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# **Biowaste valorisation through biorefinery system according to Circular Economy strategy**

The dependency on non-renewable resources represents an environmental, economic and social problem, globally affecting the planet with emissions of greenhouse gases (GHGs) and resulting in increased fuel extraction costs. The dependence on fossil non-renewable fuels is bound to increase, unless something changes, due to the increased demand for energy and chemicals induced by the fast world population growth. The production of biobased products and bioenergy should be considered as a unit of an integrated value chain of processes in the overall green bioeconomy. The EU Green Deal (EU-Green Deal, 2019) is a European political initiative promoting a new growth strategy aimed at transforming the EU into a fair and resource-efficient economy, where emissions of greenhouse gases in 2050 decrease of 30% and economic development is decoupled from resource use. Furthermore, the Green Deal enhances the EU's natural capital and protect the health and well-being of citizens from environment-related risks and impacts. The bioeconomy, a circular economy powered by nature and emerging from nature, is based on renewable biological resources and sustainable biobased solutions. Bioeconomy is fundamental for moving towards a carbon neutral EU reality and fossil free material and energy scenarios. This change of perspective promotes the shift from a Linear to a Circular structure of industrial processes, where by-products and wastes can become new secondary raw materials. To implement this transformative policy, bioeconomy promotes the concept and realisation of biorefinery. Biorefinery can represent the catalyst for systemic change to tackle holistically the social, economic and environmental perspectives. The biorefinery builds a new and synergistic relationship between technology and nature, between ecology and economy growth and belongs to Green Chemistry or Sustainable Chemistry.

Based on new biotechnological approaches, the bioeconomy maximizes the use of waste and resources, both biological, terrestrial and marine, as well as non-biological, CO<sub>2</sub> and fossil waste streams, as inputs for industrial and energy production, implementing a circular logic management to maximise opportunities of reuse, recycling and recovery (OECD, 2020).

Recently, Europe joined the 2030 Agenda for Sustainable Development Goals (SDGs, 2019), which established new targets in climate change and energy-production to ensure greater competitiveness, safety and stability of energy systems. The target defined by 2030 Agenda for Sustainable Development are: 1) GHG reduction equal to 40% of the levels of 1990, 2) at least 27% of the used energy must come from renewable energy and 3) 27% energy savings compared to current situation. To achieve these targets, biorefinery system plays a key role. Biorefinery enables the realization of Green Chemistry at the full scale, optimizing the supply chains of enhancement of biomass, ad hoc and waste, CO<sub>2</sub> and fossil waste stream, in local contexts developing integrated technology platforms and cascading use schemes.

The biorefinery process is like the petrochemical refining, but the crucial difference is the nature of the starting material; because for biorefinery is biomass, a renewable matter, for the petrochemical refinery is coal and petroleum, namely fossil resources.

Biorefinery is classified on the ground of biomass origin in first generation (1G), second (2G) and third (3G) generation biorefinery, respectively feed with ad hoc biomasses, waste biomasses and algae. This thesis focuses the attention on 2G-biorefinery for ethical, environmental, economic and social reasons.

In the present thesis two processes are considered: fermentation for L(+) Lactic acid (LA) production and anaerobic digestion (AD) for biogas production.

The study starts with the analysis of 2G-biorefinery system in EU28 and its three fundamental units: the feed biomass, the corresponding process and the resulting products. The aim is the realization of three data inventories: 1) biomass available in EU28, 2) process technical-economic-environmental feasibility and 3) generable high-added value products. The study combines bottom up and top down approaches, aimed respectively to evaluate how the fundamental units are interlaced and influenced each other and to define a sustainable biorefinery system.

According to the European Technical Guidance waste classification (2018/C 124/01) and Eurostat database, four biomass categories are evaluated: wastewaters and sewage sludge, municipal solid waste, waste from agriculture, forestry and fishing activities and waste from manufacturing of food and beverage products.

