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Federal Department of the Environment, Transport,
Energy and Communications DETEC

Swiss Federal Office of Energy SFOE
Energy Research and Cleantech Division

Final report dated 20th of February 2024

MIMAGAS - Combination of physicochemical pre-treatments of biomass to maximise biogas production

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Source: HEIG-VD, 2021

Date: 20.2.2024

Location: Bern

Publisher:
Swiss Federal Office of Energy SFOE
Energy Research and Cleantech
CH-3003 Bern
www.bfe.admin.ch

Co-financing:
HEIG-VD & Agrogaz Lignerolle SA
Grand Vailloud 10, CH-1357 Lignerolle
www.agrogaz.ch

Subsidy recipients:
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SFOE contract number: SI/502202-01

Zusammenfassung

Lignozellulosehaltige Abfälle sind in allen organischen Abfällen in unterschiedlichen Anteilen enthalten und bilden manchmal die Hauptfraktion. Die Widerspenstigkeit von lignozellulosehaltiger Biomasse (LCB) macht sie resistent gegen mikrobielle Hydrolyse, was die Effizienz der biologischen Umwandlung von organischem Material in Biogas verringert. Jüngste Forschungstrends deuten darauf hin, dass es keine einheitliche Strategie zur Verbesserung der anaeroben biologischen Abbaubarkeit von lignozellulosehaltigen Abfällen gibt, sondern dass Vorbehandlungen und Co-Vergärung in diesem Zusammenhang eine wichtige Rolle spielen.

Das MIMAGAS-Forschungsprojekt zielte auf die Entwicklung eines milden Hydrolyse-Vorbehandlungsprotokolls für Kuhmist ab, das eine Kombination aus mechanischer Einwirkung, verdünnter organischer Säure und milder Wärmeeinwirkung darstellt. Für dieses Projekt wurde ein in der Schweiz weit verbreitetes (und manchmal problematisches) Lebensmittelabwasser, die Käsemolke, als Säuremittel verwendet. Dieser innovative Ansatz konnte allen Anforderungen gerecht werden, da er sowohl Vorbehandlung als auch Co-Vergärung kombiniert.

Zunächst wurde eine Sondierungsanalyse auf der Grundlage eines aus der Taguchi-Methode abgeleiteten Versuchsplans durchgeführt, um die günstigste Kombination von Vorbehandlungen zu ermitteln, d. h. diejenige, die den löslichen chemischen Sauerstoffbedarf und den Methanertrag maximiert. Vorbehandlungen unter milden Bedingungen (d.h. Säuregehalt, Temperatur und Druck) sollen die Bildung von toxischen oder hemmenden Verbindungen verringern. Trotz des geringen Schweregrads der in diesem Projekt verwendeten Vorbehandlungen begünstigte die Hydrolyse von Gülle in Gegenwart von Molke, einem Substrat, das reich an leicht hydrolysierbaren Kohlenhydraten ist, wahrscheinlich die Bildung von Sekundärprodukten, die aus parasitären Reaktionen zwischen Zuckern (Karamellisierung) oder zwischen Proteinen und Zuckern (Maillard-Reaktion) resultieren, deren Vorhandensein die Ergebnisse verfälscht und die Schlussfolgerungen dieser Studie beeinflusst haben könnte.

In Versuchen im mittleren Maßstab gefährdeten diese Nebenprodukte, die Hemmstoffe der anaeroben Vergärung sind, ernsthaft die Stabilität des mit hydrolysiertem Substrat beschickten Fermenters, zumal die Versuche in der trockenen Konfiguration durchgeführt wurden, die die Anhäufung von Hemmstoffen begünstigt. Neben der Bildung von Inhibitoren steht die in diesem Projekt entwickelte Kombination von Vorbehandlungen im Verdacht, die Dehydratisierungskapazität der hydrolysierten Substrate zu erhöhen und damit die Auswirkungen des Wassermangels zu verstärken.

Die Umstellung auf ein halbtrockenes Verfahren unter Zugabe von Wasser ermöglichte die Stabilisierung des mit hydrolysierten Substraten beschickten Fermenters, und die wirtschaftliche Analyse zeigte, dass trotz mäßig günstiger Bedingungen erhebliche Gewinne erzielt werden konnten (+16 % bei den jährlichen Einnahmen).

Die in diesem Projekt entwickelte Kombination von Vorbehandlungen könnte daher in flüssiger Form wirksamer sein. Unabhängig von der geplanten Konfiguration erfordert ihre großtechnische Umsetzung die Entwicklung eines Hydrolysereaktors, der ein hohes Maß an Homogenität und Bedingungen (Temperatur/Hydrolysezeit) gewährleisten kann, die den Verlust von Zuckern begrenzen.

Was die mechanische Vorbehandlung betrifft, so existiert der von uns verwendete Fleischwolf bereits in einer Größe und Kapazität, die für die Verarbeitung der Dungmenge aus der Agrogaz-Anlage geeignet ist, doch müssen mehrere technische Fragen geklärt werden, bevor diese Art von Maschine in großem Maßstab für die Vorbehandlung von Dung eingesetzt werden kann. Das Hauptproblem ist der Umgang mit Steinen und Fremdobjekten, die manchmal im Dung enthalten sind und die Messer des Häckslers ernsthaft beschädigen können.

Résumé

Les déchets lignocellulosiques sont présents dans tous les déchets organiques, dans des proportions variables, et constituent parfois la fraction principale. La récalcitrance de la biomasse lignocellulosique (LCB) la rend résistante à l'hydrolyse microbienne, ce qui réduit l'efficacité de la bioconversion de la matière organique en biogaz. Les tendances récentes de la recherche indiquent qu'il n'existe pas de stratégie unique pour améliorer la biodégradabilité anaérobie des déchets lignocellulosiques, mais les prétraitements et la codigestion ont tous deux un rôle important à jouer dans ce domaine.

Le projet de recherche MIMAGAS visait à développer un protocole de prétraitement par hydrolyse douce sur le fumier de vache en combinant une action mécanique, un acide organique dilué et une action thermique douce. Pour ce projet, un effluent alimentaire abondant (et parfois problématique) très répandu en Suisse, le lactosérum de fromage, a été utilisé comme agent acide. Cette approche innovante pourrait répondre à toutes les exigences car elle combine à la fois les prétraitements et la codigestion.

Dans un premier temps, une analyse exploratoire basée sur un plan expérimental issu de la méthode de Taguchi, a permis d’identifier la combinaison de prétraitements la plus favorable c’est-à-dire celle permettant de maximiser la demande chimique en oxygène soluble et le rendement en méthane. Les prétraitements dans des conditions douces (acidité, température et pression) sont censés réduire la formation de composés toxiques ou inhibiteurs. Cependant, malgré la faible sévérité des prétraitements utilisés dans ce projet, l’hydrolyse du fumier en présence de petit lait, un substrat riche en carbohydrates facilement hydrolysables, a vraisemblablement favorisé la formation de produits secondaires, issus de réactions parasites entre sucres (caramélisation) ou entre protéines et sucres (réaction de Maillard), dont la présence a pu biaiser les résultats et influencé les conclusions de cette étude.

Lors des tests à l’échelle intermédiaire, ces sous-produits, inhibiteurs de la digestion anaérobie, ont fortement mis en péril la stabilité du digesteur nourri avec le substrat hydrolysé et ce d’autant plus que les tests ont été réalisés en voie sèche, une configuration qui favorise l’accumulation d’inhibiteurs. Outre la formation de ces inhibiteurs, la combinaison de prétraitements mis au point dans ce projet est suspectée de favoriser la capacité de déshydratation des substrats hydrolysés renforçant ainsi les effets dus au manque d'eau.

Le passage en voie semi-sèche, avec l'ajout d'eau, a permis de stabiliser le digesteur alimenté en substrats hydrolysés et l'analyse économique a montré que malgré des conditions modérément favorables, des gains substantiels pouvaient être réalisés (+16% de revenus annuels).

La combinaison de prétraitements développée dans ce projet pourrait ainsi être plus efficace en configuration liquide et, quelle que soit la configuration envisagée, son implémentation à grande échelle nécessitera de développer un réacteur d’hydrolyse capable d’assurer un haut degré d’homogénéité et des conditions (température/temps d’hydrolyse) limitant la perte en sucres.

En ce qui concerne le prétraitement mécanique, le hachoir à viande que nous avons utilisé existe déjà dans une taille et une capacité appropriée pour traiter la quantité de fumier provenant de l'installation Agrogaz, mais plusieurs questions techniques doivent être abordées avant que ce type de machine puisse être mis en œuvre à grande échelle pour le prétraitement du fumier. Le principal problème est la gestion des roches et des cailloux, parfois présents dans le fumier, qui peuvent sérieusement endommager les couteaux du broyeur.

Summary

Lignocellulosic waste is present in all organic waste, in varying proportions, and sometimes constitutes the main fraction. The recalcitrance of lignocellulosic biomass (LCB) makes it resistant to microbial hydrolysis, which reduces the bioconversion efficiency of organic matter into biogas. Recent trends in research indicate that there is no unique strategy to improve the anaerobic biodegradability of lignocellulosic wastes, but pretreatments and co-digestion both have an important role on this issue.

The MIMAGAS research project aimed at developing a mild hydrolysis pre-treatment protocol on cow manure that by combining mechanical action, diluted organic acid impact and mild thermal action. For this project an abundant food effluent (and sometimes-problematic effluent) that is widespread in Switzerland, the cheese whey, was used as the acid agent. This innovative approach could match all requirements as it combines both pretreatments and co-digestion.

First, an exploratory analysis based on an experimental design derived from Taguchi's method was used to identify the most favorable combination of pretreatments, i.e. the one maximizing soluble chemical oxygen demand and methane yield. Pre-treatments under mild conditions (i.e., acidity, temperature, and pressure) are said to reduce the formation of toxic or inhibiting compounds. However, despite the low severity of the pretreatments used in this project, the hydrolysis of manure in the presence of whey, a substrate rich in easily hydrolyzable carbohydrates, probably favored the formation of secondary products, resulting from parasitic reactions between sugars (caramelization) or between proteins and sugars (Maillard reaction), whose presence may have biased the results and influenced the conclusions of this study.

In intermediate-scale tests, these by-products, which are inhibitors of anaerobic digestion, seriously jeopardized the stability of the digester fed with hydrolyzed substrate, especially as the tests were carried out in the dry configuration, which favors the accumulation of inhibitors. In addition to the formation of inhibitors, the combination of pretreatments developed in this project is suspected of enhancing the dehydration capacity of hydrolyzed substrates, thus reinforcing the effects of water shortage.

The switch to a semi-dry process, with the addition of water, allowed the stabilization of the digester fed with hydrolyzed substrates and economic analysis showed that despite moderately favorable conditions, substantial gains could be achieved (+16% in annual revenues).

The combination of pretreatments developed in this project could therefore be more effective in liquid configuration, and whatever the configuration envisaged, its large-scale implementation will require the development of a hydrolysis reactor capable of ensuring a high degree of homogeneity and conditions (temperature/hydrolysis time) limiting the loss of sugars.

As for mechanical pretreatment, the meat grinder we used already exists in appropriate size and capacity to handle the amount of manure from the Agrogaz plant but several technical issues need to be addressed before this type of machine can be implemented on a large scale for manure pre-treatment. The main being the management of rocks and pebbles, sometimes present in the manure, which can seriously damage the grinder knives.

Main findings

- A mild combined mechanical, chemical et thermal pre-treatment of cow manure enables to increase the methane yield by about 20%;
- Applied to all agricultural anaerobic digesters, this pre-treatment could significantly increase the energy potential of swiss farmyard manures.

Contents

Abbreviations

5-HMF: 5-Hydroxymethylfurfural

AD - Anaerobic digestion

BMP - Biomethane potential

CHP - Combined heat and power

EPS – Extra polymeric substances

HRT – Hydraulic retention time

HSAD – High solid anaerobic digestion

IGT - Institut de Génie Thermique (Institute of Thermal Engineering in English)

LAB - Lactic acid bacteria

LB EPS – Loosely bound extra polymeric substances

LCB – Lignocellulosic biomass

LCFA – Long chain fatty acid

MRP – Maillard reaction products

OFEN – Office Federal de l’Energie (SFOE – Swiss Federal Office of Energy in English)

OLR – Organic loading rate

RSM - Response Surface Methodology

sCOD – soluble Chemical oxygen demand

TB EPS – Tightly bound extra polymeric substances

TS – Total solids

VFA – Volatile fatty acids

VS – Volatile solids

WRC – Water retention capacity

WHC – Water holding capacity

WWTP – Wastewater Treatment Plant

1 Introduction

1.1 Background information and current situation

The world is facing an unprecedented energy and climate crisis. In order to meet the objectives set by the Paris Climate agreement (UN Climate Change, 2015) and to support the Swiss Energy Strategy 2050 (OFEN, 2018), it is important to develop sustainable and carbon-neutral energy routes. Among those, biogas production plays an important role, as it converts organic waste into energy (bio-methane, electricity and heat) and into an organic rich fertilizer. In 2016 in Switzerland, the share of renewable energy reached 20% of the total energy consumption, and only 2.5% of renewable energy originated from biogas production (IEA Bioenergy, 2018). However, this technology is currently expanding quickly within Switzerland, as the biogas production from agricultural and industrial plants is steadily increasing annually by 4% (OFEN, 2020). These data confirm the need for the optimization of the biogas production process to contribute to the overall renewable energy production in Switzerland.

Biogas is produced by anaerobic digestion (AD) of organic matter through a biochemical process of degradation under anaerobic conditions by microbial consortia. It is an eco-friendly process and one of the most efficient methods for the conversion of biomass into methane (Sárvári Horváth et al., 2016). Biomass feedstock and agricultural wastes used in AD process are often manures mixed with corps and residual fibers. Lignocellulosic biomass conversion strongly depends on the accessibility of organic compounds to exo-enzymes produced by anaerobic microorganisms and due to their fibrous structure, important substrate fractions are not converted into biogas: only 50-70% of volatile solids (VS) are commonly converted (Angelidaki et al., 2005).

The conversion of energy from biomass feedstock and agricultural wastes is highly explored in the research field. In fact, residues from agriculture sector are one of the most promising resources for growth in bioenergy production. The impressive number of suggested methods and related publications clearly shows that the research field is very much alive (Galbe & Wallberg, 2019).

Several pre-treatments have been developed during the last decades in order to achieve better AD performances and the research is still ongoing to find the best compromise in efficiency and economic balance (Galbe & Wallberg, 2019; IEA Bioenergy, 2014). Pre-treatment of lignocellulosic biomass is commonly preceded by a mechanical action, a size-reduction step, to increase the accessible surface area for microorganisms and hydrolytic enzymes. High demand of energy for physical pre-treatment reduces economic viability for AD. This is the reason why a combination of pre-treatments is generally developed. A chemical pre-treatment is very often used utilizing strong inorganic acid (such as sulfuric, hydrochloric and nitric acids) (González-Fernández et al., 2008; Lavarack et al., 2002). These acids break down lignin and hemicellulose network and disrupt the crystalline cellulose structure. Although this strong acid pre-treatment is very efficient, it is not suitable for methane production as it is inhibited by the production of H_2S and N_2 from reducing sulphate and nitrate, respectively. Additionally, the use of strong acid would cause serious environmental concerns and the high cost of these chemicals must be considered (Hendriks & Zeeman, 2009).

In contrast, diluted acid pre-treatments and weak organic acids are less toxic, less corrosive, and less hazardous, and do not require corrosion resistant equipment. In addition, the week organic acids pre-treatments have an easier scale-up due to their inexpensive safety management procedures. Weak organic acids such as acetic, propionic, and lactic acids can eliminate the disadvantages of strong acid technologies and can be directly utilized by methanogens as substrate to produce biogas (Zhao et al., 2010). Moreover, these organic acids could be recovered from industrial effluents or production streams, to use by-products, thus reducing the need for new resources by strengthening the circular economy and sustainability. However, it must be noted that the use of organic acids for this process could interfere with the digestion process by causing an acidic stress. In AD, pH is a very important parameter as the methane-forming process is inhibited when the pH is higher than 8 or lower than 6. At lower pH the accumulation of VFAs cause the pH to decline and affect the environment of methanogens (Yuan et al., 2006).

Pre-treatments under mild conditions (i.e., acidity, temperature, and pressure) would reduce the formation of toxic or inhibiting compounds and therefore the energy consumption. The compositional and structural changes of fibrous substrates due to weak organic acid pre-treatments under mild temperature and atmospheric pressure has not been studied much previously, however; research is focusing on it (Peng et al., 2019). In a recent study, the use of acidic wastes from juice factory and sour cherry production combined with mechanical pre-treatment was investigated. It was repotrtfound that acid-containing wastes could replace industrial chemicals with an increase of biogas production (via BMP tests) up to 27% (Vazifehkhoran et al., 2019).

To develop a pre-treatment protocol and improve biogas production, for this project we are considering an abundant food effluent (and sometimes-problematic effluent) that is widespread in Switzerland, the milk whey[1]. Cheese production in Switzerland generates 1’300’000 t of milk whey annually, of which 24% is used for human consumption, 31% is processed into high value fodder and 45% is directly distributed to pigs (Bisig et al., 2015). Part of this by-product could be better valorised by using it as a fermentation medium to produce lactic acid, suitable for enhancing manure degradability, creating a concrete link between cheese production and manure management. With the proposed pre-treatment protocol, 25% of milk whey is expected to be added in proportion to the manure for the manure hydrolysis, and with these quantities, it is reasonable to assume a consequent increase in methane production of 20% (average value derived from literature). It can be assumed that an increase in revenue is also expected even if there is a reduction in digester feed because of the milk whey addition.

For improving the efficiency of the weak organic acid pre-treatment, a mechanical grinding device (such as the one used for other food applications - meat grinder), as well as mild hypothermal treatment derived from heat surplus from combined heat and power (CHP) units that burn biogas in AD plants, with a temperature up to 80 °C will be used. According to an average value derived from literature studies (see section 3.1), an increase of 20% in methane production can be expected where the heat fraction required for heating the hydrolyser should be less than 2% of the entire heat gain of the installation. The electricity fraction required for the hydrolysis pre-treatment unit (including the grinder) should be approximately 6% of the entire electricity production gain for the AD plant. The effective heat consumption will depend on the required hydrolysis temperature but will stay relatively low because of the small hydrolysis volume required.

From preliminary calculations and assumptions, an annual milk whey need between 50-70% of milk whey production can be assumed for a small cheese producer (< 10 employees). In Switzerland, about 80% of the cheese producers are small factories looking for alternative solutions to milk whey destination, while the larger processors (9 industries in Switzerland) can afford more expensive and established solutions. There are currently at least three biogas producers in Switzerland from diary wastewater (Marti & Bisig, 2011): *Emmi AG*, *Lataria Engiadinaisa SA* and the animal feed manufacturer *Gefu Oberle*. Apart from these companies and for most cheese producers, milk whey is a waste product that producers want to get rid of. For most of them, the coupling to a piggery is not deliberated choice but a constraint in order to eliminate the milk whey in an economical way, in accordance with the legislation in force.

