Identifying the Most Optimizing Methods and some Influential Conditions in Methane Yield out of Olive Wastes

(A Comprehensive Meta-Analysis on Biochemical Methane Potential Tests)

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Abstract

The organic wastes including olive oil mill residues are an inseparable part of food manufacturing processes while implying multi-faceted damages to the environment. A good quantity of research has been conducted to examine the biogas enhancement level in the anaerobic process of olive residues. Seeking the optimum pretreatment method and the co-digesting substrates, the current study has conducted aggregative research on 155 experiments out of 22 studies. The conducted meta-analysis recognized the chemical type of pretreatments as the most effective treating procedures, according to which, application of the combined alkaline and lime, followed by trace metal cobalt supplementations are recognized as the most effective methods. Furthermore, the study found intriguing results on the optimum type of olive main substrate, inoculum, digester type and effective volume as well as the superior country and year, in the anaerobic digestion of the olive mill residues.

Keywords: Methane, anaerobic digestion, treatment, olive wastes, pretreatment, co-digestion
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A Note on maps: The maps were built Using the map builder via the link: [https://mapchart.net/world.html](https://mapchart.net/world.html) by the following steps: 1. Setting countries colors, background, borders, names, 2. setting the legend by adding title and the label for each color, 3. Extracting the map. However, the map 1 is grounded by the information derived from World Olive Producer Countries, as cited in the map caption. And map 2 is grounded by the current study results in chemical subgroup: country.

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<th>Description</th>
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<tr>
<td>AD</td>
<td>Anaerobic Digestion</td>
</tr>
<tr>
<td>BMP</td>
<td>Biochemical Methane Potential</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<td>GHG</td>
<td>Green House Gases</td>
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<td>IEA</td>
<td>International Energy Agency</td>
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<tr>
<td>IPCC</td>
<td>Intergovernmental Panel on Climate Change</td>
</tr>
<tr>
<td>IRENA</td>
<td>International Renewable Energy Agency</td>
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<tr>
<td>OMW</td>
<td>Olive Mill Wastes</td>
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<td>PRISMA</td>
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<tr>
<td>SD</td>
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<td>TS</td>
<td>Total Solids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile Solids</td>
</tr>
<tr>
<td>WOS</td>
<td>Web Of Science</td>
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</table>
Map 1: the global disparity of olive product, based on the data provided by the Worlds Olive Producer Countries (WOPC, 2021)
1 Introduction

1.1 Background

As astute, sophisticated competent and experienced beings, we require to design the present and future based on the previously attained experiences and learnings. This knowledge, along with constructing the existing living structures, produces preparations to face the coming issues. On the strenuous route to uphold the economic, environmental and social developments, as the sustainability pillars, it is required to redefine and reconstruct the scientific infrastructures. To this end, and to comply with the ever-transforming patterns of economic, social and environmental development, it is necessary to revise the energy production structures. To tailor energy production structures to new circumstances and conditions of the transforming world, not only the prudent choice of energy, but the generation methods are of high significance and have to be reexamined. Transition to biofuels as clean and renewable sources of energy, though may seem a rather modern topic that started after industrialization, has a long time throughout history.

Incontestably, the history of biofuels as the human’s earliest source of energy predates the fossil and electricity sources of energy, which had been applied in the forms of vegetable oils, animal fats, crops based ethanol and wood-based methanol. (Kovarik B, 2013 quoted Bailey, D.M. 1975). In 1903, Henry Ford, designed T model car, burning organic seed oils, and asserted, “there is enough alcohol fuel in one year yield of an acre of potatoes to drive machinery to cultivate the fields for a hundred years.” Further, in 1925, he called ethyl alcohol originating from by-the-road sumacs, fruit, weeds, sawdust or any organic and fermentable vegetable, as the “fuel of the future”. During his lifetime, He had always dreamed of the epoch of the prosperity and development obtained by the organic choice of energy. Meanwhile, it was 1912 when Rudolph Diesel, the German inventor of ignition engines, maintained that the palm, peanut or castor oils, and even sun heat could work adequately well to produce energy. (Kovarik B. 2013, quoted Knothe, 2011) In fact, It was no sooner than 1925, when the energy potential of cellulose, the earth’s most ample organic substance appeared in both scientific and popular literature. (Kovarik B, quoted New York Times, 1925, 1938). Notwithstanding, the advent of industrialization, flashed the shift to fossil fuels in mid 19s, providing cheap and available sources of energy. As they ignited the industrial revolution, the organic feedstocks faded gradually. (Kovarik B, quoted Newyorktimes, 1925,1938)However, the recent drastic climate changes, climate extremes, environmental disasters, energy scarcity and the continuous need for development made us return to organic energy sources. (Guo M., 2015) Though extracting biogas from organic wastes, which is done through a set of complex microbial processes
in the absence of oxygen called anaerobic digestion (AD), was first practiced in 17th century, it was not completed until the past century. (Pennstate, 2012)

1.1.1 Major Concepts

Biofuel implies an ample number of liquid, solid and gaseous types of fuels, and applies to any combustible fuel derived from biomass, or any recent non-fossil living matter, including plant-based ethanol, plant or animal-based biodiesel and biogas. (UF, 2021) The gaseous type of biofuels or biogas which is central to this paper, consists of methane and CO2, while the fraction of methane varies between 60-80% of the produced biogas. (Based on the author's knowledge from the studied supplemented articles). Methane production level, however, is the central subject of the current paper.

Investigating methane generation and optimization implies and necessitates an adequate knowledge of the methane production process, including some key concepts. In this respect, these concepts as well as the studied parameters are defined and motivated. However, the detailed specifications of the studied parameters are provided in Table 1.

**Anaerobic digestion:** Implies a process of extracting biogas, and more importantly methane, as the end product from organic wastes, which is done through a set of complex microbial processes to the breakdown of the biodegradable materials in the absence of oxygen. (Bhatia S.C., 2014). Proteins, lipids and carbohydrates, to respectively turn to amino acids, fatty acids and sugars, are considered the essential pillars of the process. (Doble M., et al, 2005) In this process which consists of several steps, the organic matter by several reactions, via communities of bacteria and in a fermentation process is converted to methane and carbon dioxide. (Doble M., et al, 2005). Through the anaerobic process, the organic fraction is broken down to firstly the amino and then to fatty acids, and secondly to the turn to volatile fatty acids, ammonia, CO2 and other by-products. The produced acids and alcohols are ultimately converted to methane and CO2. (Thompson D.A. et al, 2018). Hence, assessing the organic fraction of the substrate is considered a determining factor in the estimation of the methane yield within the anaerobic process. Researchers use a variety of methods to measure the organic fraction of the substrates. (Estimating COD and VS have been the major methods utilized in the studied articles)

**Bio-mechanical Methane Potential:** On the transitional route toward standardization of the AD process, and optimization of the generated methane (as the end product of the AD process), a standard framework was established to define the baseline for operational parameters, as well as to
evaluate the substrate qualities and to foresee the highest of methane produced. (Hollinger, C., et al, 2016) The framework named Biochemical Methane Potential (BMP) test further determines the ultimate methane of the organic substrate assessing the impact of treating methods on optimizing biogas value. (Hollinger, C., et al, 2016). The test hence is applied as a prominent systematic technique to estimate the organic solid or liquid wastes capacity and biodegradability to transform to bioenergy. (Filer J. Et al, 2019).

**COD:** Chemical Oxygen Demand, is the amount of oxygen utilized to oxidize the organic matter of the substrate and is regarded as a measurement method to determine the organic content of the substrate. (Colin M.W. et al, 2015)

**VS:** volatile solids, is regarded as another measurement for the organic content of total solid (TS or the dry matter content of the substrate), and is measured normally as a percentage of TS in grams per kilogram. (Ejiroghene Kelly O. Et al, 2017). The VS is determined by the organic loading rate and the effective, working volume fed to the digester each day (Babae A., et al, 2011) The amount of the removed substrate organic content (shown by VS, COD) determines to what extent the substrate is biodegradable or can be decomposed to microorganisms, or to what extent it contain organic carbon to feed these microorganisms, which will turn to the end carbon products such as CO2 and methane. (Angelidaki, I., et al, 2004). Therefore the organic contents are significant in determining the methane potential.

The TS and VS contents affect the performance of the anaerobic process, and are determining for the methane yield volume, thus to improve the efficiency of this process, it is necessary to estimate VS value and promote it through a number of pretreatments. (Ejiroghene Kelly O. Et al, 2017). Moreover, the BMP is estimated by the volume of dry matter under standard conditions (1atm of pressure, 0 degree C) per mass of volatile solids with the unit: Lch4KgVS-1 (Holliger C, et al, 2016)

**Pretreatments and their functioning mechanism:** are the treating measures used to enhance anaerobic process efficiency, improving methane yield. They are able to decrease the cost for post-treatment of the substrates, however, not all pretreatment methods are successfully applied. (Ariunbaatar J. Et al, 2014). These measures turn digested to a simply digestible and fermentable sample and function in various ways depending on the type of substrate and type of pretreatment. They affect the matrix in chemical oxygen demand, phenol contents, as well as the composition of lipids, carbohydrates and proteins. (Elalami D., et al, 2020), and improve the substrates biodegradability by various mechanisms:

- **On COD:** the removed chemical oxygen demand, as a measurement for the organic material in the substrate, is regarded as the criteria for anaerobic performance. (Colin M.W. et al, 2015) Pretreatments need to enhance the removed COD and raise the soluble chemical oxygen demand (sCOD), leading to optimizing the substrate’s biodegradability, resulting in methane optimization.

- On PH: Methane yield is optimized with increasing PH, and some pretreatments are capable to raise PH level (Al-Mallah J., et al, 2016)

- On carbohydrates: given the fact that through the anaerobic process carbohydrates have to be turned to sugars, pretreatments ease this transition, and therefore increasing the sugar level, can be a sign for a smooth anaerobic process and methane enhancement. (Elalami D., et al, 2020)

- On fibre: in order for a smooth and uncluttered anaerobic process, fibre such as cellulose, hemicellulose, and lignin are required to be decomposed. Some pretreatments increase salvation and solubilization of the substrate, which helps fibre degradation, leading to disruption of the lignocellulosic tissue, and subsequently to increased methane value. (Rincon B., et. Al, 2014)

- On lipids: lipids as one significant pre-requirement for anaerobic digestion, are to be decomposed in this process. The treating measures capable of disintegrating the lipid particles into soluble materials are influential in methane yield. (Rincon B., et. Al, 2014)

**Pretreatment categories:** They undergo various categorizations in different literatures. For instance, some set them in mechanical, thermal, chemical and biological pretreatments, (Ariunbaatar J. Et al, 2014) While others have set them in more detailed categories as, mechanical, microwave, thermal, ultrasonic, dilute acid and alkaline pretreatments. (Elalami D., et al, 2020). However, the majority of the studies maintain three main categories for pretreatments: chemical, physical, biological (Anu A., et al, 2020) (Donoso-Bravo, A., 2016). The existing study, however, (merging thermal and mechanical as a physical category), utilizes the physical, chemical, biological pretreating category, and the combination of physical and chemical ones (since a good number of studies applied the combination of these categories). Figure 4 and table1, illustrates these set categories and the detailed treating measures applied. (Figure 4) (table 1).

