 2022; Hilkiah Igoni et al. 2008.) pH is recommended to adjust with lime or sodium bicarbonate, and lime cannot be added too much because of the precipitation of calcium carbonate (Dai et al. 2020; Hilkiah Igoni et al. 2008). Alkali is popular retreatment method because it lowers lignin's and phenol's inhibitory effects by causing saponification and breaks down lignin-carbohydrate linkages (Van Der Pol et al. 2014). One possible additive is ash and char along with other co-digestion substrates. However, it is uncertain whether the ash brings other benefits except the buffering. (Ezieke et al. 2022.)

1.8. Research objectives

This study is conducted, because there are plenty of material available and loading landfills. In an industrial scale methane production from oat husks could help in the disposing process and create value to otherwise waste material. End-material from the biogas production plant can be used in fertilization, which also creates value for the process. By using biomaterial and more importantly waste biomass in biomethane production, it creates more sustainable alternative for fossil fuels.

Current studies show that there are not too many papers on the topic, especially in Finland. When looking at agricultural production in Finland, other possible biomasses are limited in the biogas production from side streams, but mainly focus is in grain production due to local agriculture. One objective was to verify current results from the field of study.

However, the uniqueness of this study does give another dimension to the comparing process. First, the uniqueness is caused because of the substrates source, which is a side streams side stream. Second, the material is slightly different from oat husks themselves, which does cause somewhat different results.

It is important to follow research tradition, hence a goal is also to complement the field of research, bring more understanding to the field of study and use comparable research practices. The research objective is to figure out the methane potential of the oat husk powder. Next, this paper discusses materials and methods used in the study.

2. Materials and methods

2.1. Chemical analysis

2.1.1. Ash analysis of oat husk powder (KKJP) and hemicellulose permeate (KKJ-H-perm

Raw materials were first freeze dried to minimum water content. The total dry content (TDC) was then measured with moisture analyzer (METTLER TOLEDO HB43 Halogen). KKJP had the TDC of 97.06 % after one week of freeze drying. Samples of KKJP were then dried further in 105 °C temperature overnight. After cooling, the samples are weighted and burned in ash oven (600 °C, 5 hours). (Schmitz et al. 2020.)

KKJ-H-perm (3 %) had TDC of 94.46 % after two weeks of freeze drying. Dried KKJ-H-perm sample was dried in 105 °C temperature overnight. After cooling, the sample is weighted and burned in ash oven (600 °C, 5 hours). Further inorganics analysis is conducted elsewhere.

2.1.2. Extractives analysis of KKJP

Extractives were measured with ASE extraction technology (DIONEX ACE 200 Accelerated Solvent Extractor) with first extraction being conducted with 99 % acetone and two extractions with ultrafiltered water.

KKJP produced yellow coloured liquid of water and acetone with extractives and extracted dry mass. The extracted dry mass is used in Klason lignin measurements. The KKJP extractives liquid was dried completely with nitrogen oven dryer, and again in vacuum drying oven two times (40 °C, 1 h).

2.1.3. Klason lignin analysis of KKJP

Klason lignin analysis method is variation of TAPPI-Y222 om-02 method (Schoening and Johansson 1965). Extracted dry mass is dried to achieve 93.82 % total dry content. 72 % H_2SO_4 is cooled to 2 °C in ice-water bath and added 15 ml to the extracted dry mass

sample 1 ml at a time. Mixture is warmed to 20 °C and mixed with glass rod every 15 minutes for 2 hours. Mixture of sample and H_2SO_4 is heated to boiling temperature and let simmer for 4 hours on low heat. Mixture is left to cool overnight with a lid on.

Weighted funnels are placed on aspiration bottles and mixture of sample and H_2SO_4 is filtered twice and rinsed with filtered water. Filtration is continued with filtered water, until the water from funnels is pH 5.5. Funnels are filtered with aspiration bottle until no drops are formed, after which the funnels are placed to 105 °C overnight.

2.1.4. Hydrolysis of KKJP and KKJ-H-perm

Three samples of each freeze-dried raw material are analysed in hydrolysis. Calibration paper's (cotton linter) DC is measured to be 96.22 %. DC of KKJP is 97.06 % and KKJ-H-perm is 94.46 %.

200 µl of 72 % H_2SO_4 is added to each sample with a small glass ball. Samples are placed into vacuum oven in 40 °C for 5 mins. Samples are then placed for 2 h in ultrasonic bath, after which 500 µl of ultra-pure water is added. Samples are placed in ultrasonic bath for 4 h, and after incubation 6 ml of ultra-pure water is added.

Samples are autoclaved for 1,5 h in 115 °C and approximately 1.25 bars. Barium carbonate ($BaCO_3$) is added to samples until pH 5.4 is reached with visual measurement using indication colour (bromocresol green). 1 ml of standard liquid (5 mg/ml xylitol) is added to each sample. Samples are centrifuged 1100 rpm for 10 min and first phase is collected. Phase samples are dried completely in nitrogen dryer oven (40 °C) and after drying 200 µl of pyridine, 200 µl of HMDS (1,1,1,3,3,3-Hexamethyldisilazane) and 100 µl of TMCS (Chlorotrimethylsilane) are added. GC (PerkinElmer Gas Chromatograph Clarus® 690) is used to analyse the samples.

2.1.5. Methanolysis of KKJP and KKJ-H-perm

Two samples of each freeze-dried raw material are analysed in methanolysis. 1 ml of methanolysis calibration solution (rhamnose, arabinose, xylose, mannose, galactose, glucose, galacturonic acid, glucuronic acid) is added to each sample and evaporated to

dry. 2 ml of 2 M methanolic hydrochloric acid (HCl) are added to 4 calibration samples and each sample followed by vortex mixing. KKJ-H-perm samples are incubated in oven for 3 h and KKJP samples for 5 hours. 200 µl of pyridine is added to each sample. 1 ml of standard solution (resorcinol 0.1 mg/ml methanol) is added to calibration and KKJ-H-perm samples and 4 ml to KKJP samples. 1 ml of first phase is collected and evaporated in nitrogen gas oven at 50 °C. After drying 200 µl of pyridine, 200 µl of HMDS and 100 µl of TMCS are added. GC is used to analyse the samples.

2.2. Anaerobic digestion of KKJP

Setup is visualized in the figure 4. In this study the methane is extracted from carbon dioxide by using 5 M NaOH bubbling. Fermentation bottles are placed in incubator (35 °C, 80 rpm) and tubes are lead from fermentation bottles to lye solutions (5 M). From each lye solution the gas follows to methane collection canister, where methane replaces water. From the collection canister, another tube is lead to excess water tank, where the replaced water flows into. This means that produced biogas' carbon dioxide is collected to lye and methane is collected in the first canister.

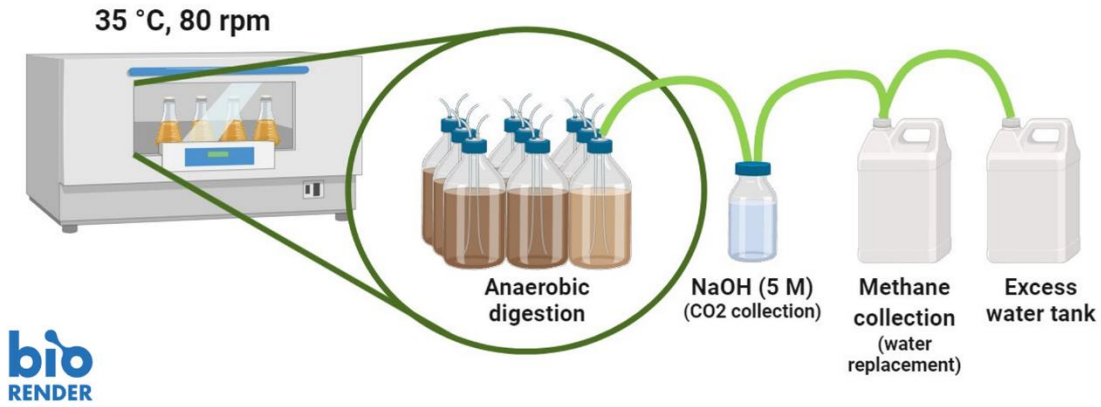

Figure 4. *Visualisation of anaerobic digestion process. Fermentation bottles are placed in incubator (35 °C, 80 rpm) and tubes are lead from fermentation bottles to lye solutions. From each lye solution the gas follows to methane collection canister, where methane replaces water. From the collection canister, another tube is lead to excess water tank, where the replaced water flows into. Illustrated with © 2024 BioRender.*

2.2.1. Raw material properties and adjusting

Fermentation includes three control digestions with only the inoculum (K1, K2, K3), three cellulose control digestions with inoculum and microcrystalline cellulose powder (Sigma-Aldrich®) (S1, S2, S3) and three sample digestions with inoculum and sample (1, 2, 3). First fermentation is conducted with KKJP, second with double feed of KKJP. Due to the novelty value of this study, no additives are added to see the methane production at natural state.