In Switzerland, so far, agricultural installations with biogas upgrading technologies account for less than 1% of the total number of digesters. Despite of this, with the hope of seeing an increase in these technologies, a solution for AD without CHP unit could be the combustion of the produced off-gas in appropriate boilers that potentially can produce between 0.1 – 0.35 $\mathrm{kWhth/Nm^3CH_{4\ produced}}$, or simply through the same heating system used for digester heating, since consumption is minimal.

According to the Swiss Overall Energy Statistics 2020 (OFEN, 2020), the amount of heat valorised externally after deduction of self-consumption for agriculture AD plants is about 50 GWh in 2020 (Table

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[1] Also referred as cheese whey in this report

1). For this reason, it is presumable that some of this heat could be used for the pre-treatment process based on the preliminary calculations.

Year	No. of biogas installations	Biogas consumption (GWh)	Heat used (GWh)	Electricity production (GWh)
2015	99	289	22.4	99.8
2016	98	331	22.9	115.8
2017	106	353	23.3	154.5
2018	11	390	47.8	138.5
2019	112	448	57.9	160
2020	119	498	50.4	175.8

Table 1 Biogas: installations, consumption, production (OFEN, Swiss Overall Energy Statistics 2020).

In general, the reactions during pre-treatment take place separately to AD reactions and specifically are as follows:

1. The grinding reduced the size of lignocellulosic biomass, essential to increase the accessible surface area and the porosity of the particles. Moreover, it reduces the crystallinity of the cellulose and improves the efficiency of the next processing step.
2. The diluted acid treatment with fermented whey results in hydrolysing the hemicellulose.

The mild thermal action can increase the enzyme accessibility to break down the chemical links and promote the hydrolytic first step in AD. For this reason, we will act on the first metabolic reactions of AD, as we will increase the amount of available reagent that will be then converted into acetate.

Based on actual data, an AD plant of 2’500 m^3 volume, fed with 20 m^3/day of manure (solid part) will have a pre-treatment sector of about 65m^3 (<3%) for the entire protocol process (whey storage, whey fermentation and manure/fermented whey hydrolysis). In addition, it will be necessary to add 5 m^3/day of fermented whey for the hydrolysis. This can result in a net gain in electricity production of about 500 MWh/year and for thermal production of about 600 MWh/year (derived from the performances of the *Agrogaz Lignerolle SA* plant). Based on the investment cost per cubic meter of the existing AD volume of *Agrogaz* plant, the complementary investment for the pre-treatment installation is estimated at less than 200 kCHF and the additional revenue is estimated to be about 100 kCHF per year.

We are not aware of such pre-treatment application at an industrial level so far leading to the development of this pre-treatment protocol. The new pre-treatment installation is intended for agricultural installations using manure as a digester feed and would be designed to be installed on the site of an existing agro-AD plant to facilitate the process and to better manage the protocol phases. The pre-treatment protocol will be integrated in the AD installations by adding a storage/fermentation tank for the whey and a hydrolysis tank to mix the liquid and the partially grinded manure next to the digester:

From preliminary calculations and assumptions, the total additional volume for the entire pre-treatment protocol corresponds to approximately 3% of the existing anaerobic digester volume. Given the simplicity of the protocol and the small volume of additional installations required, the pre-treatment protocol can be implemented in any agricultural plant, even small ones that have a heating system for their digester.

This project aims to investigate several parameters to improve the AD process and optimize biogas production. Organic acid concentration from fermented milk whey, the size of grinding grids for the mechanical action, the reaction temperature and the reaction time were investigated.

The best combination of parameters will then be applied to 50L anaerobic digesters during a period of 6 months to study any rheological digestate properties. Finally, the economic feasibility and scaling up of the pre-treatment protocol will be evaluated.

1.2 Purpose of the project

This research project aims at developing a mild hydrolysis pre-treatment protocol that can maximize the methane production from lignocellulosic material, perfectly aligned with the actual and increasing needs of renewable energy. Based in the context of OFEN energy research program (Federal Energy Research Masterplan for the period from 2017 to 2020), the increased methane production and/or energy saving for pumping and mixing will contribute to the increase of energy production from agricultural plants. In addition, a circular economy system (of efficient and neutral energy production) that binds together local small cheese producers and farmers with AD reactors will be introduced and developed.

For this project, all the expertise of the research team of '*Bioenergies R&D*' at *HEIG-VD IGT* are involved. The main research topics of the group are energy and biofuel production from wet and dry biomass, agricultural and food waste AD, biogas purification technologies and micro-macro algae utilization for nutrients removal.

As far as concerning the development of novel pre-treatments of lignocellulosic organic wastes, our team works in collaboration with the agricultural AD *Agrogaz Lignerolle SA plant*, on which our calculations are based as we have detailed information of process and parameters. Our collaboration with *Agrogaz* allows us to put research investigations with technical application together, in a continuous exchange of ideas, possible solutions and real matrices on which our studies are performed.

1.3 Objectives

Objective 1: *Exploratory study on the combined action of different types of pre-treatments*

The first objective aims at experimentally studying the effect of the following parameters:

1. The acid concentration from fermented milk whey as a chemical agent
2. The size of the grinding grids for the mechanical action
3. The reaction temperature
4. The reaction times

This target is achieved by measuring the efficiency of the combinations by measuring volatile fatty acids (VFA), soluble chemical oxygen demand (sCOD) and performing biochemical methane potential (BMP) analyses.

Objective 2: *Up-scaled anaerobic digestion (AD) tests of the most promising pre-treatment protocol*

The hydrolysed substrate will be compared to untreated manure by feeding in parallel 2 individual digesters of 70 litres capacity, to evaluate biogas production in a volume 70 times bigger than BMP tests and verify any improvement in the rheological digestate properties. Any possible impact of pH on biogas production will be investigated here, together with potential strategies of pH management and protocol optimization.

This objective will be achieved by 6-months AD tests for process stability assessment.

Objective 3: *Economic feasibility and scale up study*

The results obtained from the experiments will allows us to hypothesize the economic feasibility of the pre-treatment protocol and will give us a basis to evaluate the scale-up process for the transfer of laboratory results to the agricultural plant.

This objective will be achieved with a market study and a scale-up solution for in-field application.

2 Description of facility

The first part of the project mainly focused on the whey milk fermentation to increase the total acidity concentration. Tests were performed in 2L reactors (Fig.1) where different temperatures conditions (38°, 40° and 44 °C) were evaluated.

Figure 1: Set-up of 2L reactors for fermentation of the whey milk

After the fermentation tests, the mechanical action was tested using a grinder for the food industry with three different grids with different holes diameters (Ø 6, 13, 20 mm) (Fig. 2).

Figure 2: Food device to grind manure

The last part of the pre-treatment protocol consists on putting the grinded material at 3 different temperature levels (20°, 40° and 80°C) and for 3 different durations in time (2, 8 and 24 hours).
The hydrolysis reaction takes place in 2-liters closed bottles immersed in temperature-controlled water baths, as shown in Fig. 3.

Figure 3: Hydrolysis reactors

Nine combinations have been evaluated by measuring volatile fatty acids (VFA), soluble chemical oxygen demand (sCOD) and performing biochemical methane potential (BMP) analyses. The best combination of parameters has then been applied to 70-liter anaerobic digester during a period of 4 months to study anaerobic digestion performances and verify any improvement in the rheological digestate properties. For comparison purposes, two individual digesters were set up in parallel, one fed with untreated manure (digester 1, control), and the other one fed with the pre-treated manure (digester 2, test). (Figure 4)

Figure 4: Tandem digesters set-up

3 Procedures and methodology

3.1 WP1: Exploratory study of combination of different types of pre-treatments

This first WP aims at developing a pre-treatment protocol that will combine mechanical action, diluted organic acid impact and mild thermal action. The testing of different parameters and their impact on the efficiency of the biogas production were evaluated.

These tests were carried out at a laboratory scale through these different steps:

1. Fermentation of milk whey to increase lactic acid concentration. Lactic acid naturally present in milk whey is used as chemical agent to attack fibrous material in manure compared to water. The whey fermentation increases the acid concentration to test a possible boost of the treatment.
2. Mixing of manure and liquid agent and its mechanical grinding. The use of an enhanced version of a meat grinder reduces the size of straw material at a low energetic consumption.
3. Mild thermal treatment of the grinded material using a temperature up to 80°C, which corresponds to the level available from the combined heat and power (CHP) plant from our industrial partner.
4. Analyses of VFA, sCOD and BMP to evaluate pre-treatment performance and efficiency.

3.1.1 Design of experiments

Different parameters combinations were tested, through changing the concentration of lactic acid, the size of the grid and the reaction temperature, but always keeping “mild” values, to minimize the whey and energy consumptions. The different levels of each parameter are presented in Table 2.

Factors	level 1	level 2	level 3
Time (hours)	2	8	24
Temperature (°C)	20	40	80
Acid conc. (%)	0	1	5
Grid dimension (mm)	6	13	20

Table 2: Factors and levels used to the experimental design

To have zero concentration of lactic acid, water was used as liquid agent. In cheese whey, the concentration of lactic acid may vary, and consequently different quantities are obtained from fermentation. Since we worked with real and not synthetic substrates, we decided to use unfermented whey for the minimum concentration of lactic acid and fermented whey for the maximum level.

As far as the parameter "grid dimension" is concerned, there is not an exact zero level (which would correspond to no grinding at all). However, the 20mm-grid is quite close to the natural product while allowing a better homogenization of the substrate and in this project, it was assimilated to the zero level.

The experimental tests grid we performed is based on the method of Taguchi L9 (four factors at three levels) which consists of nine runs. Two series of tests were carried out, one in July 2022 (batch 1), the second in August 2022 (batch 2). During the latter, we performed a confirmation run (run 10), to check the quality of our model of DOE. The choice of parameters for test 10 was dictated by the trade-off between protocol simplicity and low energy consumption.

The of performed runs is presented in the following table.

Run	Time	Temperature	Acid concentration	Grid dimension
1	2	20	0	6
2	2	40	whey	13
3	2	80	fermented whey	20
4	8	20	whey	20
5	8	40	fermented whey	6
6	8	80	0	13
7	24	20	fermented whey	13
8	24	40	0	20
9	24	80	whey	6
10 (control)	8	40	whey	20

Table 3 Experimental test runs based on the Taguchi L9 method Experimental runs of second tests batch (with confirmation assay)

Experiments are performed in triplicates in a randomized order to determine the combination offering the best robustness, via an ANOVA[2] analysis. The proposed fractional experimental design was carried out in accordance with ISO/TR 12845:2010 and was analysed according to the Taguchi method (mentioned in the ISO, explained by dedicated statistical works).

Our Taguchi design is based on an orthogonal repertoire, which evaluates the effects of factors on the mean and on response variation. With an orthogonal repertoire, the design is balanced so that factor levels are equally weighted. In this way, each factor can be evaluated independently of all the others, and the effect of one factor does not influence the estimation of another.

3.1.2 VFA measurement via GC

The GC is a MG#5 (for Multiple Gas) model from SRI company (Figure 5). It is equipped with a line for the analysis of carbon compounds in solution (e.g. volatile fatty acids), in addition to the basic configuration for the analysis of non-condensable gases.

The GC consists of injectors, connected to separation columns that are connected to detectors, whose signal (in volts) is collected on a computer with dedicated software (PeakSimple). The whole system is constantly flushed with an inert gas (helium) to protect the columns from oxidation by oxygen. The columns are placed in an oven in which the temperature is programmable in time.

Figure 5: Multiple gas GC

Liquid samples are injected into the split-splitless injector (SSL). After separation with a capillary column (e.g. FFAP), the compounds are detected by an FID detector.
Samples are prepared according to an internal protocol, following centrifugation, dilution, and acidification operations. The VFA concentration is calculated based on a calibration curve obtained by using a standard solution with the main VFAs to be analysed at known concentrations.

3.1.3 sCOD measurement via titration method

The determination of the soluble fraction of COD is performed according to ISO6060. The equipment in Figure 6 allows carrying out a strong digestion of organic matter and measuring the unreacted residue by titration.

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[2] ANOVA *stands for Analysis of Variance*. It is a statistical method used to analyze the differences between the means of two or more groups or treatment.

Figure 6: COD measurement unit

Due to the high COD concentration and strong staining of the samples, a commonly used spectrophotometric measurement could not be used in this case.

3.1.4 BMP tests

The Biomethane Potential experiments were determined through Holliger et al. method (2016) using three automatic volumetric BMP systems (Nautilus, from Anaero Technology, UK – see Figure 7). The inoculum used is provided by the wastewater treatment plant (WWTP) of Yverdon-les-Bains in the form of digestion sludge. The tests were performed at 37°C and cellulose is used as positive control to check the correct functioning of the analysis.

Figure 7: Biomethane potential (BMP) reactors set-up

Methane production was performed for each individual substrate (untreated manure, fermented or not - whey alone) and for all the materials coming from the tested pre-treatment protocols. Three measurements per sample were conducted.

BMP tests can also provide a measure of substrate degradation kinetics, and this might play a role in decreasing the retention time in the digester, providing gain in terms of smaller digester design, or in terms of increased applied organic loads.

3.2 WP2: Testing of pre-treatment protocol at intermediate scale (70 litres)

The main goal of this WP was to test the best pre-treatment protocol that had been established in WP1 in 70 litres- digesters. Two individual digesters were set up in parallel, one fed with untreated manure (digester 1, control), and the other one fed with the pre-treated manure (digester 2, test), and run for 4 months. They were closely monitored (i.e., biogas flowrate, biogas composition, pH, alkalinity, ammonium concentration, VFA) in order to assess digestion performances and stability.

Besides the gain in methane production due to this pre-treatment protocol, the impact on the sludge rheology will be evaluated. When reducing the particle size of lignocellulosic substrate, it is expected that less energy is required for mixing the digester, through improving rheological properties of the sludge. Because of the better rheological properties of the pre-treated substrate, a more efficient substrate mixing is expected, which should lead to a reduction of the energy consumption per unit of methane produced.

3.2.1 Digester Operation

Initially, the digesters were supposed to be set up and operated to match the digestion process of agricultural anaerobic digesters. However, given some technical constraints, a different approach had to be implemented. Indeed, in agricultural installations with an efficient system for livestock urine collection and storage, the latter can be valorized in the digester. In a plant like Agrogaz, this liquid fraction represents up to 70% of the volume of organic matter introduced each day (Table 4). Depending on the database, the methanogenic potential of this liquid fraction may vary, as it is linked to the quantity of nutrients present in the urine, which in turn depends on the performance of the urine collection system and storage conditions. When these operations are carried out properly, the contribution of urine - in the form of slurry - to anaerobic digestion is not negligible. In addition to its contribution to the organic load, this liquid fraction helps to fluidize the digester contents and improve liquid/gas exchanges.

Feedstock	**Quantity**	**TS (%)**	**VS(%)**	**Organic load**	**Total OLR**
Manure	20 t/d	20	82	3,28 t_{VSS}/d	4,3 $kg_{VSS}/m^3/d$
Liquide fraction	45 t/j	10	80	3,6 t_{VSS}/d	

Table 4: Daily feeding of Agrogaz anaerobic digester - composition and organic loading rate

The appropriate management and storage of this liquid fraction being more difficult to achieve in our facilities, we initially chose to work without adding any liquid fraction (end of June to end of August). The absence of urine or any liquid fraction means that the substrate contains a high level of solid particles. This is the principle of **high solid anaerobic digestion (HSAD)**, also known as dry anaerobic digestion which was developed to reduce water usage and increase organic loading rate. Being operated at 10 – 25% solid content, HSAD of livestock manure allows for treatment of higher amounts of waste per volume of digester (smaller digester) and has the advantages low heating energy and less digestate (much simplified post treatment as it reduces nutrient loss in digestate and decreases the need for digestate dewatering) (Li et al., 2017). However, two major limitations existed in HSAD of livestock manure:

1) The methane production rate and methane yields are low compared to wet anaerobic digestion due to lower substrate biodegradability.

2) The lower substrate biodegradability means that the digestate exiting the digester is not only of poorer quality, but also still contains significant quantities of pathogens.

Thus, when using the HSAD configuration, the contribution of the pretreatments developed in this project should prove all the more beneficial.

- The process stability of HSAD can be improved by particle downsizing through mechanical treatment. Indeed, particle size reduction increases the mass transfer and improves the distribution of metabolites by enlarging surface area and facilitating the access of biomass and metabolites to microbes. (Basinas et al. 2021)
- Thermochemical pretreatments have already proved effective to enhance pathogens elimination during HSAD of manure. (Wu et al., 2017)
- Eventually, as HSAD suffers from limited heat and mass transfer due to moisture deficit, using this configuration was intended to favor digester 2 fed with the hydrolyzed manure/whey mixture, because the increased solubilization of the organic matter obtained through pretreatment (grinding/hydrolysis) along with the presence of whey milk was supposed to compensate, at least in part, for the disadvantages associated with the absence of a liquid fraction.

However, we will see in the next section how tricky the HSAD configuration can be to master, and how, to complete the project and finish the tests, we had to switch to a semi-dry configuration with the addition of water at the beginning of September Eventually, we performed some trials at the end of October to test the validity of hypotheses regarding pretreatment effects. (Table 5).

Période	**Configuation**	**Digester 1**			**Digester 2**		
		OLR	Estimated HRT (d)	Water fraction (% m)	OLR	Estimated HRT	Water fraction (% m)
21/06 - 02/07	HSAD	1,4	58-80	0	1,4	49-60	0
03/07 – 11/07		1,8		0	1,8		0
12/07 – 07-08		2,1		0	2,1		0
08/08 - 31/08		2,5		0	2,5		0
04/09 – 11/09	**PAUSE – Switch between configurations**						
11/09 – 01/10	Semi dry	1,5	60	43	1,5	60	33
02/10 – 20/10		1,9	48	33	1,9	48	22
21/10 – 03/11		**Co-digestion tests**					

Table 5: Operational conditions used for WP2 tests

3.2.2 Feeding protocol

With HSAD Configuration

Both digesters are fed once a day with a constant OLR from Monday to Friday, and feeding is interrupted at weekends The organic load and HRT values presented in table 5 are therefore 7- day averages, taking weekend interruptions into account.

With Semi dry Configuration

Both digesters are fed once a day with a constant OLR from Monday to Thursday, then on Friday, the feed dose is double. The quantities of liquid fraction added have been defined based on previous work (Song et al., 2022), and to match hydraulic retention time (HRT) of 49 days which corresponds to that applied at the Agrogaz plant. The organic load and HRT values presented in table 5 are therefore 7-day averages, taking weekend interruptions into account.

3.3 WP3: Economic viability and scaling up study

The results obtained from WP1 and WP2 were used to determine the effective economic gain of the process and evaluate if it is rentable in terms of energy balance. Based on the increase in methane production, the corresponding supplementary income was estimated and put into perspective with the expected investment and operating costs required by the hydrolysis over an AD plant lifetime.

The proposed pre-treatment cannot be carried outside the AD plant and the technology is thought to be decentralized to concentrate the operational actions in one site, the AD installation, where the process can be easily controlled and modulated according to production requirements.

If economically relevant, the up scaling from laboratory to real agricultural conditions will be designed to bring out the challenges and the risks of a subsequent pilot and demonstration (P&D) project.