**Co-digestion:** to compensate for the anaerobic limitations regarding the feedstock composition, the simultaneous digestion of various substrates, called co-digestion, is applied. The method possesses the capacity to upgrade digestibility via the synergic impact of the substrates and enhanced stability. (Karki R., et al, 2021)

**Inoculum:** The choice of inoculum is regarded as a determining factor of the anaerobic process, and the output biogas. Inoculum as the microorganism rich mixture added to the digester, providing necessary bacteria to drive the anaerobic process, has been one of the crucial factors affecting the methane generation through anaerobic digestion, as it can lead to different biodegradability results. (Raposo F. Et al, 2012)

**Bio-digester type:** continuous, semi-continuous and batch bio-digester is utilized, in batch digester types in which the total substrate is added in the initial step, provide simpler assays and therefore most widely used, compared to continuous set-up, in which the digestate is added in
multiple steps and subsequently the latter is more laborious and time-consuming. (Doble M., 2005).
In this study, both conditions, batch, as well as semi-continuous set-ups have been utilized within the
studied assays.

**Working volume:** Working volume, and the concentration of the substrate, has also been
considered significant operational condition. It determines the organic loading rate, indicating the
volatile solids to be fed each day to the digester (Babae A., et al, 2011) In too low substrates
loading, there will be very low metabolic activity and consequently the low amount of produced
biogas, while in high loadings, building up the volatile fatty acids can result to biogas inhibition,
however, as Raposo asserts, the choice of volume is highly dependent on the type of utilized
substrate, as the accurate estimation of the methane yield in the homogeneous materials, necessitates
a lower reactor volume. (Raposo F. Et al, 2012). Generally, it seems that the preferred bio-digester
set-up and its optimum working volume are dependent on the substrates being digested.

1.2. Specific Justification for Research

The extraction of bioenergy which is specifically accomplished through the anaerobic digestion
(AD) process and assessed through the standardized BMP test, emerges a set of consequences, which
are subject to discretion and prudence. Since this has been a costly process, one of the crucial
concerns in bioenergy generation, is economic feasibility and efficiency. (Solomie A., et al,
2010)Prior to establishment, the economic performance in the segments including biomass, labour,
transportation, water and electricity, operation and maintenance, the overhead and depreciation costs
and expenses have to be estimated. (Solomie A., et al, 2010). Furthermore, as per Thompson and
others, AD can close the loop of a circular economy by the energy recovered from organic wastes,
provided that an efficient and well-managed AD is conducted. The absence of efficiency in AD, of
the anaerobic process, can affect the generated methane level and subsequently, the cost of electricity
generated from AD. According to them, better design in the whole process and optimizing methane
generation can promote AD efficiency. (Thompson D.A., 2018)

To conduct such a costly process necessitates prior investigations in form of experiments for
higher efficiency. For efficient production of biogas, the treating procedures have to be examined in
order to decide on the most bioenergy optimizing ones. To this end, it is required to test various
forms of a biomass substrate, operating conditions multiple supplementations and any pretreatments
which may interfere with the anaerobic process. More importantly, an integrated study, aiming to
aggregate the existing tests are required to determine the optimum treating methods for a finalized
treatment.
1.3. General Justifications for Research

This set of justifications is based upon the tripled gainings the green sources of energy can provide. As an organic waste, olive residues possess capabilities of a mature feedstock, (in section 1.3.5, these characteristics are elaborated) and the potential to be transformed into biogas, meanwhile, irresponsible disposal of olive oil extraction mill wastes, brings environmental consequences, and the prudent collection and application of them can be of a great gain. Further, olive residues as biomass can compensate for the energy scarcity within the olive producing regions. The crucial role of bioenergy in global development, and the great achievements it brings, can highlight the urgent need for an integrated meta-research in biogas enhancing treating procedures on the olive organic wastes. These justifications are presented in four approaches, a detailed viewing from four perspectives.

1.3.1. Waste Management Approach

The olive oil extraction stream produces large quantities of waste. Based on the most recent International Olive Council statistics on olive and olive oil production, the world’s olive oil production within the 2018/2019 period, reached 3,262,000 T, which has been tripled compared to 1959. (IOC b, 2021). Assuming the fact that to produce each litre of olive oil, four litres of waste is generated (Vossen p., 1995), reveals that annually more than twelve million tonnes of olive wastes are generated on the global scale. This load of oil production, imposes a high load of wastes on the natural environment, affecting land, air and water.

Olive wastewater and pomace as the wastes produced by Olive industry streams can be beneficial and applied for various utilities, if retreated and recovered through engineering processes, however, they can damage the environment when disposed of unstudiedly. Normally, the world’s large olive oil producers do not disclose the exact oil extraction processes and the waste disposal manners they apply. However, abundant documents on the OMW (olive mill waste) disposal methods, unveils the destiny of OMW, when not treated and recovered for a sustainable utility. (IOC a, 2021) According to Souilem and the co-writers, OMWW (olive mill wastewater) are oftentimes discharged in evaporation ponds which make odour nuisance, which can inhibit plants growth and pollute streams when disposed on soil and water respectively. (Souilem S., et al, 2017) In fact, OMW is regarded as one of the most contaminant compounds produced out of agro-food industries. As stated, the application to the soil in long term can damage and degrade soil due to the high organic load,
phenolic compounds and fatty acids. (Souilem S., et al, 2017) The high quantity of organic matters and the phenolic property makes them resistant to biodegradation and phenolic content make them highly phytotoxic. In water, they restrict living organisms by lowering oxygen concentration, moreover, they accelerate eutrophication and algae formation by increasing phosphorous. (Souilem S., et al, 2017). Other researchers also assert although disposing OMWWs on soil has a positive effect in short, they can damage the soil structure if applied without enough study and engineering. They can subsequently damage the soil hydraulic conductivity (especially for clay soils) due to the high content of salt, and can also harm soil germination. (Barbera A.C. et al, 2013)

Based on the olive oil statistics, Europe and Mediterraneans as the main providing regions and Spain, Italy and Greece as the producers of 70% of the total world olive oil (IOC c, 2020). The countries collectively produce some 12,647,796 tons of olive product. (FAO, 2021) Through a collective look, it can be understood that the huge quantity of OMW for the producer countries and specifically to the manufacturers and the surrounding environment may produce a high pollution load and be catastrophic. In addition, regarding the industry’s fast development during the recent years, and the high probability of more growth in the near future, olive mill wastes management will be of greater concern.

1.3.2 Climate Mitigation Approach

Based on the scientific IPCC report on climate change, the Physical Science Basis, sea-level rise, ice melting and ocean acidification as the main indicators of climate change that occurred due to the warming temperature. Warming earth as a touchstone to climate change was occurred through the greenhouse effect, the phenomenon which happens upon reaching sun irradiation to earth’s surface. While the short wave energies heat the surface, the long wave energies reflect back to the atmosphere and are absorbed by GHGs and trapped by them in the lower layers of the atmosphere, and consequently less heat is returned to the atmosphere and the earth gets warmer. (IPCC, 2013)

The GHGs as the main responsible factor for the rising temperature has been a human-influenced phenomenon. (IPCC, 2013) Based on this IPCC report, the anthropogenic activities specifically since the industrial revolution onwards, have been the major sources of the greenhouse gasses, leading to global temperature rise and consequently climate change. (IPCC, 2013)

The IPCC report on mitigating climate change defines climate change mitigation as limiting the sources of climate change as well as sequestering greenhouse gases. (IPCC, 2014) As the report explicit, despite the increasing number of mitigating options, there has been a sharp rise in total anthropogenic emissions. From 1970 to 2010, the annual GHG emissions growth from 1.3% in the period 1970-2000, reached 2.2% during 2000-2010. Seemingly, the report demonstrates that the
generated CO2 out of fossil-fuel combustion accounts for some 78% of the total GHG growth in the entire period 1970-2010. This report maintains that the energy supply, industry, transport and buildings have been responsible for 35%, 21%, 14% and 6.4% of the total emitted greenhouse gases respectively. (IPCC, 2014) Based on the report, as long as the population growth and economic development as the main drivers still persist, unless the compensatory measures to reduce emissions are taken, there will be persistent emission growth. (IPCC, 2014) In this respect, the IPCC special report on renewable energy sources and climate change mitigation (IPCC, 2011 b), in a comparative assessment, presents the ranges of GHGs released out of bioenergy systems as well as the fossil-based alternatives. The evaluation implemented in transportation, electricity and heat sectors, assessing major modern bioenergy sources (agricultural by-products, lignocellulose biomass, algae and plant-based oils) as well as advanced fossil-based energy systems. In general, the bio-energies produce far less life cycle GHG emissions than the fossil-based systems, this is true for the detailed comparison between the biogas and natural gas in the transportation sector as well. Furthermore, a large discrepancy can be observed in heat and electricity sectors wherein heat sector fossil-based systems emit two, and in the electricity sector, emit ten folds more GHG compared to modern (above mentioned) sources of bioenergy. (IPCC, 2011 b). Since bio-energies correspond to far lower greenhouse gasses emissions, they possess the capacity of compensating for the climate changes imposed by high-emitting fossil fuels. In this respect, the biogas generated out of olive organic residues (as a form of bioenergy), with ways less emission released, can be a proper substitution for fossil resources, having a great role in compensating GHG growth and climate change impacts.

1.3.3 Analytical Approach
Toward sustainability goals

Paris Agreement, a United Nations Framework Convention for Climate Change (UNFCCC, 2015) agreement, signed and contracted between the parties on climate mitigation, adaptation and finance in Paris. The number of 196 member states, ratified and mandates limiting the global temperature increase, as the agreement’s main objective. (Paris Agreement, 2015) As a prerequisite, the agreement emphasizes recognizing sustainable patterns for production and consumption. The agreement further, recognizes climate change as a “common concern” to humankind. Section 1 of Article 2, explicit that in order to reduce the global climate change hazards and impacts, states have to limit the world’s temperature increase to 1.5 degrees Celsius above the pre-industrial level in the best state. (Paris Agreement, 2015) This section of the article further stresses addressing the harmful effects of climate change and minimizing GHG emissions. Section 1 of Article 2, states that to limit the long term temperature rises, parties have to preserve the allowed quantity of emissions, for rapid emission reductions. they also have to take the advantage of the best science at hand to limit
anthropogenic emissions in the second half of the century. The enforcement of Nationally Determined Contributions by Paris Agreement, however, has been a measure to pursue the countries mitigating goals. (Paris Agreement, 2015)

To pursue the agreement’s provisions in emission reductions, switching to clean and renewable sources of energy, for instance, those attained from organic wastes has been a directive strategy. Based on the International Renewable Energy Agency (UNFCCC, 2017), in order to meet the central aim of the Paris agreement, clean energies are able to commit to the 90% reductions of energy-related emission.