The industrial inoculum has 5.68 % of TS and VS of 5.66 %, leading to 99.65 % VS/TS. As the sample produces around 350 ml of CH_4 / g VS and 210 ml of CO_2 / g VS, to fill the gas volume in fermentation setup, the amount of sample is 62.56 g. The ratio of feed to inoculum is recommended 0.5. Total mass of inoculum needed is:

$$m_{inoculum} = 3.68/0.058/0.68 = 65\ g\ . \quad (1.)$$

As the density of the inoculum is 1 g/ml, the volume of inoculum in one fermentation is 65 ml. Expected inoculum methane production is 1290 ml and CO_2 production 770 ml, total of 2060 ml of biogas. In methane collection canister maximum production capacity would be 1290 ml.

In 65 g of inoculum there are 3.679 g of VS, of which the substrate VS must be half of the mass: 1.840 g. Mass of KKJP for wanted VS is 2.031 g. KKJP is expected to produce 644 ml of methane and 386 ml of CO_2, total of 1030 ml of gas. As the pH of KKJP in water is around 4, it was determined experimentally that 10 ml of 8 % sodium bicarbonate ($NaHCO_3$) / 11.4 g of KKJP sample adjusts the pH to around 7.2. In methane collection canister maximum production would be 1930 ml.

Microcrystalline cellulose is calculated similarly, and mass of cellulose is 1.950 g in digestion. In total methane is produced similarly as KKJP: 644 ml of methane and 386 ml of CO_2, total of 1030 ml of gas. In methane collection canister maximum production capacity would be 1930 ml.

In the second fermentation KKJP mass is doubled to 4.062 g and microcrystalline cellulose 3.900 g. This results to KKJP expectations of 1287 ml of methane and 773 ml of CO_2 and in total 4120 ml of gas produced; similarly to microcrystalline cellulose methane production is estimated to be 1288 ml and CO_2 773 ml.

2.2.2. Fermentation properties

Biomethane production in other studies from biomasses has been around 35-60 % of produced biogas in a time span of 25-60 days (Dai et al. 2020; Kusch et al. 2011; Wu et al. 2010). This study has different raw material, so similar result is expected, but it is possible to undercut or exceed the expectations.

Fermentation bottles are in a shaker-incubator (35 °C, rpm 80, 30 days), and pressure inside the bottle is produced by inoculum biogas production. Methane collection canisters are weighed every other day during 30-day digestion.

3. Results

3.1. Chemical analysis results

From the total DC of 98.8 % chemical analysis results were able to determine 92.4 % of which the content is as follows: ash content 6.5 %; Klason lignin content 20.0 %; cellulose content 26.8 %; (gravimetrical) extractives content 4.7 %; starch content 10.0 %; and hemicellulose content 24.3 %. Unknown content is 6.4 %. Visualization of the content ratios is represented in figure 5.

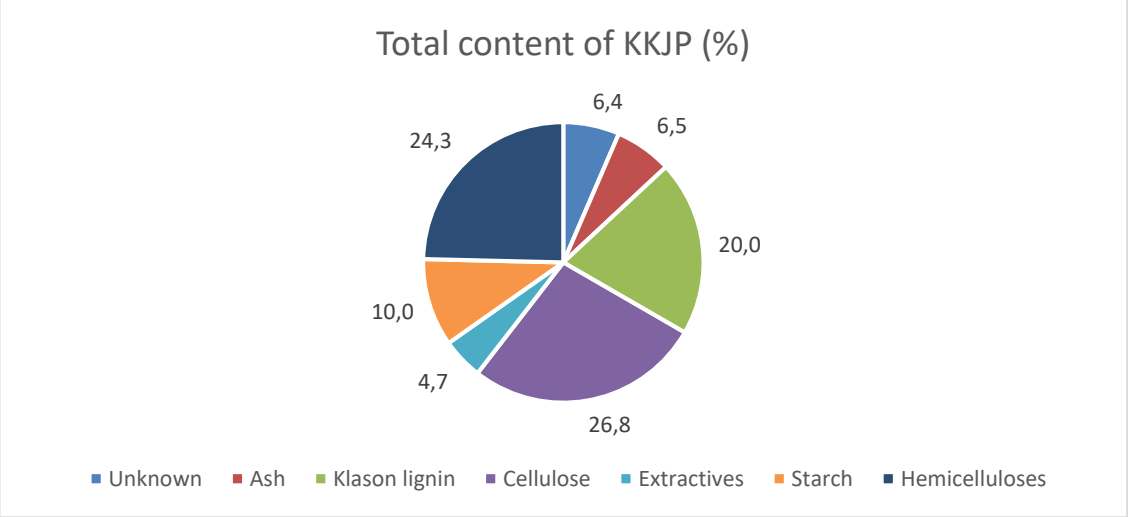

Figure 5. *Total content of KKJP of chemical analysis from DC of 98.8 %. Ash content is 6.5 %; Klason lignin content is 20.0 %; cellulose content is 26.8 %; (gravimetrical) extractives content is 4.7 %; starch content is 10.0 %; and hemicellulose content is 24.3 %.*

3.1.1. Ash analysis results

Ash and dry content are needed for the volatile solids' estimation, as well as gathering information about the consistency of raw materials. It is also needed for the methane potential estimation, as the ratios are determined by the volatile solids content. DC and ash content are seen in table 2. Ash ratio is reliable as the KKJP itself could be described as light and voluminous.

Table 2. *Ash analysis results from samples of oat husk powder 1 and 2 (KKJP-1 and KKJP-2) and oat husk hemicellulose permeate (KKJ-H-perm (dried)). Concentrated KKJ-H-perm is calculated from dried sample's results. Measured DC is measured dry content with equipment and dry content is calculated with results.*

Sample	m (g)	Measured DC (%)	Dry mass (g)	DC (%) *	Ash mass (g)	Ash content (%) *
KKJP-1	3.2484	97.06	3.2089	98.78	25.4133	6.4524
KKJP-2	3.2543	97.06	3.2144	98.77	27.5192	6.4499
KKJ-H-perm (dried)	3.0008	94.46	2.5325	84.39	26.1273	45.67
KKJ-H-perm (concentrated 2.62 %)				2.62		1.07

* Computational result

The mineral composition determines suitability of raw materials as a substrate. Mineral composition is seen in figure 6. In inoculum there are concentrations of calcium (55 090 mg/kg), iron (89 590 mg/kg), and lower concentrations of aluminium (19 760 mg/kg), potassium (27 270 mg/kg), magnesium (11 480 mg/kg), sodium (31 290 mg/kg), phosphorus (19 280 mg/kg) and sulphur (6 378 mg/kg). KKJP has concentrations of minerals in potassium (95 070 mg/kg) and phosphorus (49 520 mg/kg), and lower concentrations in calcium (24 160 mg/kg), magnesium (21 080 mg/kg) and sodium (4 751 mg/kg). KKJ-H-perm has extremely high concentrations of sodium (359 700 mg/kg) in mineral concentrations, and low concentrations of calcium (4 478 mg/kg), potassium (10 700 mg/kg), magnesium (2 863 mg/kg) and phosphorus (3 007 mg/kg).