2G-biorefinery faces social, economic, environmental and technical problems due to the huge amount of biomasses, considering biomass as secondary raw material to valorize through platform chemical and energy production. 14 biomasses are studied, and these 14 biomasses are the most representative of the four biomass categories, which have carbon content over 50% w/w and belong to carbohydrate, lipids and lignocellulose feedstock groups respectively for 43 %, 36% and 14%. The correlation biomass-process stated that lignocellulose biomasses are suitable for thermochemical, chemical and biological processes, while carbohydrate and lipid biomass are respectively suitable for biological and chemical processes. The correlation biomass-process-product assesses that among the 11 analysed platform chemicals, ethanol, propionic acid, lactic acid and succinic acid have the highest yield through biological processes, allowing 14-57.22 % market size satisfaction and 9% to 36%, biomass valorisation with consequentially waste reduction. Among the 5 considered bio-energies, biogas is the only one able to satisfy completely the market size with a surplus of 11%. The achieved results prove: 1) the fundamental contribution of biomass to chemical and energy sectors and 2) biogas fundamental role in biorefinery system. Thus, the present study focuses the attention on Lactic acid (LA) and biogas production by means of biological routes, fermentation and anaerobic digestion respectively. Before starting the analysis of LA and biogas productions, a focus on biowaste management in Italy is investigated and described. In particular, a methodology for the technical and environmental assessment of biowaste valorisation in 2G-biorefineries in Italy. Italy is chosen as case study, considering years 2016-2019. Italian context is evaluated through the following key parameters: 1) gross domestic power, 2) climate, 3) demography and 4) population density distribution. The evaluations of geo-localisation and quantitative availability of biowaste amounts aimed to define the dimension and localisation of the biorefinery plant to optimise the supply and transport chains, while the qualitative characteristic aimed to evaluate the most promising process among two different biorefineries systems: thermo-valorisation (TH) and anaerobic digestion (AD). The main finding of the study witness that AD is more sustainable energetically than TH.

Then, the thesis investigates two process to produce L (+) Lactic acid (LA) from the organic fraction of municipal solid waste (OFMSW): the simultaneous saccharification and fermentation (SSF) and separated hydrolysis and fermentation (SHF). The study was carried out at labour and technical scale, with the support of modelling by SuperPro Designer<sup>®</sup> 8.0. The aim of the study is the optimisation of SSF and SHF. In detail, for SFF the analysis includes 1) the identification of the most suitable LA strain producers: three types of *Lactobacillus sp.* and one type of *Streptococcus sp.* strains, 2) the evaluation of the necessity of autoclavation of the OFMSW and 3) the production of market value L (+)- LA. For SHF the analysis includes: 1) type and loading of enzyme and 2) solid to liquid ratios.

OFMSW is employed as source of carbon and nitrogen to carry out SSF by using for L (+)-LA production.

In SHF two enzymes are tested: Stargen and Fermgen to hydrolyze starch and proteins. Hydrolytic performance is investigated according to different solid-to-liquid ratios.

*Lactobacillus sp.* strains does not show an efficient conversion of OFMSW into LA. Whereas, *Streptococcus sp.*, liquefies the material and produced LA.

For SSF process the maximum productivity of 2.16 g/Lh is achieved at technical scale, while the highest yield of 0.81g/g of theoretically present sugars is obtained in SSF carried out at solid to liquid ratio of 5w/w.

The LA concentration achieved from 20%w/w of bended OFMSW is 58g/L. Both under sterile and not sterile conditions SSF carried out with *Streptococcus sp* A620 directly convertes OFMSW into LA without considerable production of other acids. At technical scale (72L) SSF is implemented and the downstream processing including micro- and nanofiltration, electro dialysis, chromatography and distillation produced a pure 702 g/L of L (+)-LA formulation with an optical purity (OP) of 97%.

For SHF process the hydrolysis is carried out for 1h with Stargen and sequential LA concentration after 29 hours, is 0.33 g<sub>LA</sub> /g dry OFMSW with a productivity of 3.38 g<sub>LA</sub>/L·h Furthermore, L(+) Lactic acid production is investigated from spent coffee ground (SCGC). In detail, the acid-enzymatic hydrolysis and fermentation of L (+)-lactic acid (LA) with *Bacillus Coagulans* from spent coffee ground (SCGC) is studied. SCGC, a lignocellulose residue from coffee production consists of 34.26 ± 2.67% cellulose, 7.31% ± 2.54% hemicellulose and 24.88 ± 0.11% of lignin. Sequential and combined acid-enzymatic hydrolysis are carried out respectively, at 121°C for 15 min with 1%v/v H<sub>2</sub>SO<sub>4</sub> and 14.5% SCG wet and at 52°C for 24h with 0.25 mL Accellerase 1500 per gram of dry SCG, achieving a total sugar extraction efficiency of 41.24 ± 4.53%.