4 Results and discussion

4.1 WP1: Pre-treatments characterization

4.1.1 Whey fermentation and acidity production

The part of the project related to whey fermentation is described in detail in Appendices 2.

In brief, we tested four different milk whey from four cheese producers. We reached organic acid conversion of around 75% up to 90% for one cheese factory.

We found that the 3 different temperatures tested (38°, 40° and 44 °C) on the different types of whey did not have a significant impact on the production of lactic acid for most of the samples. The purpose of these investigations was to produce as much acidity content as possible for the manure pre-treatment.

Moreover, we evaluated whether the use of already fermented whey could boost the production of lactic acid during fermentation and we found that simply fermenting the fresh whey is sufficient, because no boost is needed.

Finally, first results indicated that simply whey storage at room temperature could increase lactic acid concentration, suggesting that, in terms of energy gain, there is no need for temperature-controlled fermentation if time is not of importance. In addition, there would be no need for new equipment to facilitate the fermentation, but just a simple storage tank where cheese whey can be stored until the desired lactic acid concentration is attended. However, the storage should be insulated and heated to approximately 20 to 25ºC to insure appropriate temperature conditions regardless of weather conditions.

4.1.2 Grinding tests – Mechanical pretreatment

The mechanical action consists of the use of a food device that cuts the manure fibers with a stainless-steel knife and extrude them through a grid with holes of increasing diameters. (Figure 8)

Initially, the manure is mixed at a liquid/solid ratio of 1:4 with a different liquid, according to the experimental plan table (see Table 6): water, whey and fermented whey, for a final amount of 2.25 kg. After that, the mixture is put into the meat grinder and shredded with a specific grid, again following the experimental plan.

Figure 8 Grinding operations

For each grid used during mechanical operations, the following parameters were measured: electric power, grinding time and residual material in grinder compared to the feeding. We calculated the energy required to grind one tonne of material and the results are shown in Table 6.

Grid dimension	Grinding time	Grinding energy	Residual material in grinder
mm	**min**	**kWh/ton**	**%**
6	**3.8**	**33**	**13**
13	**3.1**	**24**	**13**
20	**1.5**	**13**	**13**

Table 6 Data from grinder action

The grinding time reduces with the largest holes dimension and the energy for grinding a certain quantity of material increases about 2,5 times with the smallest holes.

Mechanical pre-treatments are generally used to:

- Reducing the size of large particles;
- Altering cell walls to increase enzyme accessibility;
- Favoring the solubilization of biodegradable components

Depending on the technology chosen for mechanical pretreatment, each of these three objectives will be achieved to a greater or lesser extent.

The energy requirement in relation to the final particle size is one of the most important parameters describing the economical point of view of a mechanical pretreatment. As shown by the power consumption values presented in Table 6, each reduction in the grid hole size is accompanied by an increase in the energy consumed for grinding and must bring an improvement in anaerobic digestion performance.

It should be noted that, due to the interactions between the pretreatments, the order in which we carried out the pretreatments (mechanical then thermochemical) may have had an impact on the results. Thermochemical pretreatments can be preferentially carried out before mechanical pretreatments, as they might reduce the energy required for grinding.

4.1.3 Hydrolysis tests – Thermochemical pretreatment

The grinded material is put into 2-liters closed bottles and subject to 3 different temperatures (20°, 40° and 80°) and time durations (2, 8 and 24 hours), according to the DOE (see Table 5).
For the first 2 hours of reaction, all bottles were shaken every 15 minutes manually to ensure temperature homogeneity inside. Subsequently, the material was stirred after 8 hours and after 24 hours from the start.
Once the reaction time was over, the material was taken out of the bottle, spread on a tray and placed in the freezer for 15 minutes to stop hydrolysis. After this thermal shock, the sample was divided into several portions in order to perform the chemical and biological analyses. From a first sensory analysis, the material at 20° and 40°C still had the smell of manure and its colour was still brown, while the material at 80°C (runs 3-6-9) had a different smell, caramelized and its colour tended more towards black (Fig 9).

Figure 9 Hydrolysis operations

Influence of a chemical agent on pre-treatment temperature

In the absence of a chemical agent (acid or base), simple thermal pretreatment (TP) or heating pretreatment (HP) can be divided into two categories according to temperature, as shown in Figure 10: high-temperature pretreatment (HTPT, T>140°C) and low-temperature pretreatment (LTPT, T<140°C).

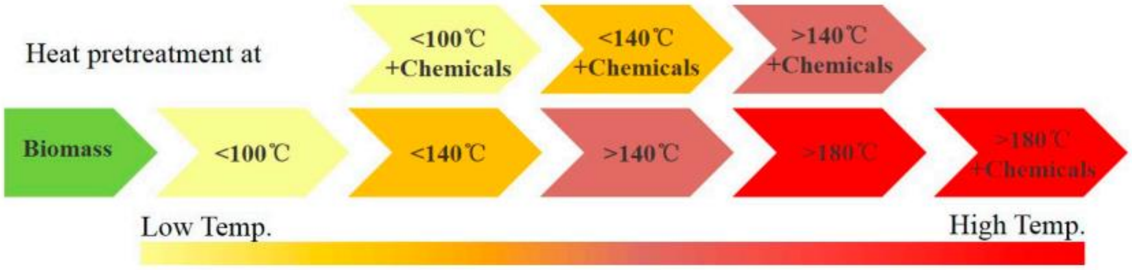

Figure 10: Heat teatment classification according to temperature interval (Wang et al., 2022)

While, HPTP is said to promote the solubilization of recalcitrant biomass (cellulose (T>150°C), hemicellulose T>200°C), it has been seen that at 80–90 °C protein and carbohydrates are easily solubilized (Appels et al., 2010, Neyens et al., 2003). When significant amount of carbohydrates and proteins are solubilized, a chemical reaction between free amino *acids* and peptides (usually from proteins) and reducing sugars can occur and produce recalcitrant products (melanoidins) that are less bioavailable for biogas production and microbial inhibitors such as furfurals, 5-hydroxymethylfurfural (HMF) and humic acids (Wang et al., 2022, Ortega-Martínez et al., 2021). This reaction, called the Maillard reaction or browning reaction, has been intensively studied in food chemistry as melanoidins give browned food its distinctive flavour.

Maillard reaction usually occur at temperature around 140°C-165°C, so that LTPT should preserve biomass from this adverse degradation. Browning is accelerated in alkaline environments but can also occur during acid thermochemical pretreatments. Caramelization generally occurs at higher temperatures (160°C-200°C) but can also be enhanced during thermochemical pretreatments.

According to figure 10, the presence of a chemical agent accentuates the impact of temperature, and this effect is probably greater the stronger the acid or base. This figure is a perfect illustration of the interaction between temperature and acidity, which must be considered in experimental design, and shows that avoiding the Maillard reaction requires the temperature to be lowered below 100°C.

In the present project, despite the use of low temperatures (80°C max) and a weak acid derived from cheese whey, we have unfortunately observed this browning phenomenon (see section 4.1.3 Figure 9), particularly in the hydrolyzed substrates used to feed the digester 2 in WP2. The mixture of whey cheese, rich in easily hydrolyzed carbohydrates (like lactose), and protein-rich manure probably forms a particularly favorable environment for the Maillard reaction and caramelization products. These compounds are coloured, mostly brown, and have high molecular weights. Therefore, temperatures below 80°C and /or reduced exposure time should be applied in future tests. When manure is heated at 80°C without cheese whey, Maillard reaction does not seem to occur which demonstrates again an interaction between factors of thermochemical pretreatment that need to be considered in experiment design. Dairy manure displays moderate C/N ratio indicating the presence of some carbohydrates, but those carbohydrates are complex polysaccharides that do not participate in Maillard reaction unless high temperature provoke their hydrolysis into simple reducing sugars.

Numerous studies have already been published on the thermal treatment of animal manure (Rafique et al., 2010, McVoitte et al., 2019, Carrère et al., 2009), including optimization studies. However, the obtained effects on anaerobic performance from HT are inconsistent and variable with the difference in pretreating biomass, temperature, exposure time, and BMP test or AD operation.

The general approach used to study heat treatments consists in combining low temperatures (50-100°C) with long exposure times (12h to 24h) and high temperatures (>100°C) with short exposure times (usually less than an hour). This approach suggests an interaction between pretreatment time and temperature.

- In their work, Passos et al (2017) observed that, in the absence of chemical agents (acidic or basic), the best methane yields from dairy cow manure are obtained with long treatments (12h - 24h) at low temperature (37°C), while short treatments (5min -30 min) at high temperature (>100°C) have no effect on methane yield.
- Conversely, McVoitte et al. (2019) worked only with short treatments (less than an hour) and thus observed no effect on methane yield below 125°C. The limitations of this approach are not, however, the only problem of this study, in which, as in many others, there is a lack of consistency in the results, with methane yields sometimes very low, or even virtually nil, including for untreated manures.

We believe that this lack of consistency and the low methane yields are partly due to the inhomogeneity of the substrates used (composition and quality), but also to the experimental conditions used when carrying out the BMP tests (substrate/inoculum ratio, initial TS value among others). We also obtained very low methane yields during this project, and this point will be discussed in detail in the next section.

It is known that anaerobic biodegradability of organic matter is related to its macromolecular composition (Amon et al. 2007; Raposo et al., 2012). It is therefore important to characterize the substrate macromolecular composition (proteins, lipids, carbohydrates, cellulose, hemicellulose, and lignin contents) (Amon at al. (2007), Angelikadi et al. (2009)) but this was not done in this project.

In conclusion, optimization of the pretreatment temperature for increased yields is often case specific and hard to control. The assistance of a chemical agent enables us to work at lower temperatures (Figure 10), but as we observed in this project, it did not prevent the formation of MRP and caramelization and that is probably due to the cheese whey used as chemical agent.

Acid pretreatment: Influence of the chemical agent

Most studies on thermochemical pretreatments use strong acids (HCl, H_2SO_4) or strong bases (NaOH), and as with the time-temperature interaction, the interaction between strong acid and temperature is evident in the procedures generally used in thermochemical pretreatments. The acid pretreatment is usually operated either under a high temperature and low acid concentration (dilute-acid pretreatment) or under a low temperature (e.g. 40 °C) and high acid concentration (concentrated-acid pretreatment). Currently, both the dilute acid and the alkaline pretreatments are the most mature technologies for commercialized application, having shown high effectiveness on several agricultural residues (Mancini et al., 2016).

More recently, weak acids such as formic or acetic acid have been successfully used in biomass pre-treatments (Cesaro et al. 2020, Peng et al., 2019) but little is known on compositional and structural changes of fibrous substrates due to weak organic acid pre-treatments under mild temperature.

The use of cheese whey as a cosubstrate for the digestion of agricultural waste is a developing practice that is already well documented (Kavacik et al. 2010, Comino et al., 2012, Hublin at al. 2012, Rico et al. 2015, Vivekanand et al. 2017). On the other hand, its use as both cosubstrate and chemicals for biomass acid pretreatment is an innovative approach. As pretreatment is efficient only when it matches substrate structural compositions correctly, the aim of this project was, among other things, to assess whether lactic acid from whey would be effective in promoting the degradation of lignocellulosic materials like acetic or formic acid.

Compared to other commonly used weak acids, the use of raw whey as an acid reagent for thermochemical pretreatment between 40 and 80°C is more complex, as it can be accompanied by numerous chemical reactions, such as the Maillard reaction mentioned beforehand. These reactions can potentially influence the thermochemical treatment and subsequently also the performance of the anaerobic digestion.

4.2 WP1 - Evaluation of pretreatments combination

The way we developed Taguchi's plan, in particular the way we defined the factors, and their levels may have led to a certain limitation in the exploitation of the results.

4.2.1 Critical analysis of Taguchi table design

First, no interaction between factors has been considered in the analysis of table L9. However, given information provided in the previous section, it is reasonable to assume that the temperature applied will influence the chemical activity and degradation of the whey. Similarly, the grinding of organic matter has an impact on the thermochemical treatment that follows it in the procedure established for this project. The importance and difficulty of considering interactions in the Taguchi design is explained in Appendix 1.

Thermal degradation of cheese whey is accompanied by lactose breakdown and the formation of organic acids: lactic, acetic, propionic, and formic acids, among others. The proportion of each of these acids, as well as the final degree of acidity, depend on the experimental conditions (anaerobic/aerobic, temperature, etc.). Just by storing the cheese whey in a temperate environment for several days it is possible to increase the acidity to various degrees. In view of these observations, we decided that our tests would not be carried out at a fixed concentration, but that it would be sufficient for it to lie within a concentration range with fresh whey as the minimum and fermented whey as the maximum.

For all these reasons, the acidity factor in the experimental design differs from the other factors. The acidity levels 1 and 5 corresponding to raw and fermented whey are subject to variation and are not fixed, as they depend on the rate of degradation and/or aging of the fermented and raw whey.

Factors like our acidity factor, must be treated as a signal factor in a dynamic plan rather than a simple control factor in a static one.

In conclusion, the difference between cheese whey and fermented cheese whey is not simply a question of degree of acidity. It is a much more complex issue, which should have been excluded from the experimental design, and should have been the subject of more in-depth upstream work during a prior screening.

4.2.2 Choice of parameters

Process evaluation with the Taguchi approach is based on the measurement of carefully selected parameters. In general, kinetics, biochemical, and solubilization parameters are the most common indicators to evaluate pretreatment performances. In this project, for each run defined by the L9 Table (see Table 7), the parameters investigated were the solubilization of organic matter (sCOD), and the methane production yields (BMP test) before and after pretreatment. Methane production rate was only evaluated quantitatively from BMP test graphs.

The results obtained on VFA are not included here, as the analysis showed that the data are not usable due to lack of consistency.

Throughout this analysis, run 1 (2h/20°C/water/20mm) will be considered as untreated sample.

Each parameter measurement provides a response, and from the set of responses, the statistical analysis calculates response characteristics (signal-to-noise S/N, means and standard deviations) according to the optimization objective (maximization/minimization) defined for the responses of each parameter. For sCOD, as well as for Methane yields, we want to response to be maximized. Statistical analysis also gives the results of the linear model.

We then plot the main effects for the three response characteristics:

- Signal-to-noise ratios (S/N ratios), as a function of control factors. Signal-to-noise ratios provide a measure of robustness.
- Response averages as a function of control factors
- Standard deviations as a function of control factors (not shown here)

All these data and graphs can then be used to determine which factor has the greatest impact on response values, and which conditions minimize process variation. (higher S/N ratio). A process designed with this in mind performs more consistently in its operating environment.

4.2.3 sCOD parameter

Measurements of sCOD for all the tests are shown in Table 7 and Figure 11, 12, and 13.

Trends observed are similar for both batches (Figure 11) and run 9 remarkably provides the maximum sCOD concentration that is the highest organic matter solubilization. When compared to untreated sample it represents an increase in matter solubilization of 105% (batch 1) and 148% (batch 2).

sCOD (mg/l)	**test 1**		**test 2**	
Run	average value	SD	average value	SD
1	29523.9	8478.0	22996.2	3871.6
2	38469.4	2239.3	31391.6	774.3
3	44252.8	3286.4	34311.8	1139.8
4	36126.8	3590.4	24968.2	257.7
5	34570.2	1159.6	30435.7	546.7
6	39560.4	4330.0	37482.7	1653.7
7	31704.8	2872.2	27053.4	1652.2
8	24917.7	1954.3	22106.9	865.8
9	60740.6	3430.7	57099.2	5560.9
10	-	-	26587.4	2170.8

Table 7 sCOD values

(white cells for water runs, orange for simply whey runs and green for fermented whey runs)

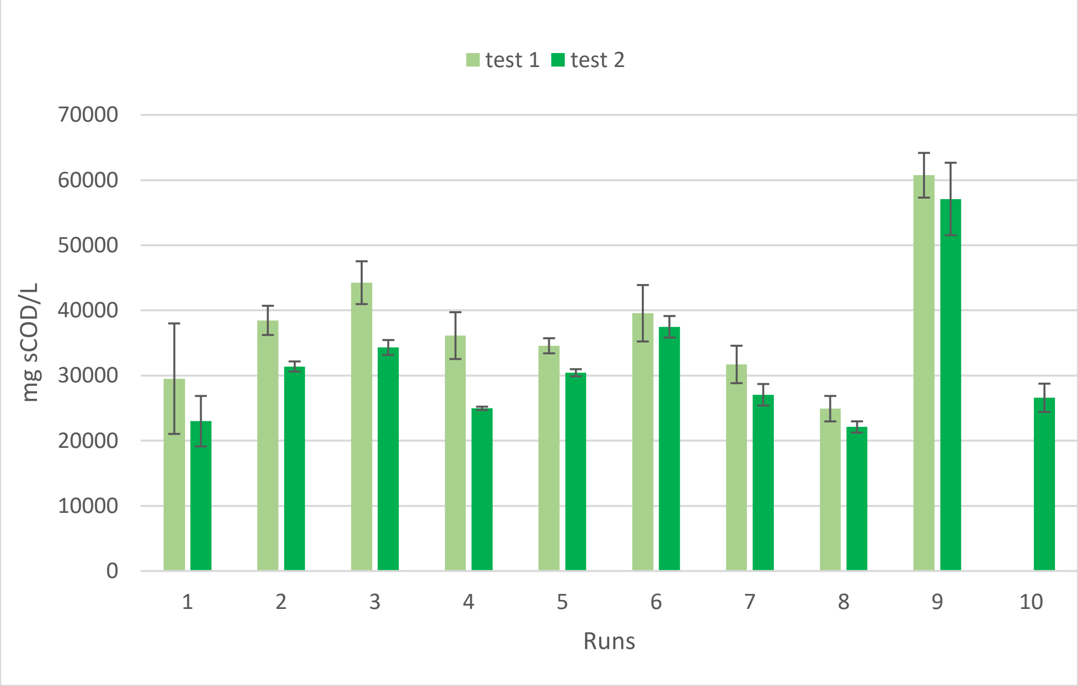

Figure 11 sCOD measurements

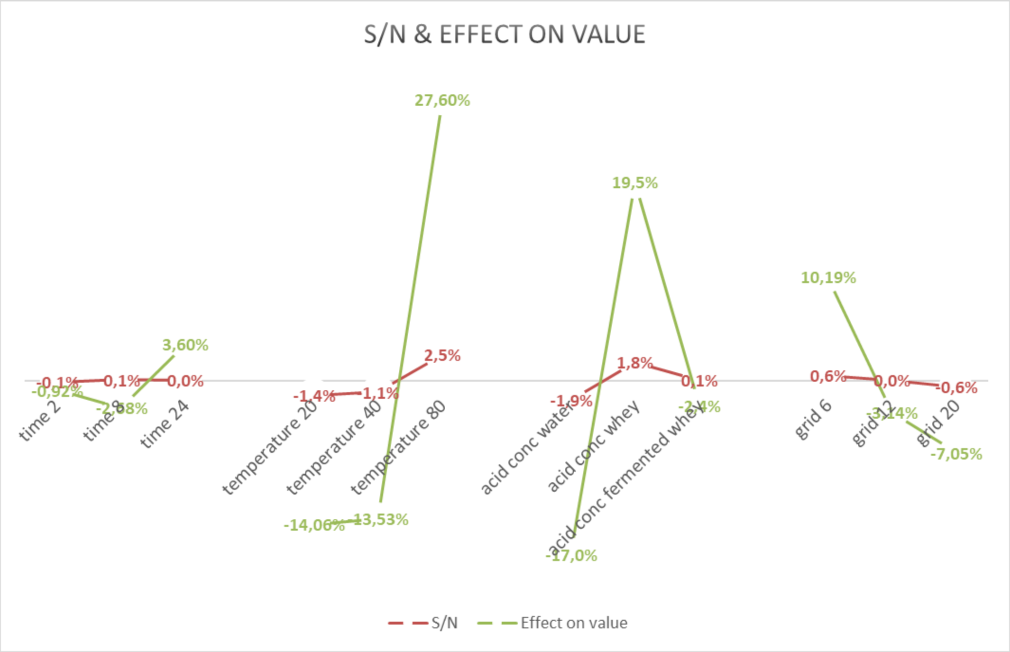

Figure 12 S/N and effect of different parameters on sCOD value (batch 1)