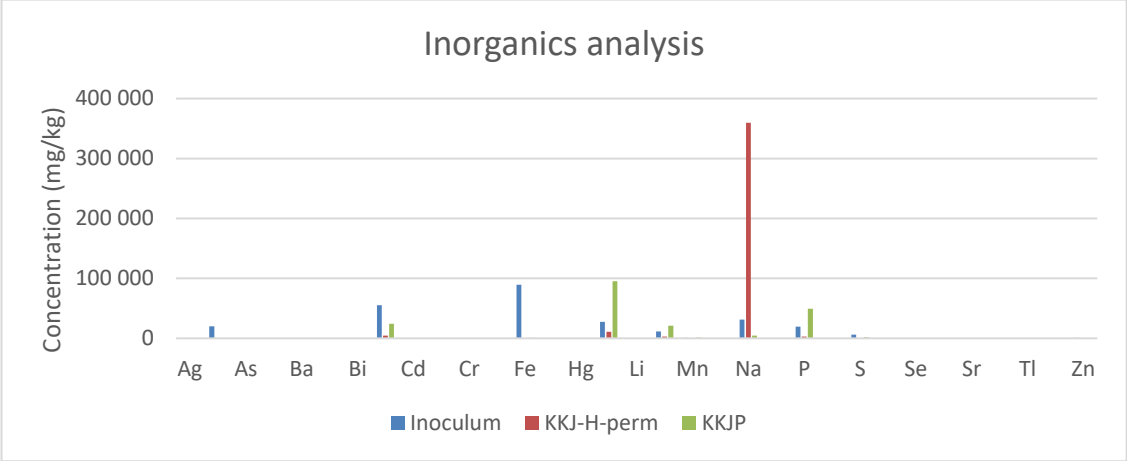

Figure 6. *Inorganics analysis, element concentrations in the ash samples. Noticeable peaks of inoculum are aluminium, calcium and iron. Peaks in calcium, potassium and phosphorus in KKJP are detectable. KKJ-H-perm results are extremely high in sodium.*

3.1.2. Extractives analysis results

Microbial suitability of the substrate can be estimated by the extractives, as it contains important proteins and other necessary material for the viability of the cells. Extracting was possible for only KKJP samples, as the dried KKJ-H-perm was expected to jam the equipment due to the total solids content. Gravimetrical results of extractives in KKJP are seen in table 3. KKJP contained on average 4.7 % of extractives.

Table 3. *Extractives analysis' quantitative results from samples of oat husk powder 1 and 2 (KKJP-1 and KKJP-2) and oat husk hemicellulose permeate (KKJ-H-perm).*

Sample	mg /10 ml	mg/g	Extractives (%)	Average extractives (%)
KKJP-1	40.5	46.74033	4.674033	4.708655474
KKJP-2	41.4	47.77900	4.777900	
KKJP-3	40.5	46.74033	4.674033	

3.1.3. Klason lignin analysis results

Klason lignin analysis was conducted to determine the possible inhibitory effects of lignin to methane production. Depending on if the substrate has a lot of lignin, it would be expected to inhibit the methane production more. Klason lignin concentration in KKJP is 20.0 %. KKJ-H-perm is considered to contain little to no lignin.

3.1.4. Hydrolysis and methanolysis results

Hydrolysis and methanolysis give information about the cellulose and hemicellulose composition of the raw materials. Methanogens use both cellulose and hemicellulose as substrate and metabolism. From the cellulose and hemicellulose the methanogens can digest further to glucose and other easily utilizable form.

Hydrolysis results show average concentrations of three parallel samples in figure 7. KKJP has highest concentrations of hydrolysis glucose (368.6 mg/g) and xylose (184.0 mg/g); lower concentrations of arabinose (35.7 mg/g), mannose (11.2 mg/g), galactose (6.6 mg/g), methanolysis glucose (100.3 mg/g), glucuronic acid (1.8 mg/g), galacturonic acid (3.3 mg/g). Rhamnose or 4-O-methyl glucuronic acid were not detected in either of the samples.

KKJ-H-perm has all results under 40 mg/g, highest of being arabinose (31.51 mg/g) and low concentrations of mannose (1.59 mg/g) and galactose (3.24 mg/g). Glucose nor rhamnose were detected.

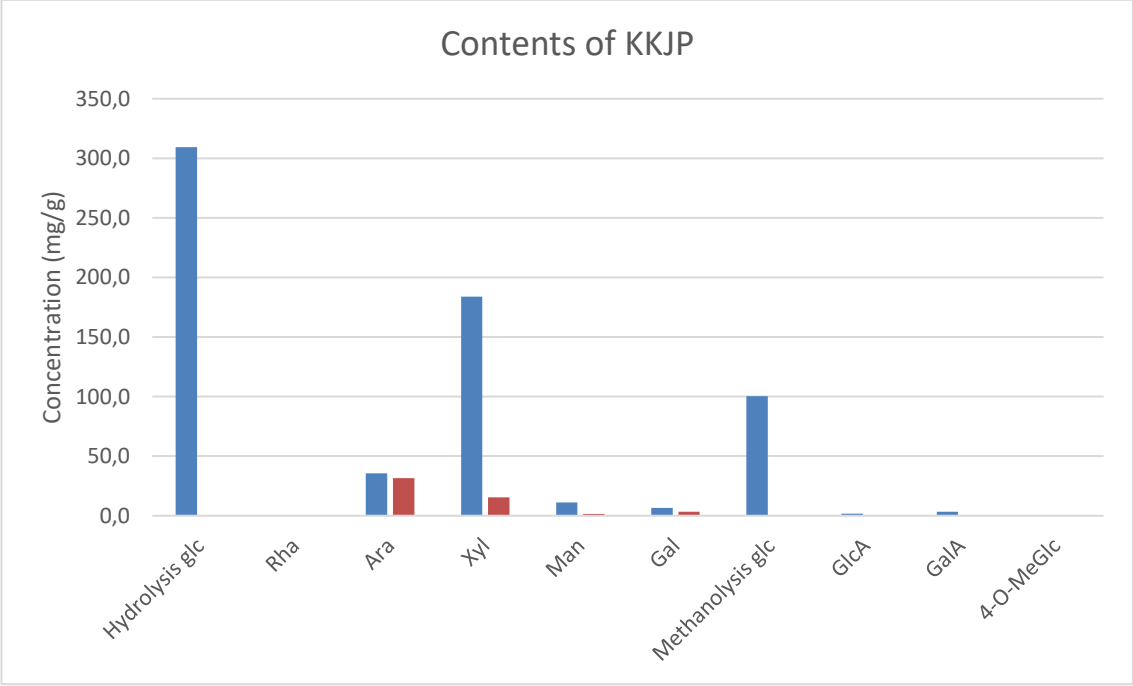

Figure 7. *Collected contents of KKJP and KKJ-H-perm from hydrolysis and methanolysis. KKJP has highest concentrations of glucose (368.56 mg/g) and xylose (184.01 mg/g). KKJ-H-perm has all results under 40 mg/g, highest of being arabinose (31.51 mg/g). KKJP is coloured blue in the figure and KKJ-H-perm is coloured red.*

3.1.5. Substrate pH

pH had to be checked before fermentation due to the acidic nature of KKJP and KKJ-H-perm. pH of KKJP and KKJ-H-perm before fermentation are seen in table 4 and 5. KKJP mixed in water shows low pH of around 4, and KKJ-H-perm has pH around 5.3 unrelated to being concentrated or not. KKJ-H-perm has low pH naturally because of the acetic acid attached to hemicellulose.

Table 4. *pH measurements of substrate: KKJP mixed with water in concentrations 0.05 g/g water, 0.10 g/g water and 0.15 g/g water.*

KKJP	pH
0.05 g / g water	4.28
0.10 g / g water	3.84
0.15 g / g water	3.89

Table 5. *pH measurements of substrate: KKJ-H-perm in different concentrations: raw material (DC 2.77 %), concentrate (DC 3.03 %), crystallized concentrate (DC >5.3 %) and dilution (DC 1.39 %).*

KKJ-H-perm	pH
raw material (2.77 %)	5.25
concentrate (3.03 %)	5.26
crystallized concentrate (>5.3 %)	5.50
dilution (1.39 %)	5.33

3.2. Fermentation results

3.2.1. First fermentation results

The first fermentation did not show expected results as seen in figure 8. Results were altered by removing measurement errors that greatly deviated from the production curve. Microcrystalline cellulose control (S2) shows gas production of 300 ml of methane in 30-day digestion in a similar cumulative curve than in paper of Kusch et al. (2011). With small production the digestion 1 showed 50 ml of methane production, but it is not certain whether or not this was due to water lapping in the beginning or measurement error. Measurements under 50 ml of methane production are considered invalid.