1.3.4 Energy Security Approach

A significant concept emphasized by the major international institutions such as IPCC, the International Renewable Energy Agency (IRENA) (2021), and the International Energy Agency (IEA) in the context of renewables and sustainability, is the security in energy. Grubb and the co-writers define it as the durability of energy supply and the solidarity against its depletion both for existing systems and coming ones. (Grubb et al, 2006). The energy security concept is perceivable via two aspects: the accessibility (stability) and reliability of the energy resources. Given the interlaced ties between energy consumption and economic growth, the accessibility and reliability of energy sources are better perceived. As IPCC, in the “renewable energy in the context of sustainable development” reports, in the long term, the scarcity and depletion of the fossil-based sources as well as decreasing their quality is probable (IPCC, 2011 a). In this respect, IEA explicits that the accelerated economic development specifically in the global South, raising the worldwide energy demand, can limit the fossil-based reserves life span (IEA, 2008). In this regard, the above mentioned IPCC report presents a metric to measure the scarcity of fossil fuels, which is the ratio between the Reserves to current Production (R/P), which determines how many years the reserve’s production at the current rate will last before depletion. Based on the estimation of the global R/P ratio, it takes 41-45 years for oil and 54-62 years for gas reserves to exhaust. The fact which jeopardizes both the accessibility (stability) and reliability of these sources, and necessitates complying with energy efficiency, and justifies a global shift to renewable green energies. (IPCC, 2011 a)

Based on these assumptions, the bio-energies possess the capabilities of compensating for this scarcity. According to the IPCC report on climate change and renewables, (IPCC, 2011b, p174) in 2008, the renewables correspond to 13% and bio-energies accounted for more than 10% of the total energy consumed, while organic wastes such as olive residues as a biomass resource can increase the share of bio-energies being substituted by fossil resources, to compensate their scarcity. (IPCC, 2011b)
1.3.5 The Chemical Specifications Approach

As described, biofuel production from olive by-products through anaerobic digestion is a technological process turning organic matters into biogas. The process treats both solid and liquid wastes into biogas mainly methane in absence of oxygen, at the same time with stabilizing and managing wastes. The mechanism is practised on large or small scales to obtain methane consisting of some 40-70% of the generated biogas. (Thompson D.A. et al, 2018)

The scholars maintain the choice of biomass as a crucial factor for optimized biodegradability and digestibility within the anaerobic process. A number of biomass chemical specifications are distinguished as the determining factors for enhanced biodegradability anaerobic digestion. The digestate’s total solid content (TS) (measuring all suspended and dissolved solid material within a liquid sample) usually varies from 20-80% within the organic agronomic feedstocks and it is assumed that the higher content of TS in anaerobic digestion, corresponds to the higher volumetric productivity of AD. (Vasco-Correa J. Et al, 2018) Based on the supplemented excel table, the TS value for olive feedstocks varies between 30-80% depending on the type, location and physical form of the feedstock.

The carbon to nitrogen ratio is regarded as the next determining element in an enhanced AD. The optimum C: N ratio for biogas yield is recognized as 20-30:1. (Dioha I.J. et al, 2013). Based on the extracted data on the supplemented table, there are varying ratios of carbon to nitrogen in olive residues (ranging between 12.1- 23.2), depending on the type and physical form of the residues. However, there is a consensus over the high content of carbon within the olive residues which provides an optimum condition for AD, especially when they are co-digested with high-nitrogen organic wastes such as animal manures (Goberna M. Et al, 2010), as well as micro-algae (Fernández-Rodriguez, M.J et al, 2019). Further, the low PH and low alkalinity of the olive mill residues are well compensated by animal residues during co-digestion resulting in higher biogas production. (Goberna M. Et al, 2010)

Additionally, during the hydrolysis, the AD first stage, proteins, lipids, and carbohydrates are required in order to transform into amino acids, fatty acids, and sugars respectively. Generally, the combination of solid and liquid fractions of olive wastes consists the in average 7.10-7.46%wt, 7.16, 13.58-14.80%wt of lipids, carbohydrates and protein respectively (Azbar N., et al, 2004) (Clemente A. Et al, 1997), the fact approves that the olive residues fulfil the chemical requirements for enhanced biogas production, applying them as biomass and searching for the optimum treating methods are justifiable.
1.4 Study Aim

A large number of researchers worldwide, studied the possibilities of turning the olive oil mill residues to biogas as a modern form of bioenergy, via the anaerobic process, using the BMP test as a set of standardized conditions evaluating the energy potentials. They further utilized some treating methods to test the ways to optimize the biogas and subsequently the methane level.

The theorem is that the whole quantity of treating measures including the physical, chemical, biological, physic-chemical pretreatment and the co-digestions work perfectly within the anaerobic process of olive mill residues and are more or less influential in optimizing methane level. However, it is required to validate the hypothesis and examine the level of efficiency for each treating method.

The paper thesis aims to aggregately investigate the conducted experiments, estimate the role of the treating methods and co-digestions within the anaerobic digestion of olive residues in optimizing bioenergy, and find the most effective ones. Thereafter, as the subgoals, the research probes for other influential conditions in the choice of olive substrate, the used inoculum, digester type and working volume and time and location of the anaerobic experiments on the olive oil by-products.

Seemingly, the following research questions are traced:

1. What is the most effective treating category in improving methane level within the anaerobic process of olive mill wastes?
2. What are the most influential treating measures within that category?
3. Which type of olive residue has the capacity of generating the highest methane level?
4. What is the optimum inoculum, digester set up and working volumes in improving methane yield in the anaerobic process?
5. What are the time and locations corresponding to the highest methane level?

To approach the objective, after general quantitative information on the global annual produced olive products, it is required to introduce the types of olive organic wastes. The investigation of the olive oil extraction industry is presented to understand different wastes resulting from various methods of oil extraction. After a detailed description of the methodology applied, eventually, through a meta-analysis on the extracted data from an ample number of scientific researches, the quantity of the produced methane is monitored and the most effective procedures and methods and conditions corresponding to the highest level of methane are found.
1.5 Olive Product

As the major agricultural yield within the Mediterranean region (consisting of the European coast, the Levantine coast, and the North African coasts, including twenty-two territories), also massively cultivated throughout South America, South Africa, China, United States, and Australia, olive fruit gets a remarkable significance over the other agronomical products. (IOCa, 2021) The olive tree, with the botanical name of *Olea Europaea*, (WCSP, 2021), is normally cultivated for olive oil and table olive, but other tree components such as the leaves and fine wood are beneficial as well. Around 90% of the harvested fruit is turned to oil and the remaining comes as table olives. (IOC a, 2021) Spain, with 9,176,929, followed by Italy with 1,945,324, and Turkey with 1,674,377 metric tones of olive produced, has been the world’s leading olive producer in 2019. (FAO, 2021). Olive oil is mainly produced in Mediterranean and European counties, and about 70% of the world’s olive oil is produced in Spain, Italy and Greece aggregately. (IOC c, 2020). Map 1 presents the global disparity of olive products (Map 1).

1.6 Olive Organic Wastes

The agricultural products when processed produce multiple types of wastes. Table olives and olive oil, as the end products of the olive industry, emerge from several processes and stages from tree to table. About 10% of the olive products reaching the olive mill are leaves, which is produced through the first stage of processing. (Goldsmith C. et al, 2015). However, olive oil, as another end product, brings various types of wastes. The high organic value of Olive Mill Wastes (OMW), the wide range of utilizations, the high nutritional, biological and technological values causes us to hesitate to regard them as real wastes, and the term by-product can a preferable term. Generally, the Olive oil extraction mill wastes are as follow: (Nunes M.A., 2016)

- Olive husk (Pomace)
- Olive wastewater
- Stones (pits) and seeds
- Other organic wastes (leaves, twigs)

The current study distinguishes the share of the olive mill organic wastes on the studied Biochemical Methane Potential (BMP) tests via anaerobic digestion. (Figure1)
1.7 Oil Extraction Methods

Oil production consists of the initial steps of defoliating, Washing, crushing, malaxation and centrifugation. Though these steps are identical between all the manufacturers, they follow a different path for the remaining process. Hence, the olive mill wastes or by-products differ in quantity and quality depending on the chosen oil extraction method. The two-phased and the three-phased as the most popular oil extraction methods are applied within the majority of the producing countries. (Christoforou E. Et al, 2016)

The three-phased or oil extraction method consists of adding water to the olives in the initial step, with the ratio of 50 L water in 100 kg olives. (Nunes M.A. 2016). In this method, apart from olive oil as the end product, olive husk and wastewater are derived as the wastes. As the method produces a high amount of water, involves a larger volume of waste (Nunes M.A. 2016). Due to the sustainability risks that the excessive water consumption and higher wastes volume imposes on the environment, some manufacturers turned to the two-phased method.

In the two-phased technology, no water is added to olive and olives are processed and centrifuged without any added water. olive husk (pomace) is considered as the main waste in this process. However, for better exploitation, efficiency and higher economic revenue of the oil production process, the olive husk (pomace) is recovered and treated for the second and third run,
and the output will be the exhausted pomace and oil (Pattara C., et al, 2010). The generated amount of wastes, as well as the composition of solid residues (pomace) and liquid wastewater, are dependent on the applied technology. (Christoforou, E., et al, 2016) (figure 2). The two-phase method as a rather new technology that is mainly practiced in Spain, is labelled as an ecological method. It not only maintains the bioactive compounds within the wastes, but saves water and reduces the olive mill wastes by 75%, and in this way, it is considered an environmentally friendly technology. (Roig A., et al, 2006)

Figure 2: Two olive oil extraction methods (adopted: Christoforou E. Et al, 2016)
2. Methodology

2.1. Structure

The existing study is accomplished based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) 2020 version, (Prisma Statement, 2020) which sets minimum items to be reported in a systematic review, and helps the authors specifically for reporting meta-analysis.

To understand the most effective methods in optimizing the methane value resulting from the anaerobic digestion of the olive residues, the quantitative data of the methane measurements after applying the treating steps were required. The present research has utilized the primary or experimental BMP tests and collects them as secondary data, to synthesize new primary data via systematic analysis to reach the aim. Applying the secondary data collection method in the next part will be justified. After extracting data out of the literature resources, and inserting it into an excel table form, the methane values were unified to millilitre per grams of organic fraction (VS or COD, …). To answer the main research question, the data were categorized into physical, chemical, biological, physio-chemical pretreatments and co-digestions to evaluate the functionality of various treating methods and to discover the most influential ones through a meta-analysis. Further, a subgroup analysis is conducted on the olive substrate, country and year of the conducted experiments to discover the substrate, country and year corresponding to the highest methane generation. Thereafter, to answer the peripheral research questions on the optimum conditions for the anaerobic process, more analyzes were conducted on the inoculum substrate, digester type and working volume of the experiments.