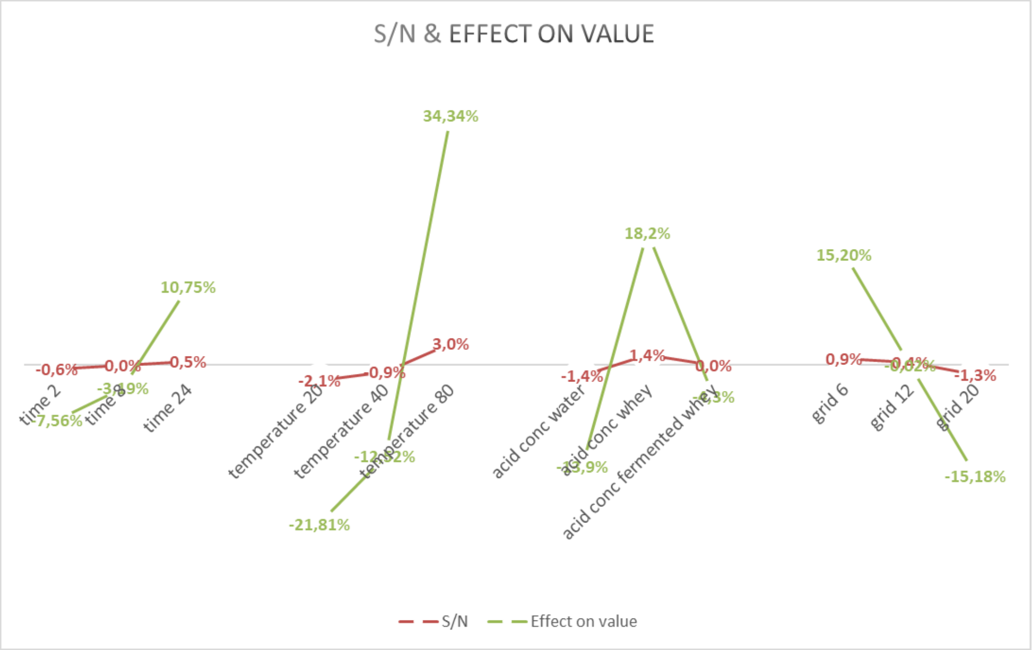

Figure 13 S/N and effect of different parameters on sCOD value (batch 2)

High consistency between batches has been confirmed by Pearson correlation test (Pearson coefficient = 0.91, slope of the linear regression line = 0.99) and student test indicates that there is no statistically significant difference between values of run 1-6-8 and runs 7 and 9. Those runs, made with water and/or over a 24-hour period, are therefore highly reproducible.

The reproducibility of the runs and process is also confirmed by the S/N graphs as there are no major variations in S/N ratio values (Figures 12 and 13). In both batches, the factor with the greatest impact on sCOD value is the pretreatment temperature. Next comes the acidity factor, which is not surprising since cheese whey and fermented cheese whey both initially contain a high level of sCOD, unlike hydrochloric acid or sulfuric acid, which are generally used in thermochemical pretreatments.

4.2.4 Methane yield parameter

The manure pretreatment protocol is proved effective in increasing the organic matter solubilization and since higher organic solubilization is often associated with high organic matter biodegradability, we could expect better methane yields, but results of BMP tests (Table 8 and Figure 14) are somehow more mixed.

BMP (NLCH4/kgVS)	**test 1**		**test 2**	
Run	average value	SD	average value	SD
1	58.3	8.5	53.7	4.8
2	118.2	8.7	85.0	8.3
3	112.2	16.6	84.8	5.7
4	123.6	1.2	74.0	20.1
5	108.7	9.1	78.0	3.6
6	49.4	8.1	46.6	1.7
7	128.5	14.1	75.0	3.3
8	43.7	24.6	45.7	3.5
9	121.2	5.7	94.6	5.9
10	-	-	92.0	16.3

Table 8 BMP values

Even though trends in both batches share similar features with cheese whey runs (both fermented and fresh) providing significantly higher methane yields than water-based runs (Figure 14), consistency between BMP batches measurements is poor as only runs with water as liquid agent (1-6-8) are statistically equal.

The poor consistency is confirmed by Pearson correlation tests (Pearson coefficient = 0.83, slope of the linear regression line = 0.43).

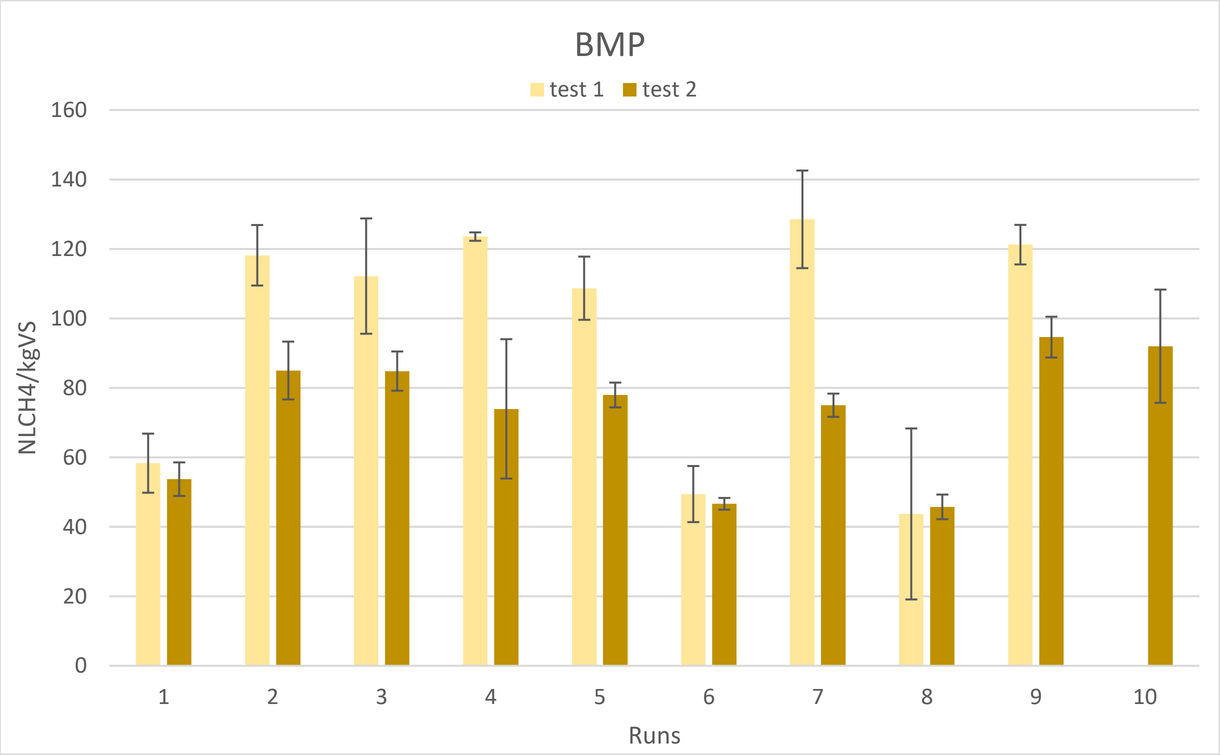

Figure 14 BMP measurement

According to Taguchi's model, only the addition of whey has an impact, and a positive one at that, on methane yields (Figures 15 and 16), but student tests indicate that the difference between whey-based runs (fresh or fermented) is statistically insignificant. Thus, BMP tests cannot differentiate between raw and fermented cheese whey.

Although characterized by the highest sCOD content, run 9 does not differ from the other runs carried out in the presence of acidity regarding methane yield.

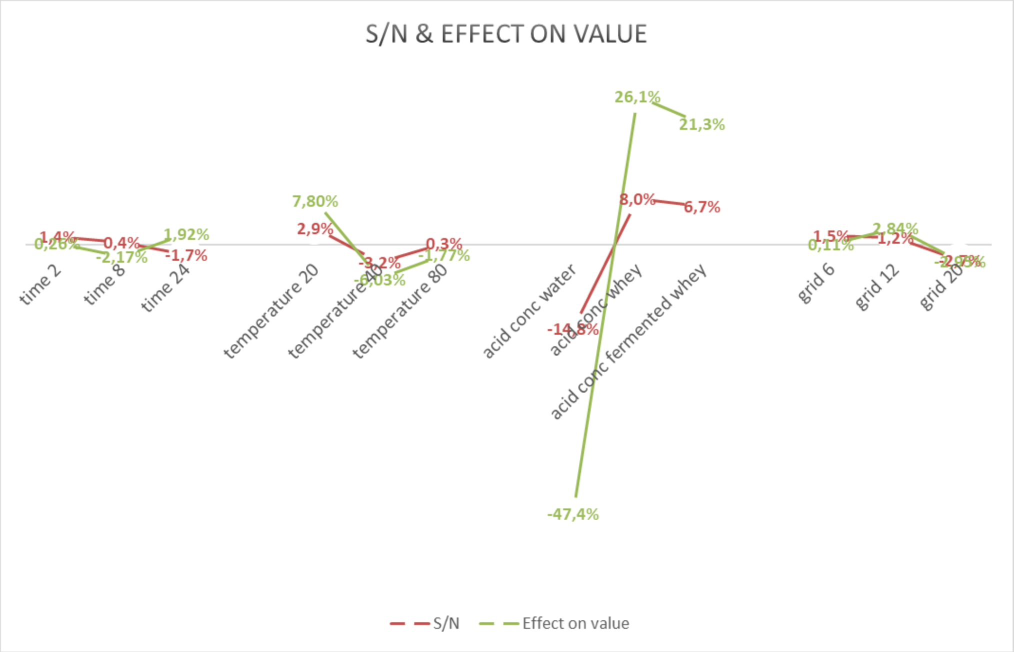

Figure 15 S/N and effect of different parameters on BMP value batch 1)

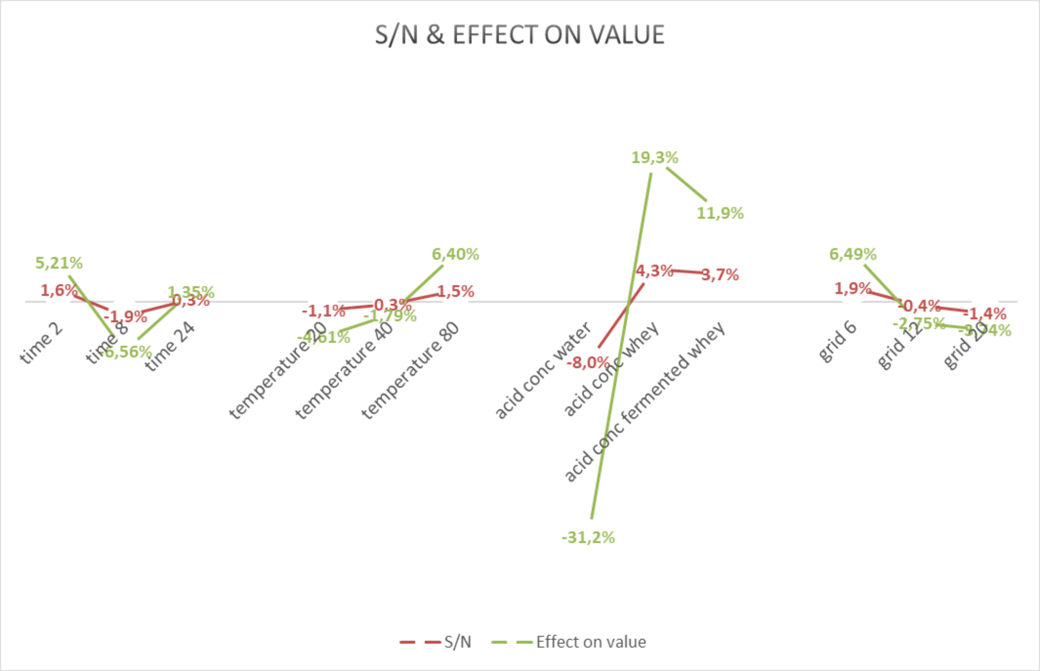

Figure 16 S/N and effect of different parameters on BMP value (batch 2)

As for the confirmation run (n°10), a good fit between Taguchi DOE model and experiment is found for sCOD (both mean and S/N VALUES) but methane yield value from model is underestimated with respect to that of run 10.

	sCOD (mg/L)		Methane yield ($NLCH_4/gVS$)	
	Value	**S/N**	**Value**	**S/N**
Experimental	26587	88,4	92	38,9
Theoretical	27940	89,0	70,7	36,6
Relative difference	-5%	-1%	30%	6%

Table 9 Comparison between DOE model and experimental data (run n°10)

While the way in which Taguchi's plan was designed and analyzed may have played a role, it seems likely that the low consistency of BMP measures is more likely due to the way in which we carry them out.

Indeed, methane yields obtained in this project are very low than those presented in the literature. Indeed, the MéthaSim v2.0 database (IFIP-Institut du porc), that collects a lot of data about agricultural and industrial biomass, reports an average BMP value of cattle manure about 230 $Nm^3CH_4/tonVS$, while we found about 70% less than this value (see Table 10).

	test 1 (WWTP)		test 2 (WWTP)	
Substrate	average value	SD	average value	SD
manure	79.4	9.5	56.4	5.3
whey	526.3	46.1	488.9	15.5
fermented whey	538.8	12.7	598.7	6.6
Run 9	121.2	5.7	94.6	5.9

Table 10 Substrates BMP values (measurement unit $NLCH_4/kgVS$)

We believed that these very low values may have hampered the correct interpretation of the results and therefore require further investigation. Initially, we attributed the cause of these values to the inoculum (from WWTP) we were using at the time, as it itself had a methane yield values lower than usual and could have been of lower quality due to technical problems at WWTP. However, the values in question (40 $Nm^3CH_4/tonVS$), although low, are still close to the recommendations in this area. (VDI 4630, Raposo et al. 2011, Holliger et al. 2016, Filer et al. 2019) (Table 11).

Parameter	**Recommended range**
Alcalinity	1,5 > $gCaCO_3/L$
Concentration	15 to 20 gVS/L
Storage	1 to 5 days at 25°C
Methane Yield	≈ 50 $NLCH_4$/ g VS

Table 11 Recommended inoculum conditions for BMP tests

Our choice to use WWTP sludge was dictated by the fact that we followed the standard protocol for measuring BMP, but we then decided to perform BMP tests with agricultural AD inoculum as it could have been more appropriate to analyze the impact of manure pre-treatment.

This was a reasonable approach as we were not testing a substrate itself in an absolute manner, but the efficiency of a treatment protocol. However, BMP value of agricultural AD inoculum is way above the recommended value, and results of BMP tests 3 and 4 performed in parallel with WWTP sludge and agricultural inoculum respectively both indicate with very low BMP values(Table 12).

	test 3 (WWTP)		test 4 (Agro AD)	
Substrate	average value	SD	average value	SD
manure	50.8	28.2	85.2	124.5
whey	450	110	458.2	30.4
fermented whey			414	61.9
Run 9	113.3	8.8	151.9	29.5

Table 12 Effect of inoculum type in BMP tests (measurement unit $NLCH_4$/kgVS)

This could mean that the manure we used at that moment of the project was of poor quality. It has already been mentioned in this report that the macromolecular composition of manure can have an impact on BMP values and cause batch-to-batch variability. In fact, low methane yields values have already been observed in the literature and have been attributed to seasonal changes in cattle feed, which in turn lead to significant variations in protein content. (Abdallah et al. 2018, Passos et al. 2017, McVoitte et al., 2019). Furthermore, fresh manure collected in winter is likely to have a higher BMP than manure used in this project collected in summer after several months' ageing.

Nevertheless, from our discussions with farmers in Switzerland, such low values are not consistent with their observations on the field, and we looked for other possible explanations relying again on official recommendations (German standard VDI 4630, Raposo et al. 2011, Holliger et al. 2016, Filer et al. 2019).

Often, great importance is attached to compliance with volatile solids guidelines (total volatile solids concentration between 20 and 60 gVS/L, volatile solids concentration in the inoculum between 15 and 20 gVS/L i.e. 1.5 to 2%, volatile solids, and concentration in the substrate below 10 gVS/L to guarantee an inoculum/substrate ratio of 2.

In this project, the volatile solids guidelines were respected but TS content were high (Table 13)

TS (% FM)	Test 1		Test 2	
Run	Average value	SD	Average value	SD
1	16,5	3,5	18,8	0,1
2	17,6	4,4	20,4	0,3
3	16,6	1,8	18,2	0,1
4	17,2	0,2	17,9	0,3
5	17,2	0,1	19,4	0,2
6	17,9	0,1	19,2	0,9
7	17,7	4,3	17,7	0,2
8	16,7	0,3	17,0	0,1
9	19,0	0,1	20,7	0,2
Average	**17,4**	-	**18.8**	-

Table 13 TS content of samples used in batch 1 and 2

Impact of substrates TS content in cow manure on BMP methane yields have been well documented by Abid et al. 2021 (Figure 17). In this study, TS concentration was adjusted at 5%, 10%, 15%, and 20% through dilution. The maximum cumulative methane yield (CMY) in cow manure was found at TS 5% (422 mLCH$_4$/gVS) accompanied by TS 10% (352 mLCH$_4$/gVS), TS 15% (318 mLCH$_4$/gVS) and TS 20% (189 mLCH$_4$/gVS). Thus, cumulative methane yield clearly decreases with increasing solid content.