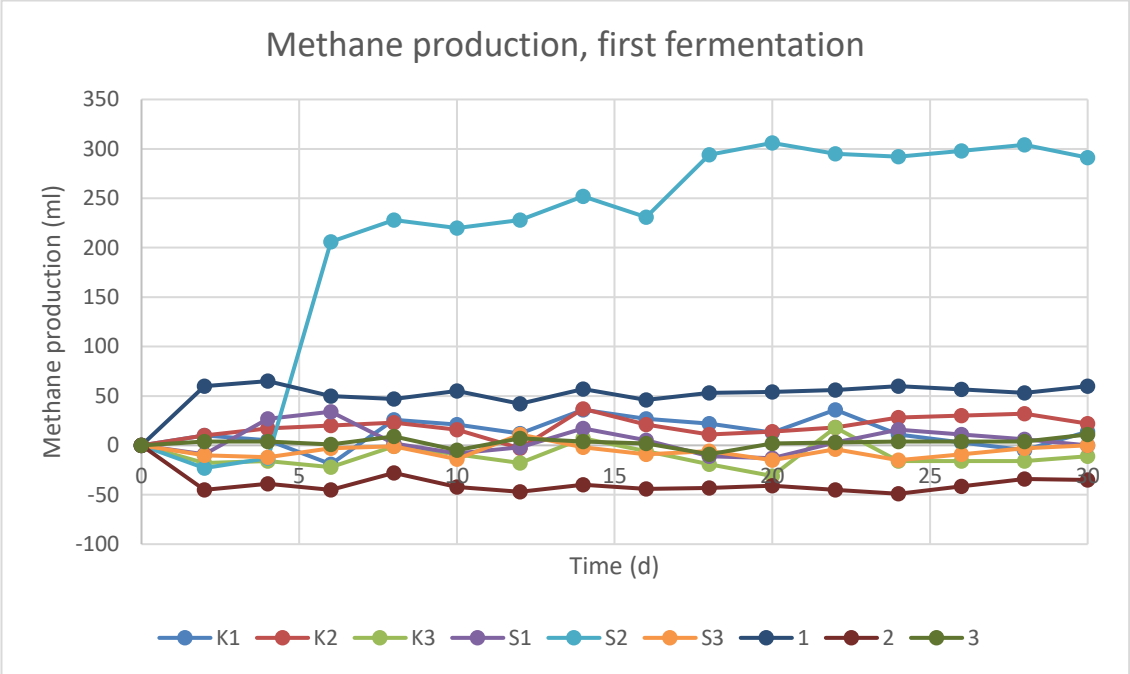

Figure 8. *Methane production of the first fermentation. Results were altered by removing measurement errors from the dataset. Detected production is seen in the digestion S2 with production of around 300 ml of methane, which falls short of expectations of closer to 1930 ml.*

Possible reasons for this were listed, and most probable reasons were considered to be leakage of the downstream process for gas or unviable inoculum. Gas leaking was tested with leak detection spray and gas pressure detector. Canisters, bottles and fermentation bottles all could hold higher air pressure than in the fermentation will be formed. Therefore, the fermentation is made again with similar setup and double concentration of raw material, if the inoculum was somehow damaged. Possible reason for inoculum functioning incorrectly is expected to be temperature control in transportation or contamination.

3.2.2. Double feed fermentation results

The double feed fermentation showed similar results to the first fermentation shown in figure 9. Results were altered by removing measurement errors that greatly deviated from the production curve. Methane production in double feed fermentation is detected in digestions S3 and 3. Possible production is seen with digestion S2 in the day 28, but it

could be due to setup lapping, as the production is the most aggressive in the beginning of the fermentation. However, either of producing digestions did not produce expected volume of methane, that was predicted to produce maximally around 1930 ml instead of 200 ml of methane gas.

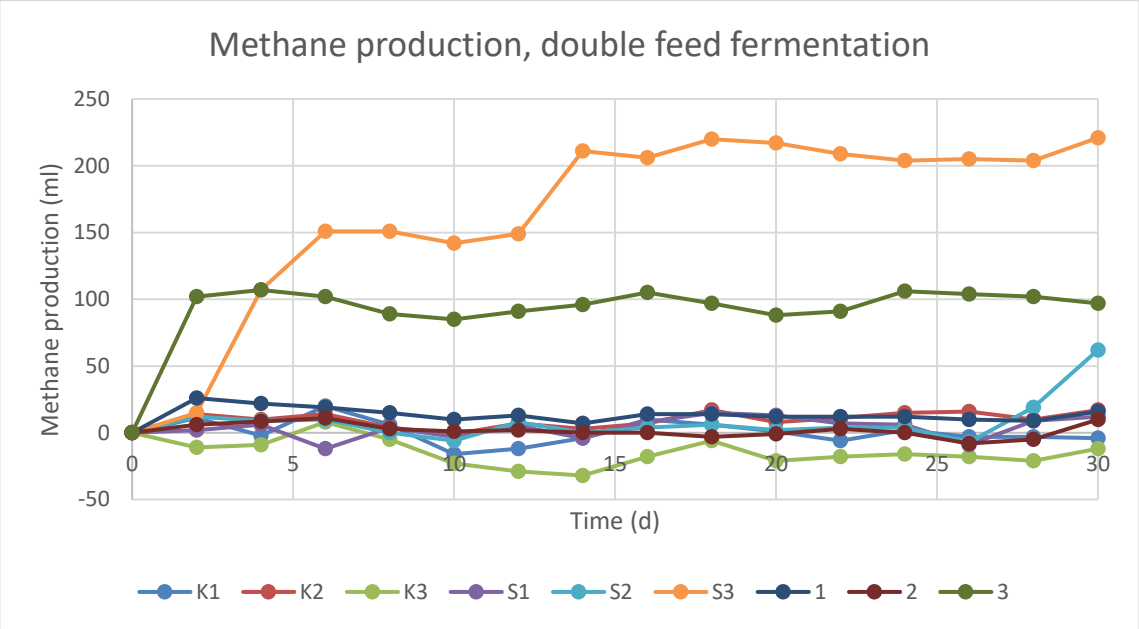

Figure 9. *Methane production of double feed fermentation. Results were altered by removing measurement errors from the dataset. Detectable methane production is shown in digestions S3 by 210 ml of methane and 3 by 100 ml of methane in 30 days, both of which fall short of expectations of closer to 1930 ml.*

Contamination is visible in digestion bottles: lighter colour in K1 and S1; possible mold growth in S2, S3, 2 and 3. It is unknown whether it has affected the methane production or not. Contamination could have altered pH in the digestion. End of the fermentation pH are shown in the table 6.

Table 6. *End pH of double feed digestion. Marked with asterisk of possible mold (*) and other (**) contamination.*

Digestion bottle	K1	K2	K3	S1	S2	S3	1	2	3
Start pH	8,0			-			8,0		
End pH	8,4*	8,3	8,3	8,8*	7,8**	7,9**	8,2	8,2**	8,2

3.2.3. Computational methane potential

Maximum methane potential can be estimated with an equation of biochemical methane potential (BMP) (BPC Instruments 2024):

$$\mathrm{BMP} = \frac{\text{total methane produced}_{\text{substrate}} - (\text{factored by inoculum ratio})\text{total methane produced}_{\text{blank}}}{\text{amount of substrate}} \quad (2.)$$

Unfortunately, without scientifically valid results the equation does not give reliable results to further analyse and discuss.