Throughout the steps of the review, from literature review, data extraction, unifying metric units, data categorization, and the systematic meta-analysis, two significant necessities of a scientific review were noticed, accuracy and objectivity. The current section elaborates on the methodology via three main categories of Structure, Data Collection, and Data Interpretation (Synthesis)

2.1.1 Why Collecting Primary Data?
New Situation, An Opportunity in Disguise
A normal situation provides the researcher with the chance to conduct the scientific experiments within the lab or field conditions, examining several options and testing different possibilities to generate primary data. As implied to the normal situation, researchers have conducted a huge number of studies on biogas potential of the olive industry wastes through anaerobic digestion, testing alternatives and variables such as the conditions of the experiment, various types of olive wastes, co-digesting substrates, and multiple pretreatments and obtained precious conclusions in form of primary data on methane enhancement methods and conditions. The pandemic situation, however, imposed a number of limitations, restricting the possibilities of a lab-based experiment to evaluate the biogas production with new variables and treatment options.

Additionally, The multiplicity of the relevant researches as the primary data, and the local disparity of the accomplished lab-based researches on the energy potential of the olive mill wastes, as well as lack of collective research on the subject to encompass the entire previous studies, made the tendency to conduct overarching, aggregative research on the entire experiments on this field and to collect and utilize these primary data to synthesize a new primary data.

### 2.2. Data Collection

To understand the best optimizing methods in methane production as the main research question as well as the secondary questions mentioned earlier, quantitative data was required. This data was acquired through internet-based literature and the Web of Science searching tool.

#### 2.2.1. Information Source

Web of Science (WOS) as a subscription-based searching tool provides access to several databases, encompassing various academic disciplines. The Institute for Scientific Information (ISI) has been the initial producer, while it currently performs under Clarivate Analytics supervision. (Clarivate, 2021) It can provide the possibility to choose the database and refine searching based on time span and language. It also provides an opportunity to filter the search based on specifications such as topic, author, journal, funding agency and DOI. This searching instrument possesses intelligent analysis capabilities and is recognized as a unifying searching instrument, enabling the user to collect and analyze the history of the database information throughout time. (Clarivate, 2021) In this respect, the user can trace trajectories of scientific research from the very beginning of the idea, up to the present time. This sophisticated searching tool furthermore, provides the researchers with the citation index of the articles, informing them about the influence of the articles. It also
makes searching between the disciplines possible and empowers the users to search for a unique scientific subject between various disciplines.

2.2.2. Searching Strategies

Understanding the most effective pretreatment as well as the co-digesting substrate to enhance methane generation, as well as the preferred substrates and optimum conditions of the anaerobic process, necessitates doing an inclusive search within the web of science searching tool. Incontestably, the words olive, methane, anaerobic digestion, and pretreatment were the primarily necessary words to cover all the aspects of the sought research. However, as the words come up with other synonyms and forms (hyphenated or spaced, etc.), which are sometimes applied depending on the authors choice, the following equivalents were also included in the search to obtain more inclusive results. Hence the words and phrases “bio-methane” or “biomethane”, “anaerobically digestion” or “anaerobically bio-digestion”, “pre-treatment” were added to the keywords “methane”, “anaerobic digestion” and “pretreatment” respectively.

Furthermore, the order and composition of the words were determining in the search results, as I conducted the search with the words “olive” and “methane” prior to “anaerobic digestion” and “pretreatment”, the result came up with 96 results including many irrelevant, off the topic studies. Accordingly, as the words “anaerobic digestion” and “pretreatment” signified over the rest of the words, more appropriate results were achieved upon prioritizing them in the search. More refinements were done to the search as well. As only the English scientific articles were sought, the search was refined to English articles to exclude the released seminars and symposium papers or studies with other languages. Eventually, the search based for the current study was conducted by the following keywords and composition within the searching tool:

- Anaerobic digestion OR anaerobically digestion OR anaerobically biodigestion
- AND pretreatment OR pre-treatment
- AND olive
- AND Methane OR bio methane

In the refine field of the result page, the language was set as:

- English

and document type as:

- Article

It was further set within the time span of:

- 1945 to 2021-05-18

and search was accomplished within the database of:
2.2.3. Eligibility Criteria

Inspecting through the Web Of Science searching instrument via the mentioned keywords, introduced 69 results. Surfing through the articles by topic and abstract, the relevant articles, those possessing a relevant research question were chosen to work on. Generally, the number of 16 articles were excluded in different stages due to possessing an aim other than assessing methane potential, or being identical with the previous ones in content but with different topics (repetitive versions) or not using an accurate metric unit for methane measurement or not presenting any value for methane produced. The number of 53 articles including 307 sample experiments were evaluated in an excel table for the assessment. However, to conduct a meta-analysis, more experiments, those samples lacking standard deviation were omitted, and ultimately, the number of 22 articles, containing 155 sample experiments were evaluated within the meta-analysis. (Figure 3)
2.2.4. Data Items

In order for a unified, consolidated general assessment, all the screened 53 articles were evaluated in excel software. The studies were sought for the following data. (Table 1). Although all the extracted items were not utilized in this research, they may be exploited for the coming researches on this field.

![Diagram of systematic review process](image)

Figure 3: The schematic release of the systematic review. Adopted from the PRISMA 2020 statement: an updated guideline for reporting systematic reviews (page M.J. et al, 2020)
2.2.5. Selection Criteria for Meta-analysis

The selected studies were scoped for many more parameters than what was utilized in meta-analysis. As the scoped parameters were not presented in a good number of studies and were missing in many studies, they were not applied within the meta-analysis for more inclusive results. About thirty parameters were scoped and extracted which are available in the supplemented excel table and may be utilized in future studies.

However, the data corresponding to the olive substrate type, the digester type and working volume and used inoculum, as well as the pretreatment and co-digested methods and their descriptions were utilized within the current study, which was widely defined as the major concepts (1.1.1) in the introduction section.

In order to conduct a statistical analysis via meta-analysis on the aforementioned parameters, however, each study sample required to be investigated in terms of the methane value, the control methane value and the standard deviations for both values as well as the number of the repetitions for each assay. The standard deviation is the essential component for the statistical analysis, showing the disparity and the spread of the samples from the average value. As Rumsey asserts, SD shows to what extent the values are close to average or is it spread over a wide range. (Rumsey, D., 2005)

Further, without the SD value, it is not possible to compare two sets of values accurately. In the present meta-analysis study, even with the close mean values for each sample and the control for methane values, the proximity of the values is not proved, rather, the SD value determines their difference.

2.2.6. Quality and Robustness Assessment

Methodology instructors recognize the systematic review and meta-analysis as the most reliable form of evidence-based studies and maintain three components for assessing the quality of a systematic review, the internal validity, the external validity, and the reporting quality. (Whiting P., et al, 2017)

- **Internal Validity, Towards Avoiding Bias in Data Sampling and Collection**
  
  The concept explains the quality of research design and conduct. Evidently, deficient design can produce bias. (Whiting P., et al, 2017) As Strene and the co-writers assume, bias is a measure to differentiate the precisely accomplished meta-analysis from others. Further, the small studies are more probable to show bias as the precision of the estimations increase when the studies are conducted on large scales. (Strene J.A.C. 2001) Moreover, as the other methodologists assume (Flather M.D. et al, 1997), the small sample size in a study can be a source of limitation. As can be seen In this research, in order to get the required answer on the highest methane promoting
experimental treatments as well as other optimum conditions, a large number of studies, (as earlier explained in detail) are applied and the conscientious screening was done for picking the most relevant sample studies, paved the way for a robust study and avoiding bias.

Additionally, the study selection and the data collection were done in absolute objectivity and particularly, was accomplished regardless of the outcome of the experiments. In other words, the study consists of both the methane promoting and methane impeding values, resulting to discover both the increasing and decreasing factors in methane production. Moreover, based on the methodologists instruction (Strene J.A.C., 2001), the applied studies have been the result of collaborative works and a cooperative effort of more than one researcher, and frequently reviewed and cited by other scientists as well.

In a quantitative study, the parameter Validity evaluates the level of the accuracy of the study. In other words, it measures to what extent the test is precise. However, as Twycross and Shields assert, along with conducting or criticizing quantitative research, the data collection instruments should be tested in terms of validity. (Twycross A. Et al, 2004) Through this part, the validity of the present study in terms of the construct, the content is attested.

In assessing content validity as one aspect of research validity, the data collection method needs to be representative of the data being measured. (Heale R., et al, 2015). As the current research tries to figure out the most bioenergy enhancing treatments on the olive wastes, it requires to probe it through a valid web-based search instrument of Web Of Science having access to all databases and the scientific and academic journals, from the oldest to the most recent relevant researches, encompassing the total experiments testing various categories of chemical, physical, biological and the combination pretreatments.

Moreover, a significant factor approving the research validity has been the fact that each value for methane (sample) has been compared to its own control value. So the estimations are calculated separately. As each article applied different methods for measuring organic fractions (COD, VS), also they had been conducted in different experimental circumstances, for greater accuracy, they were preferably compared to their own control to be assessed through meta-analysis.

Having inspected the subject from another angle, demonstrates another concept in validity. This concept defines to what extent the study is applicable to the research question. (Whiting P., et al, 2017) Here, the concept of construct validity as the very significant criteria of research accuracy helps to explain another aspect of the research internal validity. Construct validity evaluates the correlation between the concept being assessed and the criteria assessing it and determines to what extent the criteria is eligible and adherently related to the subject being measured. In fact, construct validity builds the core skeleton of the validity of a research method. (Heale R., et al, 2015)

In the current research, for determining the most effective pretreatments in enhancing the methane production level out of the olive oil by-products, also to find out the optimum substrate and other methanogenic conditions, the comprehensive, consistent and relevant keywords used (as
research variables), provided valid criteria for the research. In other words, as the applied keywords, “olive”, “pretreatment” and “methane”, “bio-methane”, covering all the aspects of the concept, and equally, were in a proper arrangement and order, provided the research with authentic criteria to find a comprehensive number of articles, therefore the construct validity of the research is inferred.

• External Validity and The Reliability of Research

The study’s external quality defines to what extent the results can be extended and generalized to other fields, contexts and situations. (Aronson E., et al, 2007) In this context, it is hard to estimate whether the results are applicable for instance to other organic substrates, treating materials and so on, and it can be answered through additional studies. However, as Heale and Twycross assert, the reliability of a searching instrument and data collection, as a quality measurement in quantitative research, is approved when the other researchers obtain the same results when they operate the test in exactly the same situation and conditions as the first researcher did. (Heale R., et al, 2015). Since the set conditions, including the searching tool, the keywords and all the searching procedures including the time span are clearly determined, identical results will be acquired provided that the test is repeated.