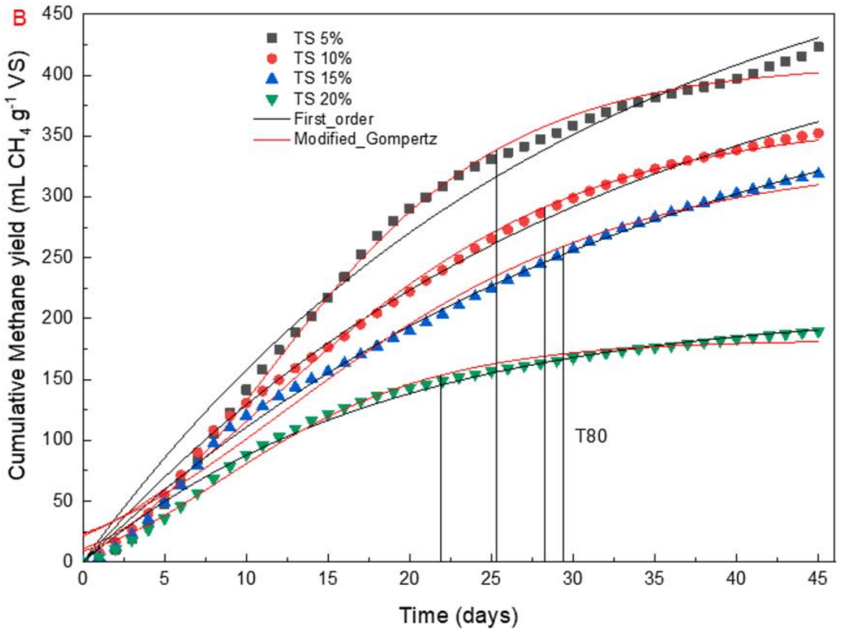

Figure 17 Influence of solid content in BMP tests of cattle manure (from Abid et el. 2021)

Although in line with BMP values of Abid et al., our results are somehow lower results, and we cannot rule out the possibility that the low methane yields are due to a combination of factors (inoculum and substrate quality, and high TS value).

In general, dilution of a substrate for BMP tests should be avoided as it might induce underestimations of the methane potential but in case of substrates with high solid content dilution is necessary to get samples with solids contents below 12%, according to the recommendations of Raposo et al. (2011) and assure an adequate mass transfer. Consequently, methane production from blank (inoculum) contributed to 70% for manure samples and 52 % for run 9 which is way too high as this contribution should be less than 20%.

For undiluted substrates, methane yield values are difficult to exploit due to low mass transfer during the anaerobic digestion process in BMP tests. It is difficult, if not impossible, to properly evaluate the different samples and therefore analyze the situation for informed decision-making. While it seems appropriate to use whey given the objectives of the project, the optimum levels of factors to be used for pretreatment are much less clear-cut. To choose the most suitable run for WP2, other aspects need to be taken into consideration.

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For a more comprehensive evaluation, we also analyzed the influence of the different protocols in increasing the rate of degradation of organic matter in the first days of anaerobic digestion.
This was made because it has been demonstrated that there are pre-treatments that increase not greatly the amount of CH_4 produced in AD but rather the rate at which the microorganisms utilize the organic matter (IEA Bioenergy, 2014 - Figure 19). This enables to work with lower solids retention times, or in other words, to load a larger number of substrates into the digester.

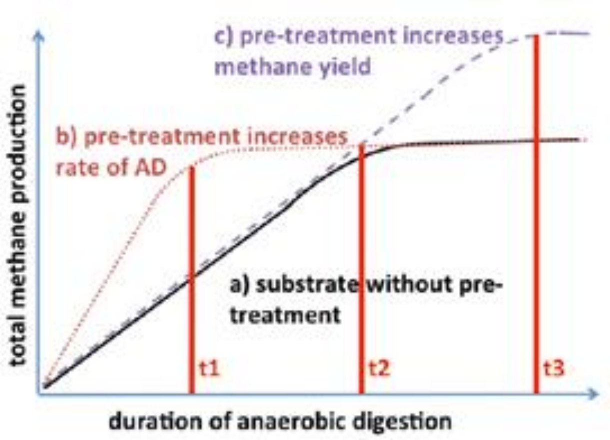
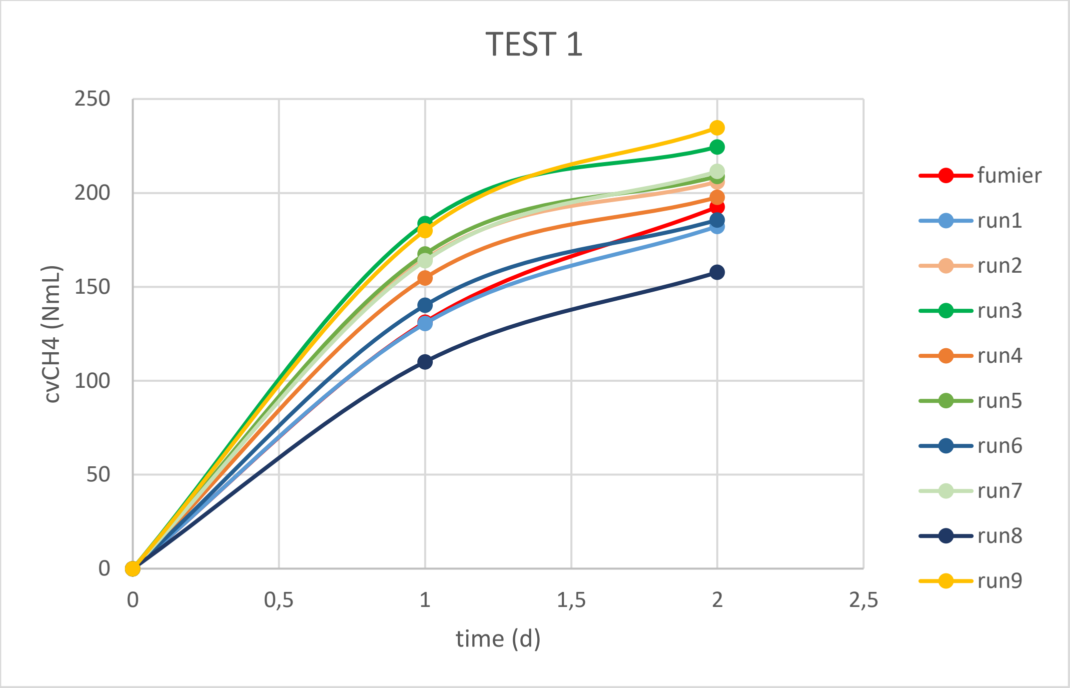


Figure 19 Difference between increased methane yield and increased methane production rate. (IEA Bioenergy, 2014)

Figure 20 shows the volumes of CH_4 produced, net of production due to the addition of the liquid part, for manure and all the different runs. From this figure, we can assume an increase in the rate of matter degradation for the mentioned runs but we prefer to remain cautious about further interpretation of the data because of the way they were obtained and processed.



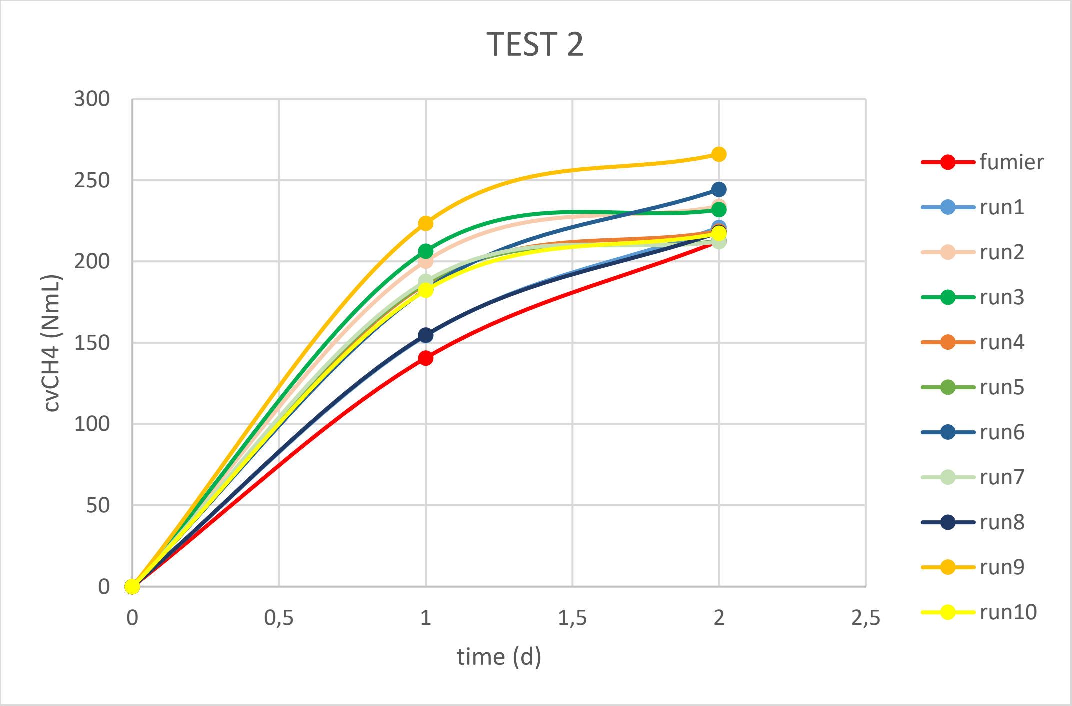


Figure 20 Volume of CH4 produced in the different runs of Batch1 and 2 after deducting 20% of the CH4 volume produced by the whey (fermented or not)

4.2.5 Conclusion: Choice of a run for WP2

Data from the exploratory study were difficult to exploit and analyze due to the experimental conditions used to perform the various tests. Performing BMP tests on substrates with a high solids content, which does not follow the recommendations for this type of analysis, generated limitations in mass transfer and low methane yields. Furthermore, the manure used for WP1 was harvested in summer, at a time when its quality is potentially at its lowest. However, as we want our pretreatment protocol to be valid for any manure type and quality, and in any AD configuration, these tests served as a reference for intermediate scale tests (WP2) carried out in HSAD configuration. In conclusion, while the way we proceeded significantly complicated our analysis work, particularly that of the Taguchi table, it did give us a glimpse of the difficulties associated with the HSAD configuration we were going to use in WP2.

The best methane yields were obtained from cheese whey-based samples (runs 2/3/4/5/7/9) but differences those samples are not statistically significant.
When considering kinetics, runs 3 and 9 seem to be the best combinations of pretreatment, thus indicating the importance of factor “temperature” for the protocol, and of these two runs, run 9 appears to be the most stable and reproducible. It is also characterized by a remarkably high sCOD and, at this stage of the project, was assumed to be the most appropriate to achieve the objectives of WP2.

In Table 14, we calculated the theoretical value of BMP by simulating the simple mixing of manure (80% w/w) and whey (20% w/w) and compared it to run 9 and manure experimental values.

Batch	Run 9	Manure	BMP yield	Manure/whey mixture	BMP yield
1	121,2	79,4	52,6	115,8	5%
2	94,6	56,4	67,7	93,4	1%

Table 14 BMP yields calculated on theoretical and measured BMP values for the 2 test batches.

Again, we note that the addition of whey significantly increases methane production compared with a substrate composed solely of manure, but the difference between run 9 and a simple manure/whey mixture is much smaller. This calls into question the relevance of pre-treatment and suggests that performance is mainly due to the use of whey, i.e. to the codigestion effect rather than to pre-treatment.

The lack of significant effect could mean that Mimagas mild pretreatments combination may not be required, which would save the AD industry from investing in unnecessary infrastructure. However, these results need to be put into perspective because, in addition to the physico-chemical characteristics of the manure used, notably its high solids content, other aspects of this project may have hampered the performance of the pre-treatment combinations, such as the way in which the Taguchi DOE was designed and analyzed.

An experimental design considering the different interactions between the factors/pretreatments would have been more appropriate for our process, and the model thus obtained would better reflect the reality of the combined pretreatments protocol in which each component influences the others. In addition, these interactions would be better assessed and characterized during prior screening using an RSM design. With a RSM design, we could also have determined the optimum experimental conditions, the robustness of which could then have been tested using an appropriate Taguchi Table.

The use of cheese whey as a chemical agent has proved more complex than that of the acids generally used in biomass thermochemical pretreatments. Cheese whey, a substrate rich in easily hydrolyzable carbohydrates, is particularly conducive to the Maillard and caramelization reactions that can occur during thermal or thermochemical pretreatment. The evidence of the Maillard reaction in whey trials, may have greatly impaired the quality and performance of hydrolyzed substrates, particularly run 9, due to the formation of recalcitrant and/or less easily digestible COD. To avoid the formation of MRP, temperature below 80°C may have been more appropriate and that could have been unraveled during prior screening.

Eventually, decision making based solely on two quantitative parameters that were not proved significant (as kinetics constants were not properly and quantitatively evaluated in this work) can be tricky. With correct experimental conditions, it would therefore be necessary to calculate hydrolysis rate of particulate organics using the first order kinetics model or modified Gompertz model.

Chemical parameters chosen for this project (Methane yields and sCOD) are largely influenced or even biased by the presence of cheese whey. Consequently, other parameters, notably physical ones, would be not only appropriate but essential for a correct evaluation of pretreatments, as we will see in the next chapter.

Despite the shortcomings highlighted, the initial Taguchi design provided valuable information. and at the end of the exploratory study, run 9, which involves the combination of the following parameters: addition of raw cheese whey, grinding at 6mm and 24 hours of hydrolysis at 80°C, appears to be the best combination of factors levels and was chosen for the tests to be carried out in WP2.

4.3 Efficiency of combined pretreatment for anaerobic digestion at intermediate scale (WP2)

The main goal of this WP was to test the best pre-treatment protocol that had been established in WP1 in 70 litres- digesters. Two individual digesters were set up in parallel, one fed with untreated manure (digester 1, control), and the other one fed with the pre-treated manure (digester 2, test), and run for 4 months. They were closely monitored (i.e., biogas flowrate, biogas composition, pH, alkalinity, ammonium concentration, VFA) to assess digestion performances and stability.

4.3.1 Rheological properties

Rheological properties of sludge play key roles in biomass management and treatment since they are being used in design of aerobic and anaerobic digesters, pumps and pipes, heat exchangers, sludge dewatering and biogas production units.

Many studies have demonstrated that rheological properties are related to a substrate's physico-chemical properties, in particular total solids (TS) content and particle size.

Feedstock pumpability

The difficulty in pumping the feedstock is influenced by the total solid (TS) content. Thus, another major concern with HSAD relates to the pumping and substrate handling devices, which add to the cost of the technology. (Vandevivere et al., 2003).

We did observe that the hydrolyzed manure/whey milk mixture is much easier to introduce into a digester using a syringe than conventional manure ground to 20 mm. It is reasonable to assume that thanks to its texture, this mixture will be easier to pump into industrial plants operating in HSAD mode. As indicated in Table 15, this is probably due to a great reduction in TS content.

Manure (D1)	Hydrolyzed Substrate (D2)	Whey/manure mixture (20/80 w/w)
22,1 ± 1,7	17,0 ± 1,0	18,8

Table 15 : TS content means of substrates used to feed the digesters in WP2 and TS theoretical value of a simple mixture of whey cheese and manure (20/80 w/w)

However, it is still difficult to evaluate the pretreatments effect as a simple dilution of manure with cheese whey also causes a reduction in TS content. During the tests we observed that simple addition of whey can also improve feedstock’s ease of insertion into the digester as any liquid agent would do by probably by bringing moisture to the substrate.

Particle size reduction during mechanical and thermochemical pretreatment as it could also greatly influence pumpability. Hence, physical parameters such as particles size distribution (PSD), not evaluated in this project, must be considered in the future. In addition, other easily measurable physical parameters such as digestate dewaterability and/or settability and could be used in the future for a more comprehensive evaluation of the pretreatments impact on rheological properties.

Reduction of particles size during physical pretreatments is also known to impact water retention capacity of substrate, an important parameter in HSAD configuration.

Digester mixing

A substrate’s rheological properties also play an important role in reactor mixing and parameters such as homogeneity, mixing energy, and heat and mass transfer depend on these properties. Mixing energy is (in first approximation) directly proportional to the apparent viscosity of the digester content (Wu, 2012).

Despite the pre-treatment of the substrate in Digester 2, we did not observe any significant reduction in power consumption for digester mixing. This is in line with the viscosity measurements of the two digestates which do not differ enough to have an impact on the power consumed during mixing.

4.3.2 Anaerobic digestion

HSAD configuration (June-August)

As indicated in the previous chapter, the HSAD configuration was initially chosen to favor anaerobic digestion in digester 2 over digester 1. However, if biogas production results are clearly in favor of digester 2 until the end of July (+30%), the difference in cumulative production between the two digesters diminishes to reach a value of 20% at the end of August (Figure 18). While this value is acceptable, the trend shown in this graph indicates a net change at the beginning of August and when we separate the cumulative production figures for July and August (Figure 19 and 20), we can see that the situation at the end of August (+10%) is becoming quite critical.

Figure 21 shows that the level of solid matter in the digestate at the output of both digesters continues to rise from June and the end of August. High solid content digestate is common in the case of anaerobic digestion of cattle manure and indicates that the rate of organic matter degradation is low. In this project, this phenomenon became more pronounced at the beginning of August, with the 3rd change in organic load. It should also be noted that, by this time, all the digesters have been renewed which means that there are no longer any traces of inoculum from Agrogaz (wet anaerobic digestion process), which composition and quality probably differ from that of the digestate produced in our digesters.

Analysis of daily biogas production (Figure 22) shows that the situation is particularly unfavorable in Digester 2 during August weekends feed interruptions. Initially, we assumed that production was lower in Digester 2 at weekends because most of the available organic matter had already been digested during the week. This hypothesis implies that digester 2 is not sufficiently nourished, which is why the OLR was increased several times. However, the fact that this phenomenon became more pronounced as the organic load increased and was accompanied by a significant rise in the solid matter content of the digestate, seems to contradict this hypothesis.

The drop in performance of digester 2 could also be due to an inhibited steady state caused by the formation of inhibitor by-products from pre-treatments and/or co-digestion which can be even more pronounced in HSAD configuration as the latter is already well known to cause the accumulation of toxic and inhibitory compounds which can disrupt or slow down methanogenic activity.

In addition, our day-to-day experimental observations suggest that the digester 2 is suffering more rapidly and more intensively from the lack of water. In fact, digester one is performing quite well in August, with an organic matter reduction rate low but in line with other studies in HSAD configuration. We suspect a problem could also lies in the reduced water retention capacity of the substrate/digestate in digester 2 because of pretreatments but as mentioned above, we do not have equipment to perform such measurements.

Anyhow, at this stage of the project, it was difficult to pinpoint a single cause to explain how the situation, which was largely favorable for digester 2, reversed so rapidly in August as many factors may be involved, whether related to pretreatments, co-digestion, HSAD configuration or due to cross-effects.

In the meantime, in the absence of a liquid fraction, the digesters content was becoming increasingly solid, making digestate removal more difficult, and when organic matter degradation rates are low, the risk of rapidly endangering the digester or entering in an inhibited steady state is high in HSAD configuration (Song, 2022). In view of all these factors and hypotheses, we decided at the end of August to switch to a semi-dry anaerobic configuration, adding a fraction of liquid (water, 22-43 % in mass) each time the digester was fed. However, the quantities of water used are much smaller than those used in wet anaerobic digestion (see Table 5).