Percentual methane production can be estimated with BMP (Domingues et al. 2015):

$$CH_4\ \% = \frac{\mathrm{BMP} \times 100}{\text{Theoretical } CH_4 \text{ potential}} \quad (3.)$$

Cumulative methane production is also predictable with an equation (Da Silva et al. 2018)

$$B(t) = B_0(1 - e^{-kt}), \quad (4.)$$

where B(t) is methane potential with time variable (ml CH_4/g_{VS}), t is the time variable (d), B_0 is the maximal methane production (ml CH_4/g_{VS}), and k is the kinetic parameter (d^{-1}). This equation could be used to estimate reversed maximum potential by cumulative production, but unfortunately due to scientifically invalid results, there are no reliable estimates possible for the equation.

4. Discussion

4.1. Insights of ash composition

According to the ash composition shown in figure 6, it is considered that KKJP is suitable for digestion process with higher concentrations in calcium (24 160 mg/kg), potassium (49 520 mg/kg), magnesium (21 080 mg/kg) and phosphorus (49 520 mg/kg). However, in the paper of Schmitz et al. (2020) the mineral results vary between weather conditions. KKJP has higher concentrations of minerals especially in calcium, potassium, magnesium, sodium and phosphorus than in the paper of Schmitz et al. (2020). KKJP can be considered as a good alternative in digestion as small inputs, but higher input volumes have risk to cause ash related problems in processes. Differences in mineral composition are exhibited in figure 10.

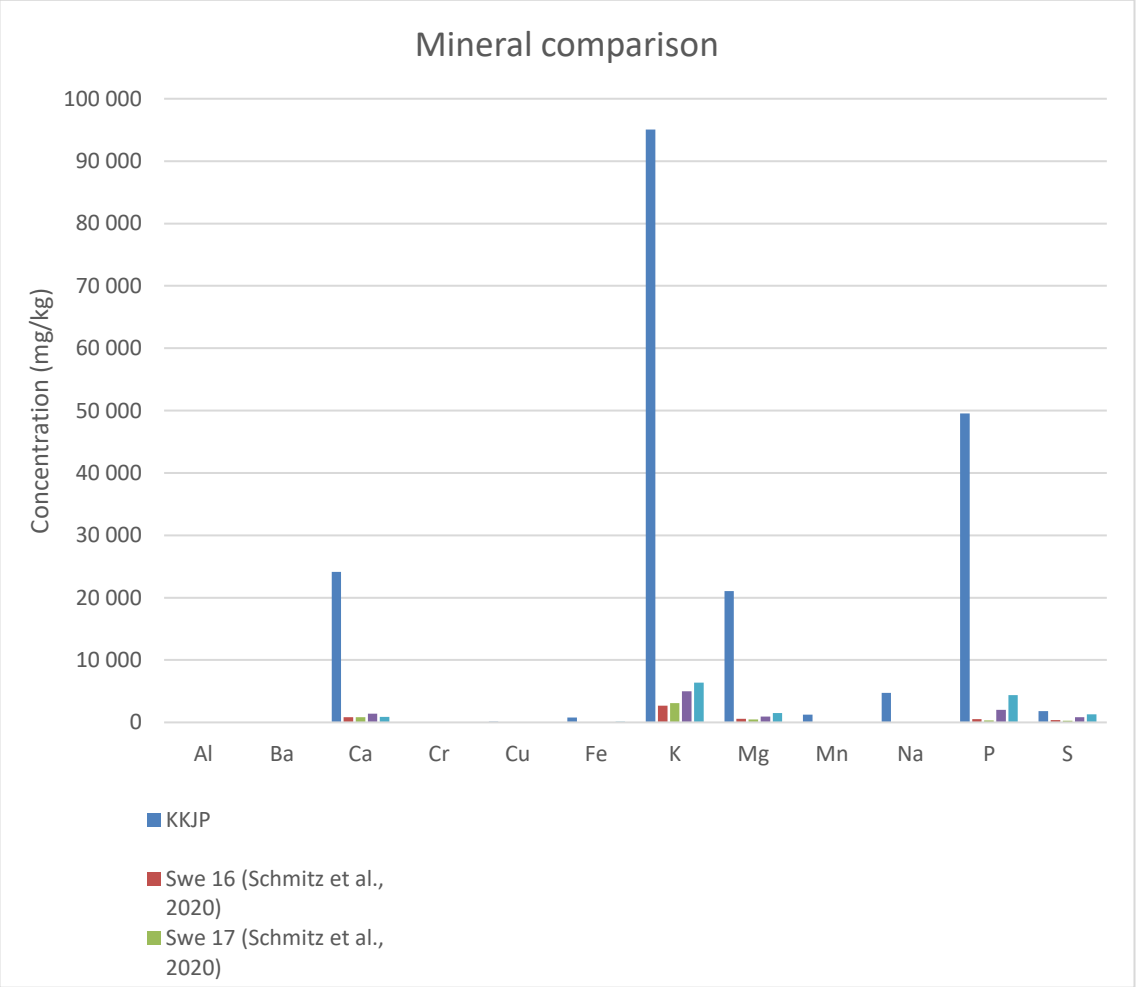

Figure 10. *Mineral comparison with results of paper Schmitz et al. (2020). Comparison is focused on the high concentrations of calcium, potassium, magnesium, sodium and phosphorus.*

KKJ-H-perm shows extremely high concentrations of sodium (359 700 mg/kg) compared to any other samples. This is possibly a cause of filtration process in the collection phase. As material it is considered harmful to digestion process and in other applications such as fertilizers. In a water strider test 90 % of the water striders died in concentration of 6 % of KKJ-H-perm, which indicates that it is toxic to be released in nature and water treatment plants do not accept it undiluted. Further investigation is considered, and according to these results, process pH alteration must be changed from $NaHCO_3$ to potassium bicarbonate ($KHCO_3$). Further digestion properties are not planned, but burning the matter to fertilizer is the next goal.

The mineral analysis revealed large quantities of sodium in KKJ-H-perm. This gave the company more insight into the process, and it is also interested in changing the processes base chemicals, as the sodium causes more question marks as the functionality is considered in any possible direction. Too much sodium is hard to put anywhere, as it is harmful to nature and difficult to process further.

4.2. Possible methane production inhibitors in digestion

During fermentation, some inhibitors are produced. Most severe ones are ammonia, sulphides, ions of light metals, heavy metals and some organic compounds. Ammonia is produced during digestion of proteins or urea, that contain large amounts of nitrogen. Sulphides are reduced from sulphates, and in methanogenesis its inhibitory effect is by adding iron salt to the solution that precipitates the sulphides. Ions of light metals have inhibitory effect as their concentration is too high, and cause dehydration to the cells, lower the activity and inhibit methane production. Heavy metals accumulate to the microbes and start to inhibit methane production as they bind to protein groups of the cells. However, as the oat is food grade, it is expected to contain minimally any heavy metals as seen in mineral analysis. (Czatzkowska et al. 2020.) Organic compounds include antibiotics, ethylene, acetylene, aliphatic compounds and phenols.

As the soluble sugars are produced in hydrolysis, furan and lignin derivatives are produced as byproducts. Main derivatives are furfural from furan derivatives and phenols and polyphenols from lignin derivatives. Furfural, phenols and polyphenols have shown

to slow the methane production but in certain circumstances produced more methane than without. (Barakat et al. 2012.)

According to Knudsen (1997) the concentration of Klason lignin is 14.8 % in oat husks. Klason lignin results of KKJP were 17.3 % and 20.1 %, which indicates that there is 3-5 % more lignin in KKJP than expected. Lignin inhibits methane production by slowing down the digestion process, as they bind to polysaccharides blocking their digestion. (Barakat et al. 2012.) It is quite difficult to prevent lignin's inhibitory properties, but some pretreatments can lower the concentration of lignin, for example enzymatically. It has been researched that with lignin the lignocellulose acts as a physical barrier in biomethane production, and shows mild inhibitory effects on methane production. (Piątek et al. 2021.)

4.3. Challenges with KKJ-H-perm

Similar material to KKJ-H-perm is hard if not impossible to find in literature. The company's process is unique and as a pilot plant its methods may vary from industrial scale processes. This study gives a lot of information about the KKJ-H-perm chemical composition, as no comprehensive experimentation was performed in the company before this study.