• Reporting Quality

To release the synthesized data, a good number of reporting guidelines are defined by the research instructors. The current study utilizes PRISMA protocol as a systematic framework for reporting in a quantitative, systematic study, the structure provides checklist-based guidelines to present the data. In this regard, Drucker and the co-writers assume, adhering to PRISMA statements has been a way to avoid bias for a promoted reporting quality in a systematic review (Drucker A.M. et al, 2016). And Whiting and the co-writers maintain that the prominent searching tools and methods and the high-quality research are not exhibited without a proper reporting guideline. Further, As the systematic type of reviews is always at the risk of bias, they have to be designed by a formal reporting protocol prior to conducting the review. (Whiting P., et al, 2017). To pattern the reporting guidelines to the current context, the study applied the PRISMA checklist protocol, to enhance the reporting quality of the existing scientific review. (PRISMA statement, 2020) (supplemented Appendix 2)
2.3. Data Interpretation (Synthesis Method)

2.3.1. Meta-analysis

To present an aggregated study over the huge number of conducted researches in the field, the paper applies meta-analysis. Meta-analysis is regarded as a quantitative and none-experimental research method, which is synthesized from pooling data from observational or experimental research with conceptually similar aims. The research method utilizes a combination of scientific research studies to synthesize a conclusion and is extremely helpful when a substantial number of studies exists. Obviously, the characteristics of the including studies is determining in the meta-analysis quality. As it builds on the results of a body of the earlier research, it is sometimes referred to as the “analysis of analysis”. (Boslaugh, SE.M., 2019). In the current study, to answer the research questions, a systematic review is done to find out all the studies conducted to answer that question. Customarily, a table is used to describe the number of all the included and excluded articles as well as the inclusion criteria, along with the reason for exclusion. The result is formulated quantitatively, which is done by determining the effect size of each study. The number includes the study outcome, the sample size and sample variability. Then the effect sizes of all the studies are analyzed to find out how many impose a positive effect and what is the average size of the effect. (Boslaugh, SE.M., 2019)

2.3.2. Meta-analysis, the Superiorities

On the tradeoffs between the independent, separate research and the meta-analysis, we approach to a new perspective. Stone and the co-writer emphasize as meta-analysis is conducted over a number of studies, it can better manifest the relation between the researches than single studies. (Stone D.L. 2017, Quote Schmidt & Hunter, 2014). Furthermore, in a meta-analysis, due to the variety and heterogeneity of the studies, the research truthfulness and the precision is fulfilled. In addition, the estimation of the publication bias in the meta-analysis can promote accuracy as well. However, as Stone and the co-writer believe, meta-analysis can undermine inconsistencies between the researches, helping to produce moderating variables to explain the gaps. (Stone D.L. 2017)

2.3.3. Data Preparation for Meta-analysis

To understand the role of each pretreatment and co-digestion in the level of the produced methane, and to estimate the positive (methane promoting) or the negative (methane reducing)
capabilities of each treated procedure as well as the favourable experimental conditions also to analyze the data through a systematic review in the meta-analysis, initially, it was required to set them in categories.


To answer the first research questions, this study has used the categories of chemical, biological and physical but adds one category of physio-chemical, as many studies had used the combined physical and chemical pretreatments. (Figure4). However, to identify the excellent substrate, the best conditions like digester type, inoculum, and working volumes, the parameters were assessed with methane values in separate tables.

Figure 4: The set categories and the applied Pretreatments and co-digestion methods
2.3.4. Data Synthesis (Statistical Analysis)

After data collection, selection, extraction and categorization, it is required to statistically analyze it to synthesize the set of data in order to identify the most favourable pretreating methods in optimizing methane production level and some of the preferred conditions for the anaerobic digestion of the olive oil by-products.

Since a good quantity of assayed studies with similar research questions, evaluating methane value after treating measures, obtained diverse results, it was required to aggregate all the results and reach a unified conclusion in a mannered system. The STATA software version 14.0 (STATA corp. College Station, TX) was applied to statistically evaluate the diverse results.

The Meta-analysis is performed on the extracted continuous data of the generated methane values of the experimental olive wastes. I applied the fix-effect or random-effect meta-analysis depending on the heterogeneity weighting to calculate pooled estimates and confidence intervals. The level of certainty is assessed using the confidence interval of 95%. To conduct a meta-analysis on the probed subjects, each study should have been provided by the methane value, the control value and the standard deviations for both, the average, as well as the number of replicates, which was the same for both the case and control samples. The mentioned values were necessary to estimate the weighted mean difference.

Unlike the categorical (qualitative) data, the current study was required to utilize continuous variables to measure our quantitative data. The Standardized Mean Difference (SMD), and the Weighted Mean Difference (WMD) variables are used to measure the continuous data. The SMD however, is applied to estimate not-unified measuring tools to standardize the effect size, while the WMD is employed for the unified ones in the quantitative data. (Andrade C., 2020) Hence, the effect size is estimated by weighted mean difference (WMD), which is the index utilized to assess the standardized, quantitative data. It was used to estimate the target community of pretreatments (including physical, biological, chemical, physic-chemical) and co-digestion categories as well as the optimal inoculum, biodigester and working volume. The results were obtained in the subgroup and overall analysis and illustrated by the forest plots. The overall p values released from the software output have been illustrative of WMD measurements.

The level of certainty is estimated through confidence interval (CI), visualizing how the effect estimation of each experiment is placed around the control value, it also determines how each effect differs from the overall effect estimate. Using the confidence level of 95% in this study shows the probability of 95% that the effect size estimations occur between the upper and lower range.

The statistical significance is estimated in the study via p-value (0.05). Though the illustrative plots are expressive on the effect size, to estimate the heterogeneity and bias, the study applies the p values in each case to assess the statistical significance. Studies are hypothetically presumed as
homogeneous and unbiased by default (null hypothesis). However, the statistical significance can
determine to what extent this is true.

The heterogeneity is estimated through I square ($I^2$). Based on Cochrane’s Q statistic (Higgins
J.P.T., et al, 2011), the $I^2$ values greater than 50% approves the heterogeneous study samples.
Furthermore, the p-value for heterogeneity can be determined in the estimation of heterogeneity. The
p values greater than 0.05 level of significance, approves the homogeneity of the samples, while the
smaller P values illustrate the heterogeneous samples.

The publication biases were assessed through Egger (Egger M. 1997) and Begg’s test and were
estimated through the software released p values for Egger and Begg’s test. As the absence of bias is
presumed, the bias p values greater than the default 0.05 of significant level are regarded as
unbiased, while the smaller p values as biased samples.

To review the methodology implications, it is worth mentioning that the meta assessments are
recognized as heterogeneous, although this is not an ideal condition, as Sedgwick (2015) assumes, it
was compensated applying the random model analysis in such cases. Further, although Strene and
the Co-writers assume that the large study sizes, which include a good quantity of samples are not
biased (2001), however, the scope of discussion may include some shortcomings in terms of
publication bias. As the study relied only on the published articles, it may have excluded some
precious studies from the scope. Moreover, as the external validity implies (reviewed in 2.2.6), the
research has to be extendable and generalizable to other contexts and fields, while it is not known to
what extent the current study results can be generalizable to other conditions.
3. Results

The study aiming to identify the most effective treating practices to promote the methane value out of the olive by-products, incorporating 155 sample experiments including 27 controls, extracted from 22 studies, came up with provocative results. The samples were stranded into the main categories of physical, chemical, biological, physio-chemical pretreatments and co-digestions, for the main assessment. The substrate, the utilized inoculum, digester type and the effective working volumes (Table 1) were also studied to answer the other research questions.

Due to the disparate and divergent positive and negative effect sizes of the samples compared to controls, meta-analysis was found as the most proper assessment method. According to Basu when there are very diverse study results, in terms of intervention, population and outcome, meta-analysis is required as otherwise, it is impossible to pool them to obtain a patterned result (Basu, A. 2017).

The Random effect model was utilized for heterogeneous and the Fixed effect model for homogeneous samples. The results are evaluated through three main test outputs, the Radial plots (Galbraith) to estimate the heterogeneity of the samples, and the Funnel scatter plots to present visual measurements of bias within a meta-analysis. (Strene J.A.C, et al, 2004) They visualized the pretreatment samples out of individual effect size (horizontal axis), by the study size (vertical axis). The publication Bias which are estimated as software output determines whether the meta-analysis has omitted the samples had to include or not, or whether it contains just positive results or contains both negative and positive ones. (Basu, A. 2017). Moreover, It is widely agreed that the evenly distributed samples, approves the absence of bias. (Basu A., 2017)
<table>
<thead>
<tr>
<th>Analyzed Categories</th>
<th>Type</th>
<th>Description</th>
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</thead>
<tbody>
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<td>Physical</td>
<td>Heating in different temperatures and durations</td>
</tr>
<tr>
<td></td>
<td>Physical</td>
<td>To various particle sizes</td>
</tr>
<tr>
<td></td>
<td>Microwave</td>
<td>In varying temperatures and durations</td>
</tr>
<tr>
<td></td>
<td>Ultrasonic</td>
<td>In different durations, powers and frequencies</td>
</tr>
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<td></td>
<td>Steam explosion</td>
<td>by a pilot scale reactor with max operating pressure of 42kg/cm²</td>
</tr>
<tr>
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<td>Separation</td>
<td>To solid and liquid phases of olive substrate</td>
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<td>H₂O₂ + Ca(OH)₂</td>
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<td>Substrates</td>
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<tr>
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<td>Two phase, three phase</td>
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<tr>
<td></td>
<td>Inoculum</td>
<td>Anaerobic (sewage) sludges, brewery anaerobic sludges, suger factory anaerobic sludges, Trace metals + macr oelements+ buffer solutions, Livestock manure + its digestion, anaerobic sludges + trace metals, bovine manure</td>
</tr>
<tr>
<td></td>
<td>Digester type/working volum</td>
<td>Semi-continuous220ml, Continuous 120ml, Continuous 400ml, Batch 100-250ml, Batch 400ml, Batch 1000ml, Batch 10000ml</td>
</tr>
<tr>
<td></td>
<td>Countries</td>
<td>Spain, Italy, Morocco, Tunisia, Greece, Turkey, Chile</td>
</tr>
<tr>
<td></td>
<td>Years</td>
<td>2007-2021</td>
</tr>
</tbody>
</table>

Table 1: The detailed specifications of the analyzed and sub-analyzed groups.
3.1. Central Results

3.1.1. Physical Pretreatments

The number of 48 (+10 controls) eligible experiments were categorized in the physical strand, including Coroca E. (2 experiments), Elalami D. (12 experiments), Serrano A. (10 experiments), Pellera F.M. (5 experiments), Rincon B. (9 experiments), Donoso-Bravo A (8 experiments), and Ruggeri B. (2 experiments), Which were conducted between 2013-2021, in Spain, Morocco, Greece and Chile, applying thermal, ultrasonic irradiation, milling, electrostatic separation, steam explosion, microwave irradiation treatments. (Table 1)

The Heterogeneity of the physical pretreatment sample design was reported as: $I^2 = 99.5\%$, $p=0.00$ (figures 6.1 and 5.1). Heterogeneity is approved due to both the observing radial plot, and the $I^2$ value (exceeding 50%). The Random effect model in the overall analysis is utilized due to the heterogeneity of the samples, which showed no helpful or improving impact on the methane produced after physical pretreatments. (Overall estimated pooled data: WMD (95% CI)= $-0.7$ ($-14.33, 12.93$), $p=0.919$) (figure 6.1).