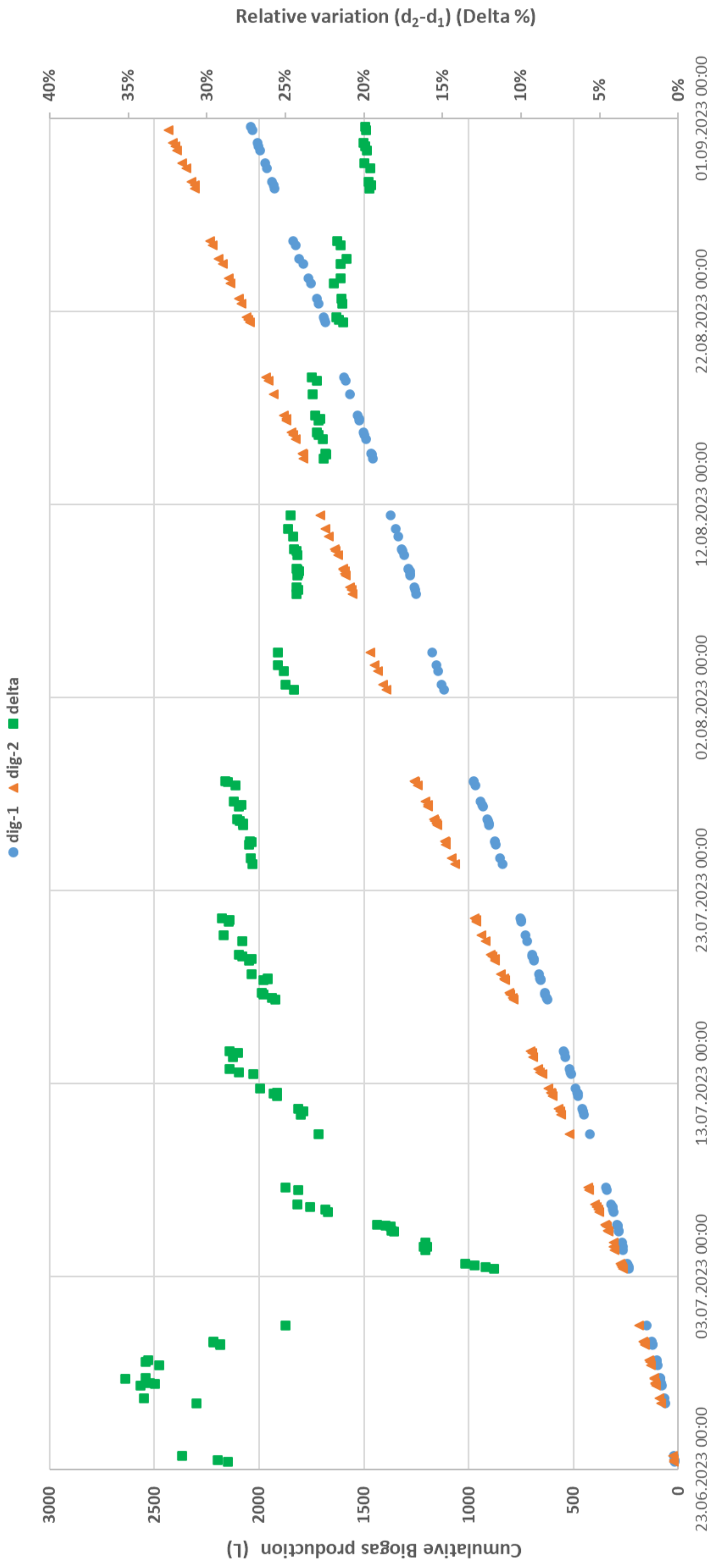


Figure 18 Cumulative biogas production for digester 1 and 2 and relative variation (d2-d1) in HSAD configuration

Figure 19 Cumulative biogas production for digester 1 and 2 and relative variation (d2-d1) July 2023

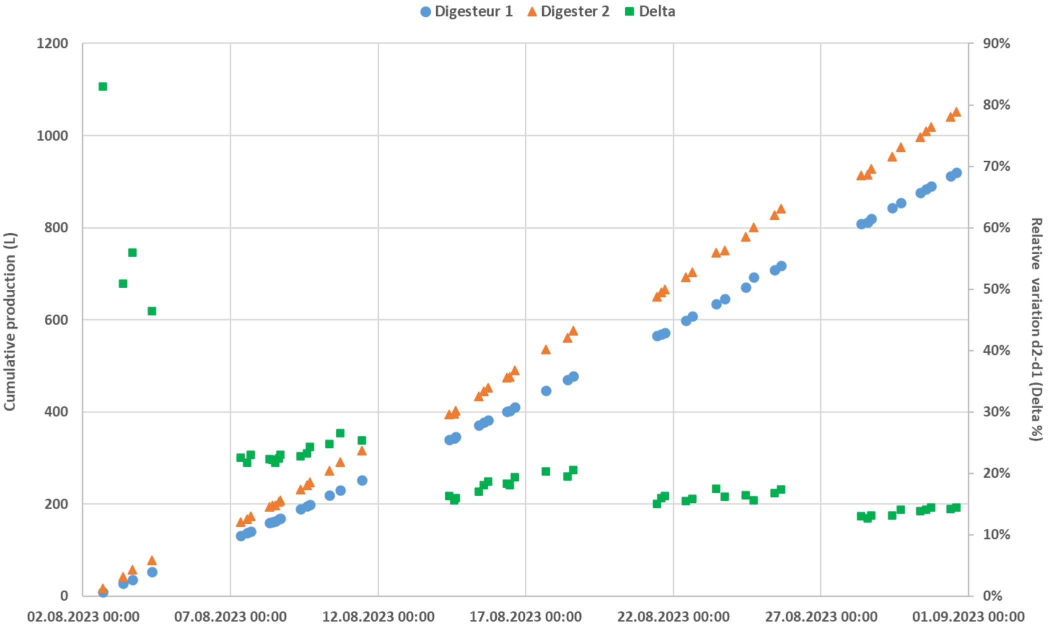


Figure 20 Cumulative biogas production for digester 1 and 2 and relative variation (d2-d1) August 2023

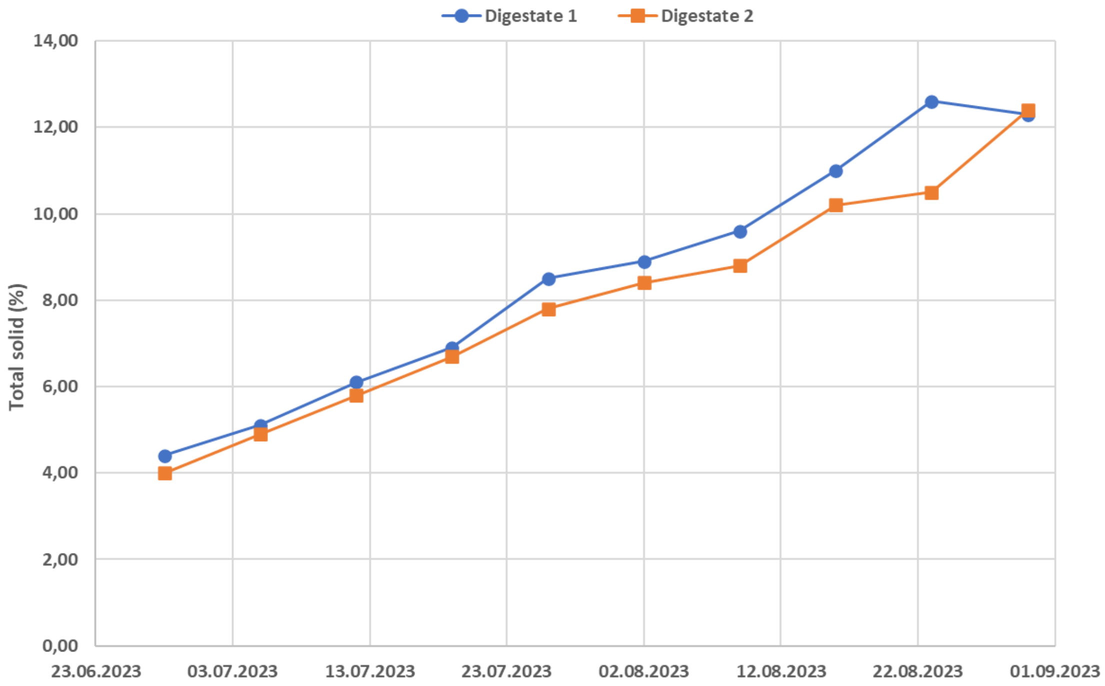


Figure 21 Digestate total solid matter content (%)

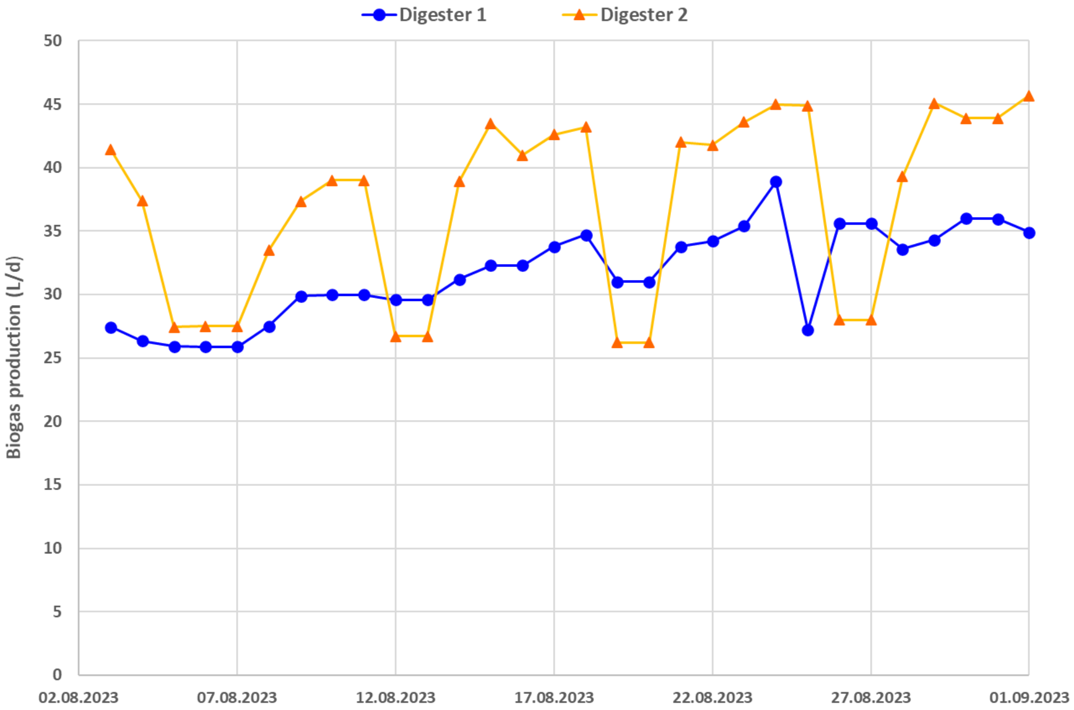


Figure 22 Daily Biogas production (L/d) (02/08 – 01/09)

Semi dry configuration (September – October)

A one-week rest period was used before switching to semi dry configuration. During the rest period, we substituted 6L of digestate with 6L of water to start diluting the digesters and decreasing level of inhibitors in Digester 2.

After the rest period, both digesters were switched to a semi-dry configuration based on the strategy described below. The aim of this strategy was to stabilize the digesters in terms of both biogas production and difference in production between the two digesters, so that reliable methane yields and organic matter reduction rates could be calculated for WP3.

1) OLR has been kept below 2.0 $kg_{VSS}/d/m^3$ from September until the end of testing in early November.

Indeed, we consider that the increase in OLR in July and August was perhaps too rapid especially when considering HSAD process. The aim at that time was to achieve an OLR like that used at Agrogaz (estimated at 4.3 $kgVSS/m^3/d$) but obtaining such a high organic load in dry process configuration was not possible, as we encountered difficulties way before reaching that value.

2) Change in feeding patterns.

To minimize the impact of weekend feed interruptions on digester 2, we choose to double the feeding dose on Fridays for both digesters.

Figure 23 shows the daily production of biogas production for the semi dry AD test. As we can see, doubling the feed dose on Friday (or on both Thursday and Friday) does not prevent digester 2 production from dropping over the weekend, while digester 1 is less affected by these changes. It is well known that adding a substrate rich in easily hydrolysable carbohydrates to one rich in protein reduces the lag phase which means that the substrate becomes much more reactive to changes in feed, and this is exactly what can be observed on this graph.

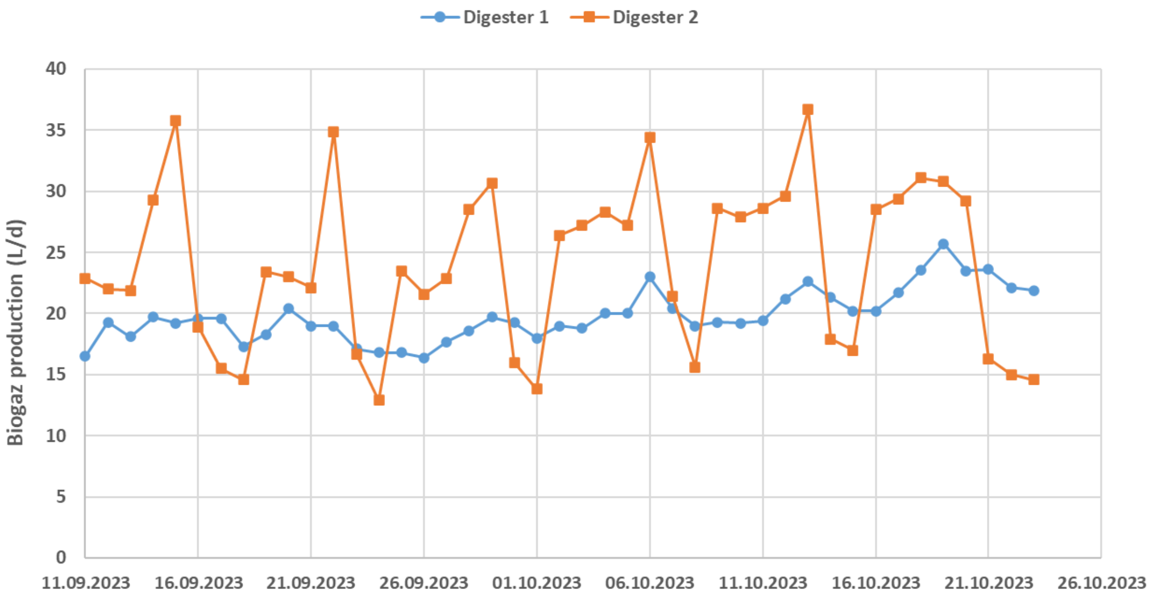


Figure 23 Daily Biogas production (L/d) (11/09 – 23/10)

This means that, compared with digester 1, it is possible to increase the organic load on digester 2, but rather than doing so excessively over 5 days, it would be better to start by regularizing the feed over 7 days. This is due to co-digestion effect as cheese whey can be treated anaerobically at relatively short HRT values (2-3 days). Theoretically, with a regular daily feeding, higher performances of digester 2 could be obtained but weekend breaks may also have limited the accumulation of VFA often observed with substrates rich in easily fermentable carbohydrates. Either way, these observations show that the way in which co-digestion is performed (feedstock composition, OLR, feeding pattern) can greatly influence anaerobic digestion performances.

The semi dry AD configuration has allowed to gradually stabilize biogas production (Figure 23) and return to favorable conditions in digester 2 (+25%) (Figure 24), even though the amount of water added was lower in digester 2 than in digester 1 (-10%). We believe that, although minimal, this quantity of water helped decrease the inhibitors concentration. It also gradually improved the quality of the digestate in digester 2, which had fallen sharply due to the HSAD configuration used in July and August, and a potentially diminished water retention capacity for substrate 2 (pre-treatment effects).

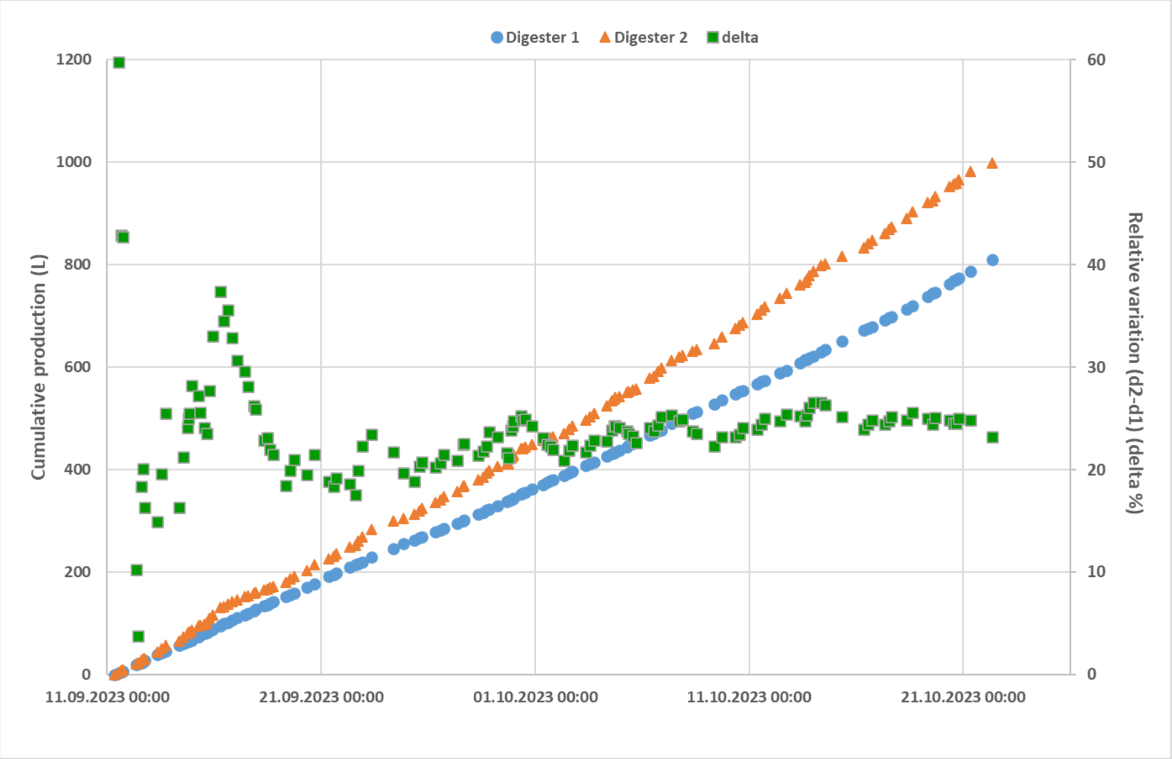


Figure 24 Cumulative biogas production for digester 1 and 2 and relative variation (d2-d1) in semi dry configuration

Nevertheless, we had to limit the organic loading rate, and ideally, we would have worked at 2.5 - 3.0 $kg_{VSS}/d/m^3$. To achieve this, we must further control the operating conditions in HS and/or semi-dry AD processes to optimize digestate quality which is a fundamental aspect in anaerobic digestion of cattle manure. On the other hand, when we finished the tests at the end of October, everything seemed to indicate that if we had continued the tests for longer, we would have had to add more water into the digesters, particularly in digester 2.