4.4. Chemical composition compared to literature

Comparing water acetone and water extractive percentages to the paper of Neitzel et al. (2023), 4.7 % of extractives is low compared to 14 % in the study extracted with only cold water. It should be considered, if the result is low due to pretreatment or as material its amount differs.

Hydrolysis results are valuated in a table 7 with papers of Schmitz et al. (2020) and Knudsen (1997). As the table shows, the results vary greatly when compared to each other. Glucose amount is high in KKJP when compared to Knudsen (1997) results, which indicates that the potential for digestion process is high in KKJP.

Table 7. *Hemicellulose results compared with other studies (Knudsen 1997; Schmitz et al. 2020).*

Hemicellulose / experiment result (%)	KKJP	KKJ-H-perm	Schmitz et al. (2020)	Knudsen (1997)
Glucose	36.9	0	-	2.0
Xylose	18.4	1.5	24.0	0
Arabinose	3.6	3.2	3.4	2.8
Mannose	1.1	0.2	0.1	0.1
Galactose	0.7	0.3	1.3	0.9
Rhamnose	0.0	0	-	-

4.5. Similar setups and microbial diversity

In the study of Dominigues et al. (2015) similar setup was conducted. Triplicate samples with volumes of 100 ml flasks, 20 ml anaerobic sludge, 20 ml cow manure suspension and 5 ml of tested matter, in this case fat suspension. (Domingues et al. 2015.) The experiment in the study of Domingues et al. (2015) was successful, so it could be considered to use smaller flasks, as this study used 500 ml flasks with relatively large airspace.

Microbial activity was not researched in this study. In literature it has been reported that the microbial diversity and anaerobic digestion depending on the total solid percentage (Li et al. 2015). As the microbial diversity or ratios between methanogen species might be relevant in this study, it was also mentioned that the results are more dependent on the substrate added to the anaerobic digestion (Ning et al. 2019). It is certain that the inoculum received might have cooled down during the transportation, which results in unexpected ratios of microbes as well as possible death of some species altogether.

Considering that main source of energy for the inoculum is glucose, KKJP is considerable candidate for digestion, whereas KKJ-H-perm has no glucose and therefore is not considered as suitable for digestion. It is considered that polymeric xylans should be used in hydrolysis method to have more accurate calibration, but these kinds of compounds are not available.

Microcrystalline cellulose control produced highest volume of gas in both fermentations. The gas volume is expected to be the same in KKJP and cellulose control. Therefore, conclusions can be drawn that KKJP is not as optimally digestible as cellulose, which is expected and the purpose of control substrate. Similar results of KKJP production could be achieved with longer digestion periods. However, both digestions stopped producing remarkable amounts of gas after day 20, so further test digestions could be shortened from 30 days to 20 days.

5. Limitations and future research

This chapter discusses problems of the practical work and reasons for unsuccessful results. The results of this study show that chemical analysis results were accurate and generalized, but digestion results lack trust. The reason why digestion results are unreliable is the low number of successful digestions. To make any scientific results viable, there must be at least three successful samples in each material. This shows that even the inoculum itself showed minimum production of methane, when there should have been some after production from the biogas production plant. Cellulose control samples showed some results, but not even nearly all of them showed consistent results of methane production, not to mention the fact that the amount of methane produced was very low from expected volumes. When control samples are unreliable, it is hard if not impossible to draw conclusions about the results of the actual research target sample.

If this experiment was to be retaken, some things would be worth reconsidering. The setup is highly experimental, which worked in theory and in some samples but in practice is not reproducible or did not show viable results. As the setup was not a readily available and researched and optimized digestor and gas sampling, it caused questions for the setup's properties and function instead of the main objective of the study – methane production.

5.1. Contamination

One major problem in the research was hygiene, as there were no sterile spaces available. This might be the biggest factor when it comes to contamination, as most of the samples showed some unknown cultivation on the surface or discoloration that indicates contamination. Also, the equipment used, pipettes, gloves, autoclave and bottles were not handled in sterile space, which might have affected the sample cleanliness. Also, the material used in the digestion were not hygienisized which might have caused the contamination as well as it was handled in the polymerization process hall and touched with possible contaminants before coming to the laboratory, not to mention air contact causing possible mold contamination. Contamination of the fermentation bottles could be

minimized by putting the raw materials used in the digestion to 70 °C for 1 h to kill some bacteria or mold before digestion process.

Another precaution would be a contamination plate test from each cultivation, so possible contaminations would be seen better and possibly recognized. This would give more information about contamination sources, and it could be avoided or minimized.

5.2. Gas collection

There were no original objectives to research gas leaking or gas diffusion through plastic equipment, which was left as an open question. The materials used for the experiment should be more planned and optimized, rather than using equipment at hand. The most interesting materials used were the plastic tubes between setup bottles, lye bottles, gas collection canisters and the different glues used for the sealing. It was made sure that the bottles are safe to be used; lye bottles were plastic that does not melt etc., and canisters were at least in theory non-diffusible of methane. However, there was no way of proving gas leaking or diffusing, as the methane is invisible and unscented gas. It was also unknown whether the plastic in any of the setup process would react with the gases in the system. In general, the results would be more accurate and usable if the digestion and gas collection was conducted with appropriate equipment.

As mentioned, the gas sampling was not optimized for pressurized system with constant low gas flow. There are questions, if the water system is operating as purposed and thus some other gas sampling options should be considered in the future studies. For example, before the experimentation gas sampling bags were considered to be more optimal and accurate version of gas sampling, because there are no forces affecting the gas more than the weight of the gas sampling bag itself. There is some leaking detected, as the valve fitting materials and adsorption of the bag walls, accelerating as the concentration reaches higher values of 1 ppm or over (Lecharlier et al. 2022). It would also have proper vents and structures for further analysis of the gas, which was impossible with the current setup. It was unknown whether the gas in gas sampling canister was methane in the first place, and if then in what purity and concentration. Gas sampling bags were not considered an option because of the financial limitations.

5.3. Methane production discussion

When looking at the results and the fact that only few of the samples could be read as "successful" results, thoughts of the number of samples is forced to come up. If there were even double the number of samples, it could be seen differently and with more viable results than now. The problem here is the size of the incubator. The incubator used in this study could only fit 12 bottles, of which now was used 9. If the number of bottles could be for example double or triple, more samples could have successfully produced some methane.

This study carried out two different concentration experiments with KKJP, with no specific results or differences between the concentrations. As the number of samples was scientifically sufficient per control and sample, more concentrations could have been added to the digestion. It is still unknown whether the amount of KKJP is enough to produce biomethane industrially, or should its concentration be more or less to produce more efficiently. Also, it should be assessed before offering to biogas production plants that does the material handle constant processes and how would the material affect the outcome. Inoculum is often used as nutrients on fields, so KKJP's effect on inoculum's spreadability and fiber leftover should be first studied before using it in practice.

5.4. Chemical analysis credibility

Trustworthy experiments in this project are nearly all chemical analyses, as they are well known and used daily in the current laboratory; ash analysis, lignin analysis, methanolysis and hydrolysis. Mineral composition was analyzed elsewhere in another company, but the results are considered to be valid.

Hydrolysis and methanolysis did not give valid answers on the first time of the analyses, which may indicate that the analysis is somewhat at flaw. It was altered by changing sorbitol to xylitol as the company was already improving their analysis, when the results came clear. However, in hydrolysis there is no calibration in polymeric xylans, but such

compounds were not found of all sugars, or they were too expensive for this purpose. Results of conducted measurements are simplified in table 8.

Table 8. *Summary of results of conducted chemical measurements. Results are shown with dried KKJ-H-perm, because the hydrolysis and methanolysis were also conducted with dry samples.*

		KKJP	KKJ-H-perm (dried)
DC (%)		98.8	94.46
Ash (%)		6.5	38.54
Klason lignin (%)		20.0	not measured
Cellulose (%)		26.8	not measured
Extractives (%)		4.7	not measured
Starch (%)		10.0	not measured
Hemicelluloses (%)		24.3	not measured
	Xylose (mg/g)	184.0	undetected
	Arabinose (mg/g)	35.7	31.51
	Mannose (mg/g)	11.2	1.59
	Galactose (mg/g)	6.6	3.24
	Glucuronic acid (mg/g)	1.8	undetected
	Galacturonic acid (mg/g)	3.3	undetected
	Rhamnose (mg/g)	undetected	undetected
	4-O-methyl glucuronic acid (mg/g)	undetected	undetected
Glucose, hydrolysis (mg/g)		368.6	undetected
Glucose, methanolysis (mg/g)		100.3	undetected

5.5. Topic recommendations of further research

Further studies of the field of study could include for example contamination of industrial biogas production plants, sterile working environments in spaces without sterile infrastructure, KKJ-H-perm properties and possible applications, gas sampling bag usage and optimization in experimental setups, downstream processing of methane in experimental setups, as well as research on KKJP and its further chemical composition.