The radial graph $(x:1/SE(b), y:b/SE(b))$, illustrates a mild downward slope from the zero starting point. The presence of $-2, +2$ lines demonstrates the $I^2$ as having a value smaller than 100%. (99.5%). A clear heterogeneity is visible due to scattered dots which are placed both between and out of the parallel lines, a mild downward slope which started from zero demonstrates the overall pooled estimated data as a small negative value ($-0.7$) (figure 5.1)

The outstanding physical method(s): However, in the physical category, through the study by Donoso-Bravo A (2015), the application of thermal hydrolysis on the two-phase olive pomace, in 148.1 °C, for the duration of 30min, provided a significant association, which notoriously increased the methane value and the meta-analysis estimated pooled data as follow: (WMD: 178.62(171.79,185.45), CI:95%) (Donoso-Bravo, A, et al, 2015) (figure 6.1)

According to the software output, bias is estimated by the Egger’s test, as (p=0.523), and Begg’s test (p=0.505), since p>0.05, no publication bias is recognized in these study samples. The funnel plot illustrating the bias (Figure 7.1), shows X axel as: WMD and Y: Se (WMD). Despite the lack of evenly distributed dots between the top and bottom, the presence of few dots in the middle and lower parts of the funnel, as well as even distribution of dots in both sides of the vertical line (overall WMD) proves the absence of bias. (Figure 7.1)
Figure 5: Radial Plots, showing Heterogeneity in the stranded samples, \( x \): 1/Se(b) (standard error for beta), \( Y \): b/Se(b)  1: physical,  2: chemical,  3: Biological,  4: Physio-chemical,  5: Co-digestions.
3.1.2 Chemical Pretreatments

The study contains 36 (+5 control) samples, consisting of the articles by Elalami D, (6 experiments), Pellera F.M. (6 experiments), Serrano A. (7 experiments), Sicilliano A. (4 experiments), Pinto-Ibieta F. (8 experiments), Ruggeri B. (5 experiments), from Morocco, Greece, Spain and Italy, during 2014-2020, applying components and methods such as alkaline, dilution, chemical reagents and trace metals. (Table 1)

The samples of chemically pretreated experiments are heterogeneous as having I² higher than 50% (I² = 100%, P: 0.00 (figures 6.2 and 5.2). Due to heterogeneity, the random effect model is utilized and the overall effect size, as the Weighted Mean Difference (WMD) was determined with 95% of CI as: (+36.28 (CI: 24.22, 48.35), P: 0.00) (figure 6.2). As the effect size implies, there has been a significant association between these samples and their control, approving the chemical pretreatments as methane enhancing.

The radial plots (figure 5.2) (x:1/Se(b), y:b/Se(b)) for the chemical group shows a steep upward slope from the zero starting point, illustrating the overall pooled estimated data as much higher than zero (36.28) in this category. The reconciled _2, +2 graphs represent that I²: 100%, showing absolute heterogeneity of the samples. The scattered dots also approve the heterogeneous samples, as well. (figure 5.2)

Publication Bias is estimated according to Egger’s test as P:0.270, and Begg’s test as: P:0.00 via software output. It is also evaluated by observing the scattered samples illustrated by the funnel plots. (figure 7.2) (x: WMD, y: Se(WMD) se: (standard error)). Although the funnel plot demonstrates bias due to dots scattered mostly on the left of the funnel, due to the authenticity of the p values over the visual funnel plots, statistical significance (demonstrated by p-values) possess priority over funnel plots. Hence, based on Egger’s test, p-value exceeding 0.05 level of significance, no publication bias is determined for chemical strand samples.

3.1.3 Biological Pretreatments

This category contains 5 samples (+2 controls), conducted by Antonopoulou G. (1 experiment) and Donoso-Bravo, A (4 experiments) in 2015 and 2019, in Greece and Chile respectively and accomplished using enzymatic treatments. Table 1 presents detailed information on the applied pretreatments.
Figure 6: The Forest Plots of treating categories: 1 Physical, 2 Chemical, 3 Biological, 4 Physio-Chemical, 5 Co-digestions, determined by: studies (ID) effect size (WMD), weight (%).
The **heterogeneity** of this category samples is approved due to overall estimation as: $I^2: 99.6$, and $P: 0.00$. (figure 6.3), and the radial plot (figure 5.3). The samples heterogeneity implies using a random effect model, according to which, the pooled effect size (CI: 95%, WMD: -61.59 (-94.15, -29.03), $p: 0.00$ (figure 6.3), showing a notable decline compared to control samples. The radial plot (figure 5.3) (x: $1/Se(b)$, y: $b/Se(b)$), with a steep downward slope of the graph, illustrates the huge decrease of the pooled estimated data from zero point, showing the overall estimated pooled data as: $-61.59$ in the biological category. However, the presence of parallel $+2$, $-2$ graphs demonstrates the $I^2$ as having a value smaller than 100% (99.6), which still approves the heterogeneity of the sample studies. Seemingly, the scattered dots out of the parallel lines proves the sample’s heterogeneity as well. (figure 5.3)

**The outstanding biological pretreatment**: none of the biological treating processes implied any significant improvement, and implied the impeding effect on the methane value generated.

The publication **bias** within the biological strand, based on Egger’s test is estimated as $P: 0.109$ and Begg’s test, $P: 0.42$ through STATA software output, which identifies no publication bias due to p-values. The presented funnel feature, (Figure 7.3) (x: WMD, y: $Se(WMD)$), confirms the absence of bias in the biological strand due to evenly scattered dots as well.

### 3.1.4 Physio-Chemical Pretreatments

The category containing 28 sample studies (+6 controls), conducted by Caroca E., (9 experiments, Spain), Fernandez-Rodriguez (6 experiments, Morocco), Elalami D. (6 experiments, Morocco), Serrano A. (2 experiments, Spain), Rincon B. (2 experiments, Spain), Ruggeri B. (3 experiments, Italy), conducted between 2014-2021. In this set of pretreating category, the following combination of treatments was applied: thermal + phenol treatment, microwave + dilution, alkaline + ultrasonic, steam explosion + dephenolization, separation + thermal, hydro-thermal, ultrasonic + reagents. (Table 1)

**Heterogeneity**: The test samples corresponding to physio-chemically pretreated olive by-products substrates, are recognized as heterogeneous due to: $I^2: 99.3\%$, $P: 0.00$ (figure 6.4), as well as the widely scattered samples on the radial plot (figure 5.4). Hence due to heterogeneity, the random effect model is applied.

The radial plot (figure 5.4) (x: $1/Se(b)$, y: $b/Se(b)$), delineated from zero with a mild downward trend, confirms a small negative value for overall estimated pooled data for physiochemical category (WMD: $-7.42$). The presence of $-2$, $+2$ lines demonstrates the $I^2$ as having a value less than 100%
(99.3%), and the scattered dots both between and outside the parallel lines proves the heterogeneity of the sample studies. (figure 5.4)

The overall pooled effect size is reported in the forest plot (CI: 95%, WMD: -7.42(-32.27,17.44), p: 0.559 (figure 6.4), which shows no positive association between the samples and the control values, meaning that the combination treatment of physical and chemical pretreatments as not being effective optimizers in the biogas and subsequently, the methane production.

**The outstanding physiochemical method:** though the overall estimated pooled data in this category shows a generally negative impact on the methane produced, a study conducted by Rincon B, (2015), (WMD: 498.00(427.13, 519.87, CI: 95%) associates with a great significance and illustrates an extremely high improving impact on methane. The study applied Hydrothermal pretreatment to the extracted liquid fraction (LF) (out of the two-phase olive pomace), which had been carried out via steam explosion, in temperature of 200 °C and reaction time of 5min, under pressure: 1.57 MPa.

**Bias:** The funnel plots illustrating the publication bias (figure 7.4) (x: WMD, y: Se(WMD), identifies no bias within the physio-chemical category, due to the even distribution of dots in both sides of the funnel. This is validated based on Egger’s test P: 0.917, and Begg’s test p: 0.430 (H0: no bias, then p values>0.05 confirms the H0), due to having p values greater than the significance level.

### 3.1.5 Co-digestions

In this category, generally, the number of 16 experiments (+4 controls) were subjected to meta assessment in STATA. The samples were collected by Fernandez-Rodriguez M.J in 2019 (b), 2020 (10 experiments), Farhat A. In 2018 (3 experiments), and Fountoulakis M.S. in 2007 (3 experiments) which were accomplished in Spain, Tunisia and Greece respectively. To improve the methane value, a number of organic materials were applied as the aiding digestates for the olive residues as the main substrate. The applied aiding substrates in the current study were various types of algae and microalgae, winery grapes, municipal sewage sludges and slaughterhouse wastes. (Table 1)

**Heterogeneity:** The study samples do not seem homogeneous as: $I^2=100\%$, P: 0.00. the released radial plot (x: 1/Se(b), y:b/Se(b)) also represents heterogeneity within the samples (figure 5.5). The random effect model is applied due to heterogeneity, the overall effect size as (WMD), determined by the forest plot (figure 6.5) for co-digestions with 95% confidence interval (CI), is -23.71(-31.87,15.55), p=0.00. (figure 6.5). Based on the random effect model result, there has not been any positive association between the co-digested samples and their control. Contrastively, the aggregated analysis of overall value for WMD, shows a negative association between them.
Figure 7: the Funnel Plots, showing the bias in the treating categories. 1 Physical, 2 Chemical, 3 Biological, 4 Physiochemical, 5 Co-digestions. Y: Se(WMD) (Se: standard error), X: WMD
The outstanding co-digestions: the most methane optimizing co-digestion has been the microalgae chlorophytes Chlamydomonas Reinhardtii, known as CHR6145, which is applied on the two-phased pomace based on an equal (1:1) ratio, (WMD: 141.00(134.60, 147.40). The study was conducted by Fernandez-Rodriguez. MJ (2019 b) The next methane promoting co-digestion recognized belong to Fountoulakis M.S. (2007), co-digesting the slaughterhouse + winery grape wastes with an equal ratio on the three-phased olive wastewater.