Thanks to the stability of the digesters, it was possible to determine the performance parameters of the digesters in semi-dry configuration (Table 16).

The organic matter reduction values are lower for digester 2 than for digester 1. This result is consistent with the fact that, since the hydrolyzed substrate has undergone several pretreatments, some of the organic matter has already been solubilized prior to anaerobic digestion and therefore cannot be measured in TS and VS. Consequently, direct comparison of these parameters between the two digesters is not possible.

Parameters	**D1**	**D2**
OLR ($kg_{VS}/m^3/d$)	1,5 - 1,9	1,5 - 1,9
HRT (d)	48 - 60	48 - 60
TS in (%)	22,3 ± 1,6	17,0 ± 1,0
TS out (%)	11,7 ± 0,3	10,7 ± 0,2
TS removal efficiency (%)	46,8 ± 11,7	37,1 ± 9,2
VS in (%)	69,7 ± 0,7	70,2 ± 0,3
VS out (%)	69,0 ± 0,8	69,5 ± 0,6
VS removal efficiency (%)	48,1 ± 14,0	37,7 ± 10,4
NH_4^+ out (g/L)	1,64 ± 0,11	1,68± 0,19
CH_4 content (%)	56 ± 3	60 ± 3
Methane yield (L/kgVS)	86,6 ± 22,6	131,1 ± 31,6
Methane yield (L/kgFM)	13,5 ± 2,5	15,6 ± 2,8

Table 16 Operation performances for anaerobic digester 1 and 2 in semi dry configuration.

4.3.3 Discussion

During the first phase of WP2, carried out in HSAD configuration, we observed a drastic drop in the performance of digester 2 during the month of August. The deterioration in digestion efficiency in Digester 2 is partly attributed to the rapid accumulation, in the HSAD configuration, of anaerobic digestion inhibitors, in particular the Maillard reaction products identified in the hydrolysed substrates in the previous chapter.

From September onwards, the switch to semi-dry mode with the addition of water to the digesters stabilized Digester 2 and brought it back to production levels significantly higher than Digester 1. It would therefore appear that, contrary to our expectations, the wet configuration is preferable for the hydrolysed substrate. This is consistent with the remarkable performance of digester 2 in July, probably due to the inoculum used to start up the digesters. This inoculum, derived from wet AD, had also been diluted prior to start-up, providing a large quantity of water throughout July.

The addition of water diluted the digestate and reduced the concentration of inhibitors (chemical equilibrium), but also rebalanced other physico-chemical parameters in digester 2 that had been significantly impacted by pretreatments and the presence of MRPs in the hydrolyzed substrate. These parameters include moisture content and water distribution which are major parameters for optimization of dry and semi dry anaerobic digesters.

Hydrophobicity, water content and distribution

Although these parameters were not directly measured in this project, based on the observations and results of the WP2 tests and the data in the literature, it is possible to explain the differences in behavior between digester 1 and digester 2, why digester 1 seems to perform particularly well in the HSAD configuration.

With an average TS content of 17.0%, hydrolyzed substrate has a higher moisture content than manure, which is why it was initially assumed that this substrate would be more suitable for dry digestion. However, some of this moisture comes from the liquid agent (whey), which undergoes several transformations during pre-treatment. The moisture provided by the whey is therefore different from that which would be provided by simply diluting the substrate with water. What is also important in this project is the way in which moisture and water are distributed in the digesters.

There are different types of water in AD which are mainly distinguished by type and intensity of their physical bonding to solid particles.

- Bound water (hydration, vicinal and interstitial water) which is held chemically, physically, or both;
- Free water which is the remaining water content that behaves as the bulk water and acts as a solvent.

During dry and semi-dry anaerobic digestion process, it has been shown that vicinal and hydration water remains intact, and only free water and interstitial water are increased. The destruction of the bond between organic matter fractions and moisture accelerates the transformation and it of mechanically/physically bound moisture into free moisture and the higher the reduction of mechanically bound moisture the better the biomass dewaterability.

Enhanced digestate dewatering is highly desirable and substrate with higher hydrophobicity might dewater more easily (Wu et al., 2017a). Thus, any process that can destroy the bond between organic matter and moisture and/or increase substrate surface hydrophobicity will help dewatering during AD.

Free water and some of the interstitial water can be removed through mechanical dewatering/pretreatment while hydration water can only by removed by thermal energy (that is thermal pretreatment).

In general, proteins are held responsible for changes in hydrophobicity. (Zhang et al., 2020). For instance, heat treatment at 80°C cause protein denaturing (change in surface function) and can alter the protein structure resulting in an increase of the substrate hydrophobicity.

On the other hand, pretreatment methods can crack cell walls of biomass which allows the organic matter present inside the cell to be released into the aqueous phase. Released matter also known as extracellular polymeric substances (EPS) is typically composed of biopolymers such as polysaccharides (PS), proteins, humic acid substances (HAS), nucleic acids, and lipids.

Release of EPS prominently exposes the surface for the microbial cell disintegration process and by shortening the hydrolysis process it can improve anaerobic digestion (Dhar et al. 2012). EPS also play an essential role in aggregate flocculation, settling, and dewatering during anaerobic digestion (Sam et al., 2022). The role of EPS in the dewatering is complex because it depends on the 3D structure of the polymer network in which water can be trapped and on EPS hydrophobicity/hydrophilicity surface properties. Thus, results in the literature are not always consistent with each other, because the nature of the EPS (proteins, polysaccharids, lipids, …) varies according to the substrate and bacterial populations involved but, in general, high release of EPS cause a decrease in dewaterability as negative charged EPS in biomass colloids can bind and entrap a large amount of water. Again, any process/pretreatment that can disrupt polymeric network and/or change increase EPS hydrophobicity, especially that of proteins, will improve digestate dewatering.

In addition to mechanical and thermal pretreatments, the presence of cheese whey and MRPs, can also influence the hydrophilic/hydrophobic character of substrates. Indeed, studies showed that some MRPs in whey milk can modify the functionality and digestibility of products so that the Maillard reaction does not only change the colour and aroma of hydrolyzed substrates but also impacts bioactivities, such as antioxidant activity and hydrophobicity.

As substrate 1 has only undergone coarse grinding, it is assumed that the vicinal water and most of the bound water is still clinging to the organic particles, whereas in hydrolyzed substrate 2, the pretreatments have greatly modified water distribution and hydrophobicity in the substrate and subsequentially in the digester.

This is in line with our experimental observations where we noted that digestate 2 was more compact than digestate 1 and suggests that MIMAGAS pre-treatment is effective for dewatering.

The aim of this section was to demonstrate that, in addition to the increase in methane yield, other parameters can be considered to measure the effectiveness of the pretreatment combination developed in this project. Water content and distribution, substrate and digestate dewaterability and hydrophobicity, among others, can greatly impact anaerobic digestion process and efficiency and should be evaluated in the future.

The impact of the combined pretreatment of this project is complex to analyze due to the many components (mechanical, thermal and chemical) involved. Drawing a parallel with the study carried out by Li et al. (2020) with free nitrous acid (FNA) (Figure 25), we can assume that the combined pre-treatments:

- Cause EPS release in hydrolyzed substrates.
- Partially destroys EPS network structure in hydrolyzed substrates.
- Alters protein structure in EPS.
- Reduce bound water and viscosity and promotes hydrophobicity.
- Enhance biomass dewatering during AD.

In wet anaerobic digestion, reducing vast amounts of biomass by improving sludge dewaterability is the best path to reduce the burden of subsequent sludge transportation and disposal. Enhanced digestate dewatering is still highly desirable for dry anaerobic digestion but we assume that excessive dewatering through pretreatments can also aggravates local accumulation of inhibitors (like MRPs) and must be properly controlled.

Furthermore, as we have already indicated, while digester 2 was entering in an inhibited steady state in August, digester 1 was performing just fine in HSAD configuration probably because its need for water was less pronounced as substrate 1 was less dewatered.

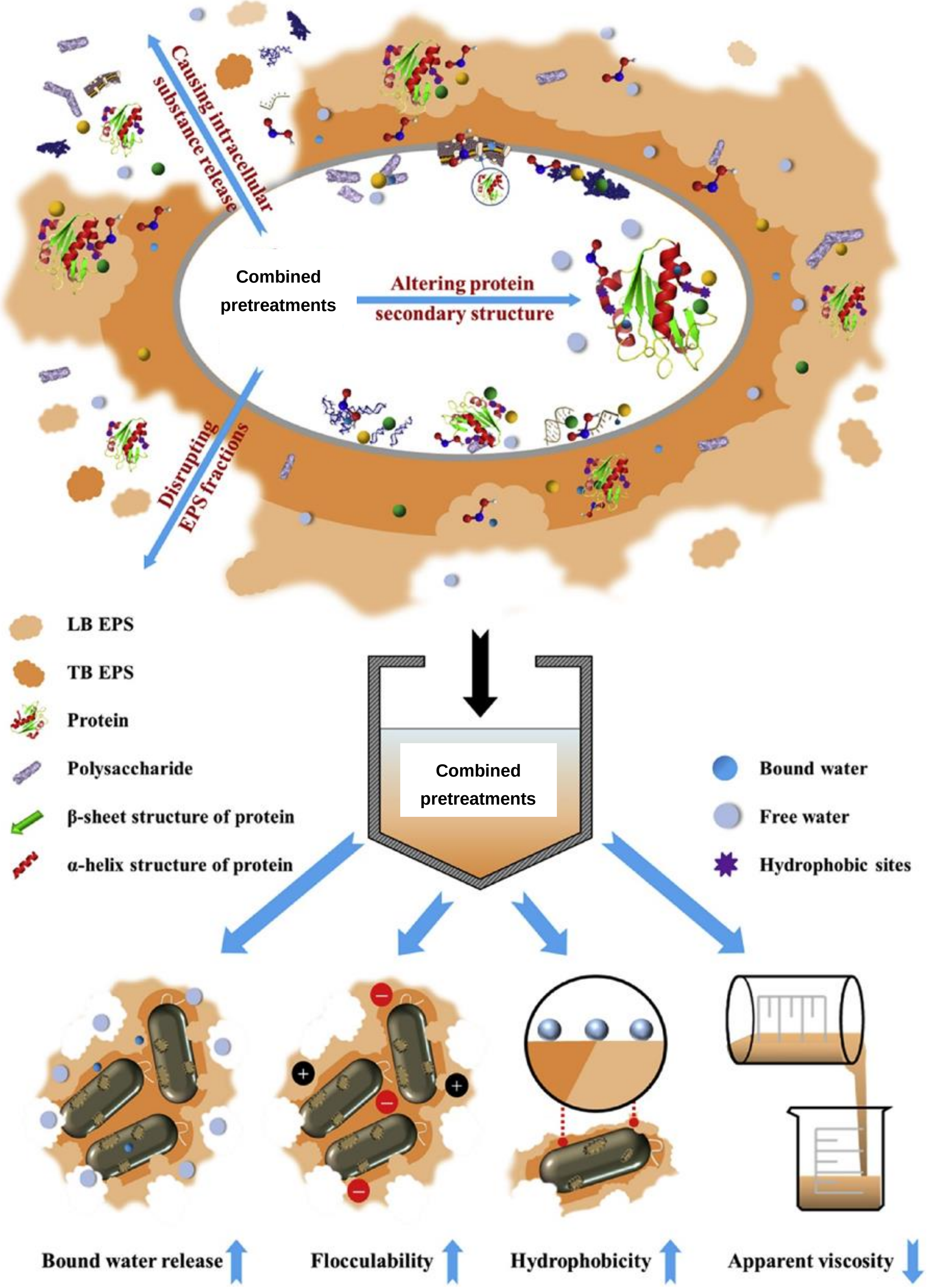


Figure 25 Possible mechanisms for combined pretreatment improving dewatering (Adapted from Li et al., 2020)

Impact of Maillard/caramelization reaction for WP2 tests

Thermal pre-treatments enhance the hydrolysis rate of organic matter during anaerobic digestion because they induce the solubilisation of complex particulate matter, so it is more rapidly and completely consumed during the anaerobic digestion process. Since HT increases carbohydrates and proteins solubilization in a great extent (Wilson and Novak, 2009), Maillard and caramelization reactions are more likely to occur during the pretreatment (Barber, 2016; Gonzalez et al., 2018).

Although browning reaction is known to occur often during biomass pre-treatment, substantive research still lacks in this field (Yang et al., 2022).

As mentioned before in this report, both Maillard and caramelization could form inhibitory compounds, such as furfurals and hydroxymethylfurfural (Bolado-Rodríguez et al., 2016; Monlau et al., 2014), or complex compounds, such as melanoidins, humic acids and caramelans. that are difficult to degrade anaerobically (Barber, 2016; H. Carrere et al., 2010). It is important to consider the problems inherent in such reactions for anaerobic digestion.

Biomass pre-treatments favours the conversion of particulate matter into soluble biodegradable organics which can theoretically lead to an increase of methane yields and methane production rate. Still, Dwyer et al. (2008) found that hydrothermal pre-treatments of sludge at temperatures higher than 150 ◦C highly increase organic matter solubilization, but not methane conversion during subsequential AD. In other words, sCOD can increase considerably during thermochemical pretreatment, but a fraction of this sCOD cannot be exploited during subsequential anaerobic digestion.

Similarly, in this project, we observed that run 9 corresponds to the greatest solubilization of material, but the BMP tests showed that this solubilization efficiency does not involve an increase in methane yield. To illustrate this point, based on the data obtained, we have plotted the methane yield versus sCOD for all the samples measured in WP1. The results are shown in Figure 26 (batch 1) et Figure 27 (batch 2).

In the case of pre-treatments, when Maillard and caramelization reactions occur, two phenomena with opposite trends can occur: the conversion of particulate matter into soluble biodegradable organics with the consequential increase of methane yields and methane production rate and the formation of soluble but recalcitrant compounds leading to an increase in the soluble COD (Gonzalez et al., 2018).

Ortega-Martínez et al. (2021) reported that the thermal pretreatment transformed almost 30% sCOD into refractory compounds owing to the occurrence of Maillard reaction at high temperatures.

Here, the recalcitrant fraction is estimated at around 25% of sCOD is recalcitrant to anaerobic digestion. In the absence of kinetic data clearly demonstrating increased hydrolysis and/or methane production rates, it is It is likely that the conditions of the run 9 are not optimal for enhanced.

The formation of MRPs, greatly favored by cheese whey, must be limited as much as possible even in wet AD, not only because some of them are inhibitors of anaerobic digestion, but also because it implies the consumption of sugars and proteins which then become unusable in AD and also for digestate nutrient recovery after anaerobic digestion. This may require lowering the temperature or reducing the exposure time of the pre-treatment, but to hedge effectively against this phenomenon, it may be advisable to remove the cheese whey from the thermal pre-treatment step which implies that cheese whey would be then only considered as co-substrate.

In addition to reducing the severity of thermal pre-treatment, it may also be necessary to reduce the severity of grinding, as grinding can also promote the Maillard reaction through extra cellular polymeric substances release.

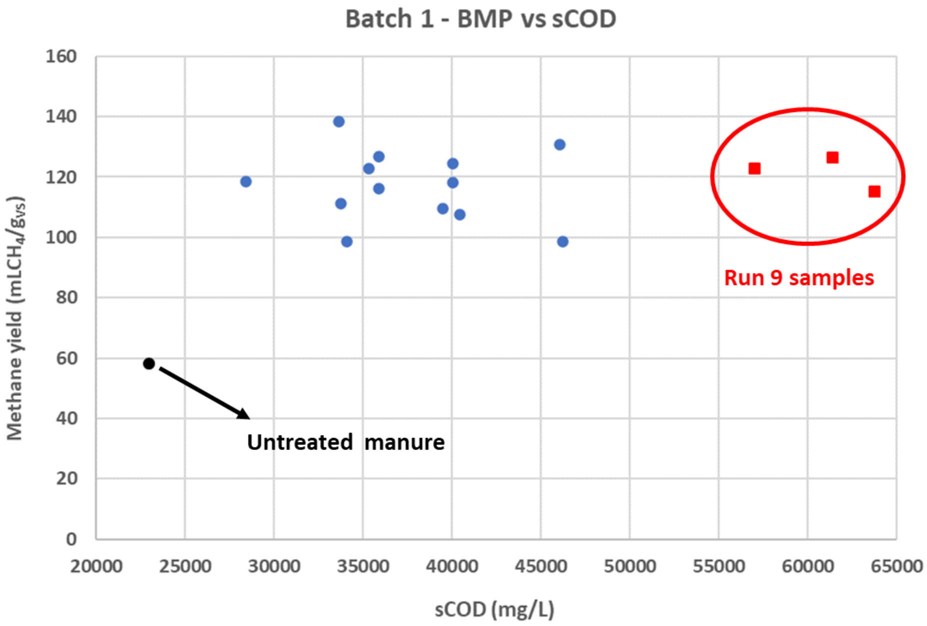


Figure 26 BMP methane yields vs sCOD value (batch 1)

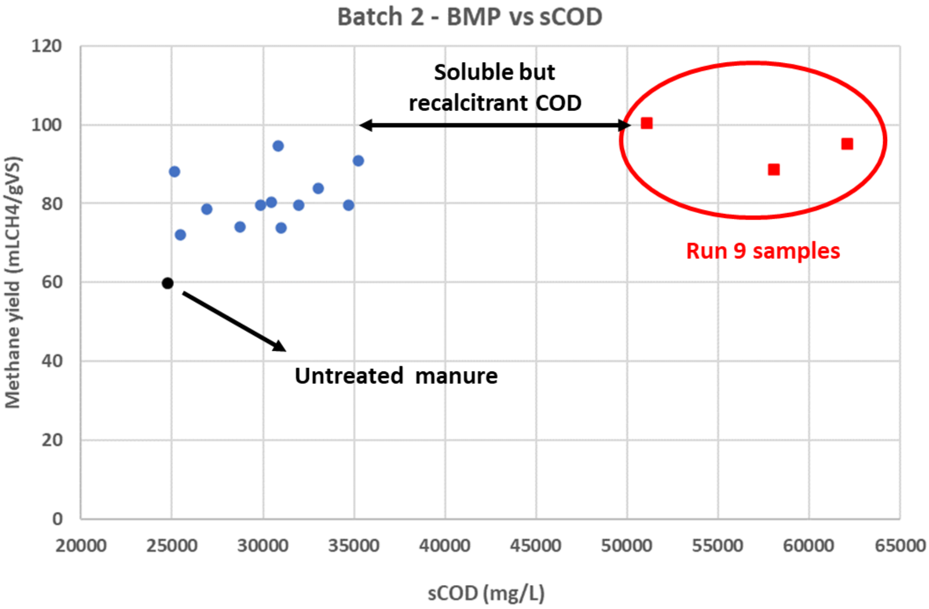


Figure 27 BMP methane yields vs sCOD value (batch 2)

4.3.4 WP2 Conclusions

Contrary to our expectations, the use of the HSAD configuration did not prove beneficial for the hydrolyzed substrates. We assume that our combined pretreatments cause an excessive dewatering process during AD. While this effect is much sought-after in wet AD, it might be detrimental in HSAD configuration where water is already lacking and diffusion limited, allowing local accumulation of inhibitors.