Fermentations are carried out both at the laboratory (2L) and technical (50L) scales and no scale effect is observed.

At 72L scale, LA yield per gram of sugar consumed and per dry gram of SCG were 0.956 ± 0.015, 0.18 ± 0.63 respectively. Downstream processing results in 786.70 g<sub>LA</sub>/L and 99.5% optical purity.

After the evaluation of L(+)Lactic acid from OFMSW and SCCG carried out by fermentation route, the thesis investigates the sequential production of L(+)-LA and biogas from organic fraction municipal solid waste (OFMW).

LA is produced from OFMW using a *Streptococcus sp.* strain A620 (optimized at the begging of the study in this thesis) by means of two fermentative pathways: separate enzymatic hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Via SHF a yield of 0.33 g<sub>LA</sub>/g<sub>FW</sub> (productivity 3.38 g<sub>LA</sub>/L·h) and via SSF 0.29 g<sub>LA</sub>/g<sub>FW</sub> (productivity 2.08 g<sub>LA</sub>/L·h) are reached. Fermentation residues and OFMSW are tested as feedstocks for anaerobic digestion (AD) (3 wt% TS). The following biogas yields are achieved: 0.71, 0.74 and 0.90 Nm<sup>3</sup>/kg<sub>VS</sub> for OFMSW and residues from SFF and SHF respectively.

The innovation of the approach consists in considering the conversion of OFMSW into two different sequential products through a biorefinery system, therefore making economically feasible L(+)-LA production and valorising its fermentative residues.

A economic and energy analysis is performed to complete the technical study of L(+)-LA and biogas productions in singular and combined process from OFMSW and SCG

Four scenarios are evaluated and compared: **Scenario IA** exclusive fermentative production of LA by means of simultaneous saccharification and fermentation (SSF), **Scenario IB** LA production carried out with separated hydrolysis and fermentation (SHF), **Scenario II** exclusive biogas production by means of anaerobic digestion. **Scenario III A-B** for sequential fermentative LA production and biogas by means of SSF and SHF from OFMSW. **Scenario IV** LA production by means of SHF from SCG. The integrated biorefinery process is compared to single processes for either L(+)-LA or biogas production. The economic evaluation, considering catchment areas from 2000 to 1 million inhabitants, is based on data from real biorefinery plants and carried out using SuperPro Designer<sup>®</sup> 8.0. The consistency of the approach is assessed through a set of composite indicators. The integrated biorefinery system is investigated from three main perspectives: 1) economic feasibility of producing LA and biogas, 2) the effect of process scale and 3) energy consumption/requirement. The present study proved that an integrated biorefinery system contributes more to optimal use of energy and material flows than single processes both for the sequential production of two market value products and optimisation of waste management. Profitability was achieved for catchment areas bigger than 20,000-50,000 inhabitants.

Finally, the present thesis focused the attention on the optimisation of AD. In detail, the key role of inoculum in mesophilic anaerobic digestion (AD) of organic fraction municipal solid waste (OFMSW) was studied. Two inocula are tested, one coming from the mesophilic digestate of wastewater activated sludge (WAS) and the other one from the mesophilic digestate of cow-agriculture sludge (CAS). Both inocula are anaerobically cultivated for three different periods: 0, 5 and 10 days and then inoculated in OFMSW considering three substrate-inoculum ratios (S:I) 1:2; 1:1; 2:1. First order kinetics and Gompertz modified model are applied to define disintegration rate, lag phase and maximum biogas yields. Energy sustainability index was calculated to define which configurations were suitable to be scaled-up. Then multi criteria decision aid was performed to outrank the AD configurations tested. The AD configurations with the best performances are: AD performed with S:I=2:1 with CAS cultivated for 5 days, AD performed with S:I=1:1 and 2:1 with CAS cultivated for 10 days and AD performed with S:I=2:1 WAS cultivated for 10 days

The present thesis is developed according the Circular Economy pillars: technical feasibility environmental sustainability and economic profitability and according to SDGs goals to promote the passage from Linear to Circular Economy. The main finding of the present study is the valorization of organic waste, from negative concept of waste to second renewable source to produce high added value product as L(+) Lactic acid and bioenergy as biogas