Bias: The publication bias is estimated, (x: WMD, y: Se(WMD) according to Begg’s test P=0.390, and Egger’s test P= 0.747. Due to the higher values of p than the 0.05 level of significance, as well as the representing funnel plot (figure 7.5), no bias is recognized within the co-digestion treated samples of olive wastes.
3.1.6. Analyzing Inoculums and Digester Type/ Effective Volume

The meta assessment to evaluate the effect size concerning the inoculums as well as the digester type and working volume, accomplished totally on 132 experiments (and 27 controls) from Spain, Italy, Morocco, Tunisia, Greece, Turkey and Chile from 2007 to 2021.

The inoculum types consist: anaerobic (sewage) sludges, brewery anaerobic sludges, sugar factory anaerobic sludges, trace metals + macro-elements + buffer solutions, livestock manure + its digestion, anaerobic sludges + trace metals and bovine manure. (Table1), and the digester type/ working volume consist categories: the semi-continuous 220ml, continuous 120ml, continuous 400, batch 100-250ml, batch 400ml, batch 1000ml, batch 10000ml (Table2).

The overall homogeneity assessment of the test: \(I^2: 100\% \ p:0.00\), indicates the heterogeneous sample experiments. The radial plot \((x: \frac{1}{\text{Se}(b)}, y: \frac{b}{\text{Se}(b)}\)) illustrates the extreme heterogeneity of both samples, due to coinciding -2,+2 graphs on one, as well as the scattered dots. (Figure 8.1) The random-effect model was conducted due to heterogeneity. Furthermore, the publication bias evaluation of both studies which is shown through funnel plot \((x: \text{WMD}, y: \text{Se(WMD)}\)) is assessed through the Begg’s test \((p:0.00)\) and Egger’s \((p:0.033)\), indicates publication bias for this set of studies (figure 9.2)

The outstanding inoculum: However, the highest effect sizes of the applied inoculums are recognized in the experiments by Sicilliano A. Using livestock manure + its digestion as inoculum, \((\text{CI: 95\%}, \text{WMD: 320.00 (319.84, 320.16)}, \ P:0.206)\), and Elalami D., Using the sugar factory anaerobic sludges as inoculum, \((\text{CI:95\%}, \text{WMD: 36.26(9.32, 63.21)}, \ p:0.00)\), which corresponds to the highest levels of methane produced. (Figure10,1).

The outstanding bio-digester type/working volume: the random effect model test indicates that the samples with the continuous setup and 120ml of volume, corresponding to the highest level of methane produced. In this regard, the effect size by weighted mean difference: \((\text{CI: 95\%}, \text{WMD: 85.60(62.16,109.04)}, \ p:0.784)\), followed by the continuous set up with 400ml of working volume \((\text{CI:95\%}, \text{WMD: 36.26(9.32,63.21, p:0.784)})\), has shown the most effective digester setup and volume in terms of the generated methane. (Figure10.2)
Figure 9. Meta visualization of effect sizes in weighted mean difference by: 1. inoculums, 2: digester type by effective volume. Forests: Study(ID), effect size(WMD), weight(%)
Figure 8: 1. Heterogeneity $Y: b/\text{Se}(b)$, $X: 1/\text{Se}(b)$, and 2. Bias visualization of inoculum and digester type/effective volume $Y: \text{Se}(\text{WMD})$, $X: \text{WMD}$
3.2. Analyzing Subgroups

3.2.1. Chemical Sub-Classes

The meta-analysis inspection for the most effective treating procedures on the organic olive wastes recognized the chemical categories as the most optimizing in methane value. However, a subgroup analysis on this category was needed to identify the noblest practices and operations in this category.

The analysis consists of 6 subgroups: Alkaline + dilution (6 experiments), Reagents (10 experiments), Trace metals (various) (7 experiments), Alkaline + Lime (4 experiments), Trace metals (cobalt) (8 experiments), and Dilution (1 experiment), and totally contain 36 experiments (+5 controls) (Table 1) (figure 11), and conducted by Elalami D, Pellera F.M., Ruggeri B., Serrano A., Sicilliano A., Pinto-Ibieta F.

The overall I-square value as released via forest plot reveals the heterogeneity. ($I^2$: 100%, $P=0.00$), approving the heterogeneity of the analysis in all the sample groups.

The random effect model which was applied due to the heterogeneity of the samples represents appealing results for the chemical subgroups by determining the effect size as the weighted mean difference (WMD). The highest value, belongs to chemical subgroup Alkaline + Lime, as: (CI: 95%, WMD 285.00 (250.37, 319.63), p:0.00), followed by Trace metal (cobalt): (CI: 95%, WMD: 69.35 (49.27, 89.43) p: 0.00), and Alkaline + Dilution group: 47.70(35.00, 6.41) p:0.00. (Figure 10).
Figure 10: forest visualization of chemical type subgroup, Study(ID), effect size(WMD), weight(%)

Figure 11: the forest visualization of the WMD by subgroup substrates. Study(ID), effect size(WMD), weight(%)
3.2.2. Substrates

Having analyzed various pretreatment categories (physical, chemical, biological, physiochemical) as well as the co-digestions in a meta-analysis, and recognizing the chemical category as the most influential pretreating category in optimizing methane value, introduces the opportunity to seek more detailed information. The subgroup analysis on the utilized substrate in the chemical group reveals intriguing results. In this group, two types of olive wastes as the main substrate to produce biogas through the anaerobic digestion and the BMP test were utilized, olive pomace and its combination with wastewater.

Based on the conducted meta subgroup analysis, olive pomace as shown generates a way higher methane value than its combination with wastewater. The result illustrates the effect size for pomace (CI: 95%, WMD: 53.08 (40.27,65.88), p:0.00), while the combination of Pomace + wastewater illustrates the effect size as (WMD: -73.10 (-140.71, -5.49), p:0.034). The positive value of WMD for pomace and the negative value for the combination (pomace + wastewater), approves a great variation between them. (Figure 11). In evaluating heterogeneity, however, the forest plot reports the ($I^2$: 100%, p: 0.00) and ($I^2$: 99.2%, p:0.00) for the pomace and pomace + wastewater respectively, and the overall $I^2$ as: 100% P:0.00, and since the overall substrate samples having p-values less than the significance level (0.05), and $I^2$ above 50%, they are proved to be heterogeneous.

![Figure 12: Forest Plot corresponding to effect sizes of subgroups](image)
3.2.3. Country, Year

The subgroup analysis on the time and location of the methane production out of olive residues discloses more appealing facts. The relevant experiments with chemical optimizers, conducted in 2014, 2016, 2017, 2018 and 2020, among which, the assays conducted in 2016, followed by 2020, corresponds to the highest and 2014, 2017 and 2018 assays to the lowest methane values. (Figure 13.1) However, the subgroup analysis on the experiment's location represents Italy, followed by Morocco and Spain respectively corresponding to the higher values for the methane produced (positive methane values), while Greece corresponds to negative values for the produced methane. The fact can unveil a probable correlation between the geographical specification as well as the climatic conditions and more importantly, the soil quality of the cultivated olives, on the amount of the generated bioenergy through an anaerobic process. (Figure13.2) (Map 2)
3.3 The Outstanding chemical Methods, The Particulars

The released visualizations, as well as the synthesized data out of the current meta assessment, reveal that the experiments applying the Alkaline + Lime correspond to the highest methane values. The 4 experiments (excluding control), utilized hydrogen peroxide (H2O2) in a fixed amount of 0.05 g/g COD, as well as the changing amounts of calcium hydroxide (Ca(OH)2, referred to as slaked lime) with different fractions from 15 to 35 g/l which was added at the start of the process. However, in the third experiment, corresponding to the highest methane value, (WMD: 320.00 (319.84, 320.16, P:0.00) in addition to the first step, the amount of 0.125 g H2O2/g COD was added after 15 minutes and the same amount of hydrogen peroxide was added 30 minutes after the start of the process. Furthermore, the experiments utilized olive pomace from the two-phased oil extraction system as the substrate, and the livestock manure as the inoculum, and were conducted by Sicilliano A. And the co-writers in 2016 in Italy.

Moreover, according to the conducted meta-analysis, the studies by Pinto-Ibieta F., applying different fractions of trace metal cobalt, can be recognized as the second-best methane enhancing procedures. Meanwhile, the assays were conducted in 2016 in Spain and applied the olive pomace from a two-phase olive oil extraction as the main substrate.
4. Discussions

The paper aimed to detect the most influential treating methods amongst the applied experiments accomplished via the anaerobic digestion of the organic olive wastes acquired from the oil extraction process. The study encompassed a comprehensive number of studies (155 samples and 27 controls out of 22 studies) with the almost shared aim of boosting the biogas and subsequently the methane value through various procedures. Having set these methods in five categories, an encyclopedic meta-analysis on the categorized treating methods was conducted. It further discovered the optimum olive substrate, the utilized inoculum, digester setup and working volume in improving methane value through the meta assessment.

4.1. Study Findings
(And the relevance to previous studies)

4.1.1 Chemical Treatments

The meta assessment recognized the chemical pretreatments as the most effective amongst the rest of the treating categories. A subgroup analysis, however, disclosed that the combination of the alkaline + Lime, or hydrogen peroxide (as alkaline) and calcium hydroxide (as lime), followed by trace metal cobalt pretreatments have been the most effective methods in generating methane. (Figure 10).

As explained, the highest methane was produced via alkaline (H2O2) + lime (Ca(OH)2) pretreatments (figure 10). Comparing this to the previous experiments finding may show an intriguing consistency. In research conducted to examine the bio-digestibility potential of a perennial grass (Pennisetum hybrid) applying the alkaline (NaOH), the alkaline pretreatment proved to have a significant effect on improving methane (21%) compared to untreated substrate, (Kang x., et al, 2018).

As the mechanism of the pretreatment functioning explained earlier in the introduction section(1.1.1), decomposing lignocellulosic matrix within the substrate can have a great role in improving biodegradability and optimizing methane value in organic biomass. (Elalami D., 2020). In this respect, based on the mentioned study, (Kang x., et al, 2018), the lignocellulosic tissue limit the conversion and efficiency rate, leading to insufficient biogas yield. The alkaline pretreatment,
however, is deemed to remove the lignin barrier and disrupt the cellulosic structure resulting in a higher methane level. (Kang x., et al, 2018)

Though the biogas-optimizing-potential of NaOH as the alkaline substance, had been tested in several studies, and its effectiveness in improving methane value was approved, there have been few alkaline tests using H2O2.