Given the conditions under which we carried out the BMP tests (WP1) and the operating conditions used in WP2 (frequent OLR changes, inhibited state of anaerobic digestion in digester 2, change of configuration), we do not have reliable data on a possible reduction in the minimum HRT for hydrolysed substrate.

While switching to semi dry AD allowed the stabilization of the digesters and provided usable data for WP3, doubts remain as to the effectiveness of the combined pre-treatments approach in particular, the benefits of including cheese whey in thermal pre-treatment are questioned.

In view of all these considerations, and despite the stabilization of digester 2 in semi-dry configuration, run 9 is probably not the most appropriate choice for the tests at intermediate scale, and better results could be obtained with an optimization work.

4.4 WP3: Process scale-up and economic viability

4.4.1 Process scale up

Our strategy for combined pretreatments scale-up is mainly focused on the mechanical pretreatment and the thermochemical pretreatment.

As for mechanical pretreatment, the meat grinder we used already exists in appropriate size and capacity to handle the amount of manure from the Agrogaz plant. The main obstacle to large-scale implementation is the management of rocks and foreign object, sometimes present in the manure, which can seriously damage the grinder knives. It will therefore be necessary to develop a protection system for the knives. On the other hand, the grinder to be used on a large scale must be equipped with an efficient drive system adapted to cattle manure, so that the substrate can be shredded without the intervention of an operator. With this in mind, we have started collaborating with an industrial meat grinder manufacturer to develop a scale-across approach.

As for thermochemical pretreatment, additional work is required for a scale-up process, as the way we have carried out substrate hydrolysis cannot be envisaged for a large-scale application.

Before considering large-scale implementation, we need to determine the optimum temperature for pretreatment and the type of reactor that will enable pretreatment to be carried out on a high solid content biomass.

The ideal approach to thermochemical pretreatment should limit the loss of sugar and protein and the formation of MRPs, while ensuring a high degree of homogeneity in the hydrolyzed substrate, despite its high solids content. Indeed, like the digestion process, pretreatments are strongly impacted when substrates high-solids substrates (dry pretreatments) and appropriate design of pretreatment reactors is required to achieve appropriate mixing and transfer conditions and to ensure correct homogenization of the substrate.

The approach we are considering here is based on the know-how developed in chemical pre-treatment of lignocellulosic materials and the use of helical ribbon stirring reactor in pilot and full-scale cellulosic ethanol production plants. (Figure 28). These reactors have been long and successfully used for high viscous fluids mixing in petrochemical industry and pulp manufacture in paper industry and their use for the pretreatment of high solid content lignocellulosic materials has recently been reported. (Zhang et al. 2015)

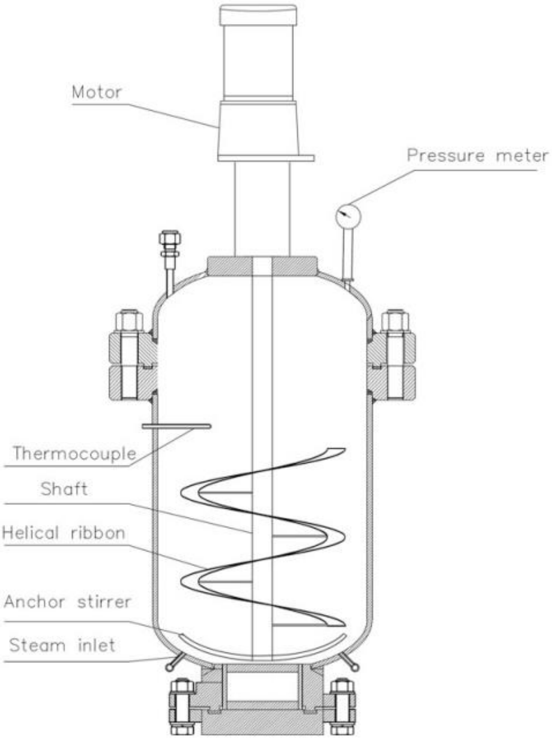


Figure 28 Schematic Diagram pf pretreatment reactor with the helical ribbon stirring (from He et al. 2014)

Before they can be implemented on a large scale, pre-treatments should meet at least three fundamental requirements:

1) Low energy input

   The high energy consumption of grinding/shredding machines inevitably increases biogas production costs. Consequently, low energy consumption meat grinder used in this project offers an economically viable alternative to reduce cost at an acceptable level.

2) Satisfactory pretreatment efficiency at low severity

   Pre-treatments under mild conditions (i.e. at moderate chemical loading, temperature, and pressure) like the one developed in this project impose less stress on reactor, reduce damage and corrosion risks and are supposed to limit sugar loss. However, as clearly demonstrated in this report, the use of whey milk as chemical agent causes degradation of digestible biomass through the Maillard reaction between easily hydrolysable whey sugars and manure proteins. To avoid this phenomenon, it may be necessary to lower the temperature below 80°C, which would further reduce the severity of pre-treatment. However, to guarantee substrate quality for anaerobic digestion, it might be worth considering using cheese whey only as a co-substrate i.e. not including it in the heat treatment.

3) Continuous operation for high productivity
   Productivity is the key factor for large scale implementation. While meat grinders can work continuously and be scaled-up easily, the helical ribbon reactors with high solid loading are still challenging when applied to a large scale because the knowledge of rheological properties of lignocellulosic biomass, and mass and heat transfer in the reactor is still limited.

4.4.2 Economic viability

In our economic analysis, the evaluation of electricity consumption for manure grinding (150 MWh/year) was based on data from an industrial grinder capable of processing the 20 t/day of manure from the Agrogaz plant in less than 3 hours with the highest degree of severity (equivalent to our laboratory's 6mm grate). This is 20 times more electricity than would be required to implement the other components of the combined pretreatment system on a large scale.

Despite certain advantages, the cost associated with the use of additives/chemicals can outweigh the benefit derived from biogas plant revenues. Therefore, a compromise between cost and methane yield optimization must be found. In this respect, the use of a by-product such as cheese whey makes sense as only transportation cost would reduce the benefits.

The economic analysis we have carried out shows that if we manage to control and optimize the way we do pre-treatment, the gains could be substantial (+16% in annual revenues after a 3-year amortization period), even more in a plant like Agrogaz, anaerobic digestion takes place in the wet process, which seems favorable to our process at the moment.

However, in view of all the scientific evidence presented above, before considering industrial-scale implementation, it is essential to determine and optimize the pretreatment operating conditions that will maximize biomethane yield, reduce hydraulic retention time and minimize the formation of MRPs and other DA inhibitors. To achieve this, it may be necessary to re-evaluate the severity of the pre-treatments in the developed protocol. In the case of mechanical pre-treatment, this could also greatly reduce costs so that minimization of grinding duration must be a priority.

5 Conclusions

This research project aimed at developing a mild hydrolysis pre-treatment protocol that can maximize the methane production from lignocellulosic material, by combining mechanical action, diluted organic acid impact and mild thermal action.

First, an exploratory analysis based on an experimental design derived from Taguchi's method was used to identify the most favorable combination of pretreatments, i.e. the one maximizing soluble chemical oxygen demand and methane yield. Pre-treatments under mild conditions are designed to reduce the formation of toxic or inhibiting compounds. However, in this project, the use of cheese whey, a substrate rich in easily hydrolysable carbohydrates (sugars), as chemical agent has proved to enhance the caramelization and Maillard reactions that can occur within sugars and between sugars and proteins. The formation of secondary products, resulting from these parasitic reactions may have biased the results and influenced the conclusions of this study.

Although the Maillard reaction is known to occur often during biomass pre-treatment, substantive research still lacks in this field. In this context, the MIMAGAS results are important because they add to the findings of the scientific community on the subject.

During the first phase of the intermediate scale tests, carried out in dry configuration (also known as high solid anaerobic digestion HSAD), a drastic drop in the performance of digester fed with the hydrolyzed substrate was observed. The deterioration in digestion efficiency in digester is attributed to the rapid accumulation, in dry configuration, of anaerobic digestion inhibitors. In addition, we assume that Mimagas combined pretreatments cause an excessive dewatering process during AD. While this effect is much sought-after in wet AD, it might be detrimental in dry configuration where water is already lacking and diffusion limited, enhancing the local accumulation of inhibitors.

This suggests that our pre-treatment protocol could be more efficient in wet AD configuration than HSAD configuration but tests with hydrolyzed samples free of by-products should be done for confirmation.

Whatever the configuration envisaged, our process can only be applied on a large scale if we fully master it, i.e. if we manage to limit or even eliminate parasitic secondary reactions (Maillard/caramelization). This could involve reducing the severity of the physical components of the pretreatment or eliminating the chemical component by removing the cheese whey from the thermochemical pretreatment and using it only as a co-substrate.

Furthermore, we have observed that the effectiveness of pretreatments can only be properly assessed if other digestion parameters are also considered, such as water distribution, a fundamental parameter for dry AD, and if rheological properties are further characterized, in particular to determine whether or not the pretreatment combination is suitable for any type of AD configuration.

Eventually, the economic analysis carried out on the results obtained in semi-dry configuration showed that despite moderately favourable conditions, substantial gains could be achieved (+16% in annual revenues After a 3-year amortization period).

6 Outlook and next steps

Pre-treatment full control and optimization

The next step will be to control and optimize the combination of pretreatments in order to avoid the presence of inhibitors resulting from the parasitic chemical reactions highlighted in this project, and to be able to precisely quantify the increase in methane yield and the potential reduction in hydraulic retention time by means of reliable kinetic analyses.

Based on this information and rigorous characterization of rheological properties, we will be able to take a clear position on the benefits of pretreatment for wet, semi dry or dry AD.

Towards pretreatment on an industrial scale

As for mechanical pretreatment, the meat grinder we used already exists in appropriate size and capacity to handle the amount of manure from the Agrogaz plant. The main obstacle to large-scale implementation is the management of rocks and pebbles, sometimes present in the manure, which can seriously damage the grinder knives. It will therefore be necessary to develop a protection system for the knives. On the other hand, the grinder to be used on a large scale must be equipped with an efficient drive system adapted to cattle manure, so that the substrate can be shredded without the intervention of an operator. With this in mind, we have started collaborating with an industrial meat grinder manufacturer to develop a scale-across approach.

The intensive energy consumption of grinding/shredding pre-treatment methods inevitably increases the biogas costs. Although the use of a meat grinder with low-energy input seems to be a promising economic alternative to reduce energy consumption to acceptable levels, every minute of processing is costly, and minimizing processing time will be our priority.

For thermal pre-treatment, we will focus on two possible approaches. The first is based on helical ribbon stirring technology which can be effective in treating substrates with a high solids content. The second is to overcome the problems associated to high solid contents substrates by adding the liquid part of manure. In both cases, deeper knowledge of the rheological properties of proximate formulation would help in designing an appropriate pretreatment.

It should be noted that, due to the interactions between the pretreatments, the order in which we carried out the pretreatments (mechanical then thermochemical) may have had an impact on the results. Thermochemical pretreatments can be preferentially carried out before mechanical pretreatments, as they might reduce the energy required for grinding. We plan to answer that question before large scale implementation.

7 National and international cooperation

This project has not been the subject of any national or international collaboration beyond that with the company Agrogaz Lignerolle SA.

8 Communication

Part of the results of this project were presented in the frame of the "Journée de la recherche sur la bioénergie en Suisse" on the 25th of April 2023 at the Eventforum in Bern.

9 Publications

The results of this project has not yet been published.

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11 Appendix

APPENDIX 1: Critical analysis of the Taguchi Table Design – Importance of Interactions

Designing a suitable experience plan requires to understand the various pretreatments used, to better appreciate the possible interactions and the levels to be chosen.

By considering interactions between factors, we could refine our results and increase the robustness of our models. However, as soon as we include interactions between factors, the number of experiments to be carried out increases considerably. It is impossible to take them all into account, and we must limit ourselves to the most important interactions and leave out those that are negligible. This requires a thorough understanding of the characteristics of the substrates and pre-treatments applied. However, as there are still gaps in the knowledge about pretreatment, specifically its application to dairy cow manure, prior screening might be required.

Figure A1-1 shows the graphical representation of the model used in this project (approach without interactions) and that of a model with interactions that could be used to evaluate our pretreatment combination.

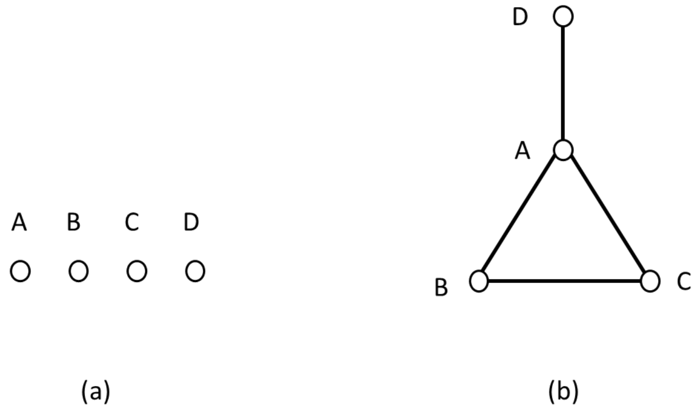

Figure A1-1 : Graphic representation (a) of the model with interaction used in the MIMAGAS project (b) of a possible model with interactions

Since the second model contains both joint (e.g. AB and AC) and disjoint (e.g. AD and BC) interactions, the standard table to be used for this model is a L81 table, i.e. the complete design. While the Taguchi method allows many variables to be studied with a small number of experiments, it is easy to see that this type of experimental design is nevertheless not suitable for studying three-level models with disjoint interactions.

If we neglect the AD interaction and one of the joint interactions, we can then use a standard L27 table, but this remains inadequate for us, as the realization of a standard L27 table would require 3 series of BMP tests, i.e. 6 series in total if we wish to repeat the experimental design.

Most industrial problems can be solved using small, standard tables with less than twenty trials. However, in research, there are cases, such as ours, where standard experimental designs are not suitable.

When facing this situation, the first thing to do is to format the problem to make it compatible with standard Taguchi tables. In our case, we could have reduced the number of experiments by reducing the number of levels for each of our factors. This approach would be reasonable as the relevance of certain levels is disputable.

However, we will still have to deal with the interactions and if curvature is suspected in a model, a 3-level approach is needed to locate the curvature in the response surface.

Thus, it is sometimes necessary to transform standard tables to adapt them to the problem to be solved. For example, if we cannot reduce the number of levels, then we need to consider creating our own Taguchi design from the standard tables, but this approach requires in-depth knowledge of experimental designs.

On the other hand, there are other types of design perfectly suited to the study of complex response surfaces, requiring far fewer tests for this type of model. For instance, Response Surface Methodology (RSM), which has been found to be reliable and effective for the design of experiments in bioprocesses, can used to study process optimization (Olatunji et al, 2023) (Sathish et al., 2016).

Taguchi methods are often preferred when the focus is on robustness and minimizing variation, and as such, it is an essential step for process scale up. In this project, RSM method could have been used for prior screening to determine interactions among the control factors and predict the optimum process conditions for the experiment. Prior screening work is time consuming, but it would have help us defining the Taguchi experimental design in a more efficient way especially when it comes to interactions, and it would have saved us time and energy thanks to a better understanding of the process.

APPENDIX 2: Effect of fermentation on lactic acid production

A lactic acid solution can be produced by a simple fermentation of milk whey with lactic acid bacteria (LAB). Four types of milk whey coming from small local cheese factories will be fermented in three different temperatures; 38, 40 and 44 °C, for approximately 29 hours to increase lactic acid concentration. The results will then be compared to evaluate the difference between the milk whey types and the effect of conversion. Milk whey fermented slowly at room temperature for a period of 1 month will also tested to evaluate whether the difference in lactic acid concentration can establish energy gains.

A2-1 shows the lactic acid concentration produced during 29 hours of fermentation of the four different types of milk whey at different temperatures: 38°C, 40°C and 44°C.

Figure A2-1: Lactic acid concentration of four types of milk whey during 29 days of fermentation at different temperatures; 38°C, 40°C and 44°C (n=3)

The milk whey from Villars-Burquin, Bory and Gros de Vaud comes from conventional farming, while that from Pomy comes from organic farming. The lactic acid concentration in all milk whey increased exponentially during the fermentation period for all tested temperatures reaching a maximum between 0.9 - 1.3%.

A2-2 shows the conversion percentage of the produced lactic acid based on initial lactic acid concentration already present in the milk whey.

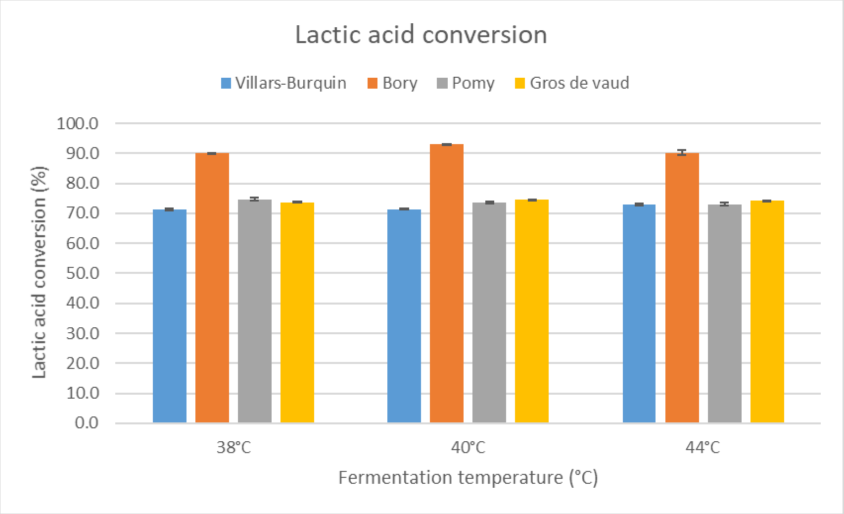

Figure A2-2: Conversion to lactic acid concentration at the end of the 29 days fermentation at different temperatures; 38°C, 40°C and 44°C (n=3)

The milk whey sampled from the factories of Villars-Burquin, Pomy and Gros-de-Vaud present a similar lactic acid conversion of around 72-75%. On the contrary, the milk whey from the cheese factory of Bory shows a conversion of 90-93%.

To evaluate whether the need of a temperature-controlled fermentation is required for a maximum lactic acid production, milk whey already fermented for 1 month at room temperature was mixed with fresh milk whey in different fractions, 0, 5 and 10%. Tableau shows the experimental conditions and the results obtained.

	Test A	**Test B**	**Test C**
Milk whey fermented at room temperature for a period of approx. 1 month (%)	-	5	10
Fermentation temperature (°C)	38	38	38
Initial lactic acid concentration (%)	0.379	0.438	0.528
Final lactic acid concentration (%)	1.265	1.263	1.235
Conversion of lactic acid (%)	70.0	65.3	57.2
Final lactic acid concentration (g/L)	15.180	15.156	14.820

Tableau 1: Conditions of milk whey (Villars-Burquin) fermentation experiment using a fraction of already fermented milk whey (a period of 1 month), and lactic acid concentration results

The use of already fermented milk whey does not show any significant effect on the production lactic acid when compared to the results shown in A2-2.