6. Conclusions

This study determined KKJP's and KKJ-H-perm's composition chemically, properties and KKJP's behavior in anaerobic digestion with industrial inoculum. The goal was to determine the methane potential of KKJP as a substrate in anaerobic digestion.

Methane production is conducted using anaerobic digestion with industrial inoculum and KKJP as a substrate. Detected production of digestion control S2 was approximately 300 ml of methane, and in double digestion methane production of digestion control S3 was 210 ml of methane and sample 3 by 100 ml of methane in 30 days. Compared to maximum potential of 1930 ml digestions did not exceed predicted methane production.

The biogas collected to gas collection canister does not exceed any expectations, which means that this digestion process requires repetition with different concentrations of inoculum, feed and sodium bicarbonate in this setup or changing of the setup altogether. The inoculum only should have produced double the volume of biomethane with realistic expectations of 1200 ml, where biomethane production was not visible at all. Current setup and resources do not make sterile working possible, which also would require some rethinking when it comes to contamination.

As majority of digestions had not produced biogas in visible volumes. Problems with the water's ability to withstand pressure are thought to be the main cause of this accident. The biogas volumes were low even after doubling the feed, which could show that the setup itself is not suitable for pressure sensitive digestion. Biogas as any other gas cumulates pressure well, which could mean that the gas pressure remained in the digestion-bottle-to-lye-bottle air gap. The biogas could be collected with a gas sampling bag, as they are relatively inexpensive and easy to use as no further training is required.

As a substrate, KKJP holds potential for biogas production according to literature and other studies as well as the chemical analysis. Thus, it should be researched more widely and with successful setup.

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References

Adnan, Ong, Nomanbhay, Chew, & Show (2019) Technologies for biogas upgrading to biomethane: A review. *Bioengineering* **6**:92.

Alekhina, M., Mikkonen, K. S., Alén, R., Tenkanen, M. & Sixta, H. (2014) Carboxymethylation of alkali extracted xylan for preparation of bio-based packaging films. *Carbohydrate Polymers* **100**:89–96.

Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H. & Kougias, P. G. (2018) Biogas upgrading and utilization: current status and perspectives. *Biotechnology Advances* **36**:452–466.

Barakat, A., Monlau, F., Steyer, J.-P. & Carrere, H. (2012) Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. *Bioresource Technology* **104**:90–99.

Bouxin, F., Marinkovic, S., Bras, J. L. & Estrine, B. (2010) Direct conversion of xylan into alkyl pentosides. *Carbohydrate Research* **345**:2469–2473.

BPC Instruments (2024). AMPTS III biochemical methane (BMP) / biogas potential handbook, version 1.0, pp. 26–27.

Buerstmayr, H., Krenn, N., Stephan, U., Grausgruber, H. & Zechner, E. (2007) Agronomic performance and quality of oat (Avena sativa L.) genotypes of worldwide origin produced under Central European growing conditions. *Field Crops Research* **101**:343–351.

CH-Bioforce Oy. Home. <https://www.ch-bioforce.com/>. (Accessed 11.12.2023).

CH-Bioforce Oy. Cellulose. <https://www.ch-bioforce.com/cellulose/>. (Accessed 11.12.2023).

CH-Bioforce Oy. Hemicellulose. <https://www.ch-bioforce.com/hemicellulose/>. (Accessed 11.12.2023).

CH-Bioforce Oy. Lignin. <https://www.ch-bioforce.com/lignin/>. (Accessed 11.12.2023).

Chanakya, H. N., Srikumar, K. G., Anand, V., Modak, J. & Jagadish, K. S. (1999) Fermentation properties of agro-residues, leaf biomass and urban market garbage in a solid phase biogas fermenter. *Biomass and Bioenergy* **16**:417–429.

Chopda, R., Ferreira, J. A. & Taherzadeh, M. J. (2020) Biorefining oat husks into high-quality lignin and enzymatically digestible cellulose with acid-catalyzed ethanol organosolv pretreatment. *Processes* **8**:435.

Costa, K. C. & Leigh, J. A. (2014) Metabolic versatility in methanogens. *Current Opinion in Biotechnology* **29**:70–75.

Czatzkowska, M., Harnisz, M., Korzeniewska, E. & Koniuszewska, I. (2020) Inhibitors of the methane fermentation process with particular emphasis on the microbiological aspect: a review. *Energy Science & Engineering* **8**:1880–1897.

Da Silva, C., Astals, S., Peces, M., Campos, J. L. & Guerrero, L. (2018) Biochemical methane potential (BMP) tests: reducing test time by early parameter estimation. *Waste Management* **71**:19–24.

Dai, X., Hua, Y., Liu, R., Chen, S., Li, H., Dai, L. & Cai, C. (2020) Biomethane production by typical straw anaerobic digestion: deep insights of material compositions and surface properties. *Bioresource Technology* **313**:123643.

Demirbas, A. (2006) Biogas potential of manure and straw mixtures. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* **28**:71–78.

Demirel, F., Germec, M., Coban, H. B. & Turhan, I. (2018) Optimization of dilute acid pretreatment of barley husk and oat husk and determination of their chemical composition. *Cellulose* **25**:6377–6393.

Deutschmann, R. & Dekker, R. F. H. (2012) From plant biomass to bio-based chemicals: latest developments in xylan research. *Biotechnology Advances* **30**:1627–1640.

Domingues, R. F., Sanches, T., Silva, G. S., Bueno, B. E., Ribeiro, R., Kamimura, E. S., … Tommaso, G. (2015) Effect of enzymatic pretreatment on the anaerobic digestion of milk fat for biogas production. *Food Research International* **73**:26–30.

Edlund, U., Ryberg, Y. Z. & Albertsson, A.-C. (2010) Barrier films from renewable forestry waste. *Biomacromolecules* **11**:2532–2538.

Ezieke, A. H., Serrano, A., Clarke, W. & Villa-Gomez, D. K. (2022) Bottom ash from smouldered digestate and coconut coir as an alkalinity supplement for the anaerobic digestion of fruit waste. *Chemosphere* **296**:134049.

Guo, H., Zhang, M., Chen, Z., Shen, Y., Lv, J., Xu, X. & Yu, H. (2021) The metabolic process of methane production by combined fermentation of coal and corn straw. *Bioresource Technology* **337**:125437.

Hansen, N. M. L. & Plackett, D. (2008) Sustainable films and coatings from hemicelluloses: a review. *Biomacromolecules* **9**:1493–1505.

Hilkiah Igoni, A., Ayotamuno, M. J., Eze, C. L., Ogaji, S. O. T. & Probert, S. D. (2008) Designs of anaerobic digesters for producing biogas from municipal solid-waste. *Applied Energy* **85**:430–438.

Karmanov, A., Kocheva, L., Borisenkov, M. & Belyi, V. (2023) Macromolecular hydrodynamics and fractal structures of the lignins of fir wood and oat husks. *Polymers* **15**:3624.

Knudsen, K. E. B. (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology* **67**:319–338.

Kurth, J. M., Op Den Camp, H. J. M. & Welte, C. U. (2020) Several ways one goal—Methanogenesis from unconventional substrates. *Appl Microbiol Biotechnol* **104**:6839–6854.

Kusch, S., Schumacher, B., Oechsner, H. & Schäfer, W. (2011) Methane yield of oat husks. *Biomass and Bioenergy* **35**:2627–2633.

Kärkönen, A. & Koutaniemi, S. (2010) Lignin biosynthesis studies in plant tissue cultures. *JIPB* **52**:176–185.