Lime (Ca(OH)2) effectiveness in the anaerobic process of olive wastes is revealed within the current study. (Figure 10). Further, Zhang and others (2017) utilized lime mud (a lime saturated substance) to improve the anaerobic digestion of sewage sludges. They found out that the synergic effect of lime in the process occurred through the accelerating effect on the first stage (hydrolysis) of the process, and figured out using lime mud pretreatment resulted in twice higher methane yield compared to untreated samples. As they assert, the alkalinity and lime elements are essential in microorganism growth and favour methane yield. As explained in the introduction, those pretreatments helping to remove VS as the organic fraction favour methane value. Seemingly, as per Zhang and the writers, lime content was effective in reducing the VS content leading to raised methane amount. (Zhang J., et al, 2017). Applying Ca(OH)2 pretreatment on the sugarcane bagasse had an identical result. According to the researchers, this pretreatment affects the lignocellulosic texture by degrading it, leading to biogas optimization and higher methane yield. (Mustafa A.M., 2018) The facts confirm the consistency of the current and the mentioned studies in the effectiveness of lime, further, it can be inferred that efficient impact of lime pretreatment, may involve many organic biomasses and is not limited to olive organic wastes.

Furthermore, Ruggeri and the co-writers (2014), in their study, applying chemical, physio-chemical and physical pretreatments, obtained notable results. They applied dilution, as well as the reagents such as FeSo4, MnSo4, FeCl3, CaCo3, ultrasonic, ultrasonic + CaCo3 and ultrasonic + FeCl3 pretreatments to evaluate methane value through an anaerobic digestion process on olive by-product. Among the total of eleven experiments, those with calcium compounds (CaCo3 and ultrasonic + CaCo3) correspond to the higher methane values. Given the fact that the presence of lime (Ca(OH)2) pretreatment has been an effective one, it may be inferred that the Calcium containing reagents can be more influential over the other reagents.

- Olive Substrate

Another noteworthy finding of the current study has been the supremacy of olive pomace compared to olive wastewater as well as the two-phase olive pomace to the three-phase, as the main substrate in the anaerobic process. (Figure 11) As it is described (introduction chapter(1.7)), three-
phase oil extraction system implies adding water at the initial step, imposing a huge burden of pollutant wastewater to the environment, it consumes a large quantity of water, which can be demanding due to the water issues in many olive oil-producing countries. (Roig A. Et al, 2006)

Hence, applying the two-phase olive pomace brings a triple win. It associates with less consumed water, less olive wastes, and based on the current scientific research finding, it corresponds to the higher functionality in the amount of produced methane as well.

The priority of pomace over the wastewater may relate to the presence of excessive water in wastewater, (as stated in the introduction (1.7), wastewater results from three-phased oil extraction method, which implies added water at the initial stage of oil extraction), lowering the substrate’s density, limits the substrate’s capability in methane production. This can be observed in those experiments with dilution pretreatment as well, which correspond to a lower methane value compared to the control samples. A study conducted by Ruggeri and other researchers (2014) which applied dilution, as well as other chemical pretreatments, revealed a far lower methane generation after dilution pretreatment compared to the untreated sample. (figure 10). The same result can be seen in comparing the study by Elalami, D (2020) who applied the combination of alkaline + dilution, to the study by Siciliano A., (2016) that applied the combination of alkaline + lime, (figure 10).

• **Subgroups Country, Year**

The subgroup analysis of the current study also found Italy followed by Morocco, as the highest methane rate locations, and Greece with the lowest. (Figure 12). The fact may disclose a correlation between the geographical dimensions, climatic conditions, and more remarkably, the soil quality of the cultivated olives and the quantity of the generated bioenergy. It also found that the highest methane rates are associated with the year 2016 followed by 2020.

The findings are hypothetically tested through weighted mean difference, heterogeneity and bias assessment. The recognized heterogeneity of the study can be originated from the diverse geographical locations of the conducted tests, it may also originate from the various applied olive products and the cultivation conditions, as well as the experiments circumstances, or other detailed specifications. (Goldsmith C. et al, 2015). More importantly, since the study is the first of this kind, an exclusive meta-analysis on the bioenergy enhancing methods on the olive by-product, it is impossible to be compared to the other studies, although as mentioned some of the study findings approve the previous ones.
4.1.2 The Outstanding Pretreatments in other Categories

• As shown (in figure 7.4), in the physio-chemical category, the hydrothermal pretreatment on the liquid fraction of the two-phased olive pomace (unlike other treatments in this category), corresponds to an extremely high methane yield. As determined by other studies, (and in line with what was expressed in 1.1.1), the capability of hydrothermal pretreatment in optimizing biogas value is due to the destruction of the substrate’s lignocellulosic structure and improving the hydrolysis of hemicellulose. (Ran G., et al, 2017) Ran and the co-writers obtained identical results using this type of treating measure to the washed vinegar residues at the temperature of 160 °C. (Ran G., et al, 2017) In addition, the hydrothermal pretreatment on the sugarcane bagasse, with the same influence on the cellulosic tissue of the substrate, (as explained in section 1.1.1 as well), led to an eminent VS removal and subsequently, a noticeable methane increase. (Mustafa A.M., 2018)

• However, in the physical category, through the study by Donoso-Bravo A (2015), the application of thermal hydrolysis on the two-phase olive pomace, in 148.1 °C, for the duration of 30min, provided a significant association, and notoriously increased the methane value.

Typically, thermal and hydrothermal pretreatments possess the capacity to solubilize organic macromolecules (lipids, proteins and carbohydrates). (Passos P. Et al, 2015) In line with what is stated in the introduction (1.1.1) on the removed VS), The VS solubilization increased by 20 folds and 9 folds after thermal and hydrothermal pretreatments respectively, which happened due to solubilizing the lipid, protein and carbohydrates molecules, leading to higher methane capacity. Based on Passos and colleagues study, the thermal and hydrothermal pretreatments on microalgae were able to significantly raise produced by 72% and 28% respectively. (Passos P. Et al, 2015)

• The most methane optimizing co-digestion has been the Microalgae chlorophytes Chlamydomonas Reinhardtii, known as CHR6145, which is applied on the two-phased pomace based on an equal (1:1) ratio.

4.1.3 Inoculum and Digester Type /Effective volume

The existing study found using livestock manure + its digestion followed by sugar factory anaerobic sludges as the inoculums corresponding to the highest methane values.

The superiority of livestock manure as inoculum to the olive organic wastes correlates to its stabilizing effect on C: N ratio in olive wastes digestion. As elaborated in the introduction (1.3.5), the optimum carbon to nitrogen ratio for smooth digestion is 20-30:1. Based on the research (Goberna M., et al, 2010), the nitrogen contained digestates such as animal manure, which are inherently low in organic carbon, can be amended by organic wastes (such as olive residues), containing abundant
organic carbon. This will balance the C: N ratio, compensating for the carbon deficiency of manures and nitrogen deficiency of the (olive)substrate, leading to a higher methane yield. (Goberna M., et al, 2010)

The analysis also found that continuous digesters with the effective capacity of 100 millilitres, followed by continuous 400ml digesters produce higher biogas in the anaerobic process. Generally, the current study result shows that the continuous and semi-continuous digesters have a higher capacity in methane yield compared to batch setups. This is consistent with what Koufi and the co-writers found. They tested olive mill waste as the main substrate, co-digested with poultry manure in various ratios, both in batch and semi-continuous mode, and found the 70:30 ratio of OMW to poultry manure, as the best mixture ratio and semi-continuous setup, as the superior digestion setup comparing to batch systems. (Koufi S. et al, 2015).

As per Raposo and others, the composition of the substrate can determine the choice of ideal working volume, as the methane potential for the homogeneous materials is more accurately determined in lower working volumes. Investigating a wide range of literature, they found that the most common and efficient assays in terms of anaerobic bio-digestability were those conducted in lower than 1 litre of working volume. (Raposo F. Et al, 2012) Moreover, the current study finding in associating the highest methane yield to the 100ml reactors approves what Raposo and others who found the lower working volumes (100-200 ml) correspond to the higher methane yields. (Raposo F. Et al, 2012)

4.2. Concluding Remarks

The current study tested the supplementary practices and conditions of optimizing methane as the end product of the olive by-products via anaerobic digestion. As described earlier, olive is cultivated through the vast areas worldwide, covering parts of all the continents, from Mediterranean regions to African coasts, and from South America to China and Australia. As mentioned in the introduction, the world’s annual olive oil production reached 3.262.000 T in 2018-2019 (which is tripled compared to 1959) (IOC b, 2021), and as foreseen, the amount of 3.1 million metric tons of olive oil is consumed globally during 2020-2021 (Statista, 2021). Further, assuming that in order to produce one litre of olive oil, 5-6 litres of olive is used (FAO, 1985) (Vossen p., 1995), and seemingly, for each litre of olive oil, at least 4 litres of waste is generated. Based on these assumptions, the volume of 12.4 million metric tons of olive wastes will be produced till the end of the current year which will be added to the previously-generated olive wastes. The facts strongly substantiate logics behind the
climate mitigation, energy security, waste management and olive chemical specification approaches which were scoped at the beginning of the paper, and subsequently back up managing these wastes as biomass, and adjusting methods and supplementations to optimize the bioenergy value, in order to treat this huge quantity of wastes, spread worldwide.

The current research provided the ground for more studies to examine the chemical pretreatments (and specifically hydrogen peroxide and calcium hydroxide) on other organic feedstocks in order to find out whether these types of pretreatments are preferable for them. Further, other combinations of organic inoculums can be tested on the olive wastes to evaluate biogas values. Moreover, more studies can disclose the reasons for the direct relationship between the lower digester’s effective volume and higher methane value. In addition, comprehensive research may be conducted on the possibilities and operational circumstances of bio-fertilizer application of the olive mill residues following anaerobic digestion, where the supplemented excel table can be adopted as a base for the mentioned fields.

The End
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Appendixes

1. The extracted data in an excel table, the meta-analysis basis
   The Supplementary Excel Table

2. The patterned reporting guidelines
   PRISMA statement checklist 2020

3. The Forest illustrations presenting the effect sizes.
   The Forests
5. References


Flather M.D, Farkouh M.E., Pogue J.M., (1997), Salim Yusuf, Strengths and limitations of meta-analysis: Larger studies may be more reliable, Controlled Clinical Trials, Volume 18, Issue 6, Pages 568-579, ISSN 0197-2456, https://doi.org/10.1016/S0197-2456(97)00024-X.


IOC b, (2021), World’s Olive Oil Production has Tripled, https://www.internationaloliveoil.org/worlds-olive-oil-production-has-tripled/ accessed: Mar 2021


Penstate Extension, (2012), A Short History of Anaerobic Digestion, Pensilvania State University, College of Agricultural science, retrieved at: https://extension.psu.edu/a-short-history-of-anaerobic-digestion