Laine, C., Harlin, A., Hartman, J., Hyvärinen, S., Kammiovirta, K., Krogerus, B., … Vähä-Nissi, M. (2013) Hydroxyalkylated xylans – their synthesis and application in coatings for packaging and paper. *Industrial Crops and Products* **44**:692–704.

Lau, C. S., Tsolakis, A. & Wyszynski, M. L. (2011) Biogas upgrade to syn-gas (H2–CO) via dry and oxidative reforming. *International Journal of Hydrogen Energy* **36**:397–404.

Lecharlier, A., Carrier, H. & Le Hécho, I. (2022) Characterization of biogas and biomethane trace compounds: a critical review of advances in in situ sampling and preconcentration techniques. *Analytica Chimica Acta* **1229**:340174.

Lehtomäki, A., Huttunen, S. & Rintala, J. A. (2007) Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: effect of crop to manure ratio. *Resources, Conservation and Recycling* **51**:591–609.

Li, D., Liu, S., Mi, L., Li, Z., Yuan, Y., Yan, Z. & Liu, X. (2015) Effects of feedstock ratio and organic loading rate on the anaerobic mesophilic co-digestion of rice straw and cow manure. *Bioresource Technology* **189**:319–326.

Liu, H. W., Walter, H. K., Vogt, G. M., Vogt, H. S. & Holbein, B. E. (2002) Steam pressure disruption of municipal solid waste enhances anaerobic digestion kinetics and biogas yield. *Biotech & Bioengineering* **77**:121–130.

Luonnonvarakeskus. Satoarvio 2022, tilanne 19.9.. <https://www.luke.fi/fi/tilastot/satotilasto/satoarvio-2022-tilanne-199>. (Accessed 5.12.2023).

Mikkonen, K. S., Laine, C., Kontro, I., Talja, R. A., Serimaa, R. & Tenkanen, M. (2015) Combination of internal and external plasticization of hydroxypropylated birch xylan tailors the properties of sustainable barrier films. *European Polymer Journal* **66**:307–318.

Mosier, N. (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* **96**:673–686.

Møller, H. B., Sommer, S. G. & Ahring, B. K. (2004) Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy* **26**:485–495.

Ning, J., Zhou, M., Pan, X., Li, C., Lv, N., Wang, T., … Zhu, G. (2019) Simultaneous biogas and biogas slurry production from co-digestion of pig manure and corn straw: performance optimization and microbial community shift. *Bioresource Technology* **282**:37–47.

Oliveira, J. P. D., Bruni, G. P., Lima, K. O., Halal, S. L. M. E., Rosa, G. S. D., Dias, A. R. G. & Zavareze, E. D. R. (2017) Cellulose fibers extracted from rice and oat husks and their application in hydrogel. *Food Chemistry* **221**:153–160.

Petersson, A., Thomsen, M., Hauggaardnielsen, H. & Thomsen, A. (2007) Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. *Biomass and Bioenergy* **31**:812–819.

Petzold-Welcke, K., Schwikal, K., Daus, S. & Heinze, T. (2014) Xylan derivatives and their application potential – mini-review of own results. *Carbohydrate Polymers* **100**:80–88.

Piątek, M., Lisowski, A. & Dąbrowska, M. (2021) The effects of solid lignin on the anaerobic digestion of microcrystalline cellulose and application of smoothing splines for extended data analysis of its inhibitory effects. *Bioresource Technology* **320**:124262.

Pohjanlehto, H., Setälä, H., Kammiovirta, K. & Harlin, A. (2011) The use of N,N′-diallylaldardiamides as cross-linkers in xylan derivatives-based hydrogels. *Carbohydrate Research* **346**:2736–2745.

Rasi, S., Veijanen, A. & Rintala, J. (2007) Trace compounds of biogas from different biogas production plants. *Energy* **32**:1375–1380.

Redaelli, R. & Berardo, N. (2007) Prediction of fibre components in oat hulls by near infrared reflectance spectroscopy. *J Sci Food Agric* **87**:580–585.

Reis, A., Pinto, P., Evtuguin, D. V., Neto, C. P., Domingues, P., Ferrer-Correia, A. J. & Domingues, M. R. M. (2005) Electrospray tandem mass spectrometry of underivatised acetylated xylo-oligosaccharides. *Rapid Comm Mass Spectrometry* **19**:3589–3599.

Rosado, M. J., Rencoret, J., Marques, G., Gutiérrez, A. & Del Río, J. C. (2021) Structural characteristics of the guaiacyl-rich lignins from rice (Oryza sativa L.) husks and straw. *Front Plant Sci* **12**:640475.

Schmitz, E., Nordberg Karlsson, E. & Adlercreutz, P. (2020) Warming weather changes the chemical composition of oat hulls. *Plant Biol J* **22**:1086–1091.

Schoening, A. G., & Johansson. (1965) Absorptiometric determination of acid-soluble lignin in semichemical bisulfite pulps and in some woods and plants. *Svensk Papperstid 68* **18**:607.

Shahbandeh, M. (2024) Statista. USDA Foreign agricultural service 2023, oats production worldwide from 2015/2016 to 2022/2023 (in million metric tons)**. <https://www.statista.com/statistics/1073536/production-of-oats-worldwide/> (Accessed 5.12.2023).

Skiba, E. A., Budaeva, V. V., Ovchinnikova, E. V., Gladysheva, E. K., Kashcheyeva, E. I., Pavlov, I. N. & Sakovich, G. V. (2020) A technology for pilot production of bacterial cellulose from oat hulls. *Chemical Engineering Journal* **383**:123128.

Soleimani, M. & Tabil, L. (2014) Evaluation of biocomposite-based supports for immobilized-cell xylitol production compared with a free-cell system. *Biochemical Engineering Journal* **82**:166–173.

Sun, Q., Li, H., Yan, J., Liu, L., Yu, Z. & Yu, X. (2015) Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. *Renewable and Sustainable Energy Reviews* **51**:521–532.

Tong, X., Smith, L. H. & McCarty, P. L. (1990) Methane fermentation of selected lignocellulosic materials. *Biomass* **21**:239–255.

Torres-Castillo, R., Llabrés-Luengo, P. & Mata-Alvarez, J. (1995) Temperature effect on anaerobic digestion of bedding straw in a one phase system at different inoculum concentration. *Agriculture, Ecosystems & Environment* **54**:55–66.

Valdebenito, F., Pereira, M., Ciudad, G., Azocar, L., Briones, R. & Chinga-Carrasco, G. (2017) On the nanofibrillation of corn husks and oat hulls fibres. *Industrial Crops and Products* **95**:528–534.

Van Der Pol, E. C., Bakker, R. R., Baets, P. & Eggink, G. (2014) By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio)chemicals and fuels. *Appl Microbiol Biotechnol* **98**:9579–9593.

Welch, R. W., Hayward, M. V. & Jones, D. I. H. (1983) The composition of oat husk and its variation due to genetic and other factors. *J Sci Food Agric* **34**:417–426.

Wu, X., Yao, W., Zhu, J. & Miller, C. (2010) Biogas and CH4 productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource Technology* **101**:4042–4047.

Yadvika, Santosh, Sreekrishnan, T. R., Kohli, S. & Rana, V. (2004) Enhancement of biogas production from solid substrates using different techniques—a review. *Bioresource Technology* **95**:1–10.

Yang, L., Ge, X., Wan, C., Yu, F. & Li, Y. (2014) Progress and perspectives in converting biogas to transportation fuels. *Renewable and Sustainable Energy Reviews* **40**:1133–1152.

Yu, P., Maenz, D. D., McKinnon, J. J., Racz, V. J. & Christensen, D. A. (2002) Release of ferulic acid from oat hulls by Aspergillus ferulic acid esterase and Trichoderma xylanase. *J Agric Food Chem* **50**:1625–1630.

Yuan, T. & Sun, R. (2010) Cereal straw as a resource for sustainable biomaterials and biofuels, pp. 1–7. Elsevier.

Zhang, R. (1999) Biogasification of rice straw with an anaerobic-phased solids digester system. *Bioresource Technology* **68**:235–245.