TECHNOLOGICAL ROADMAP ON ENZYMATIC PRE-TREATMENT FOR FRUIT AND VEGETABLE WASTE USAGE IN BIOGAS PRODUCTION: SHORT AND MEDIUM STAGES.

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ABSTRACT

Brazil is a large fruit producer but faces high rates of food waste. A promising solution is converting organic waste into biogas, contributing to reducing greenhouse gas emissions. Enzymes can break down plant residues into glucose, an essential process in biogas production. To maximize the efficiency of this process, it is important to identify and compare different enzymatic pretreatment methods. The Technology Roadmap methodology allows this comparison to be structured and comprehensive. Thirty-five patents related to enzymatic pre-treatment to produce biogas from fruit and vegetable waste were identified. Each patent stands out for its choice of substrate and the need for thermal and pH adjustments to operate efficiently. European countries and China are the leading players, with Novozymes of Denmark and the National University of Ireland Galway among the primary holders. European patents have global reach, while Chinese patents remain predominantly domestic, possibly due to local climate and energy priorities.

Keywords: Technology Roadmap. Pretreatment. Enzyme. Fruit waste. Biogas.

1 INTRODUCTION

Brazil witnesses the estimated production of 45 million tons of fruit per year, the third largest producer in the world in this segment[1]. However, such abundance is followed by the rate of waste production. It is estimated that 30% of total food production is wasted, equivalent to approximately 1.3 billion tons[2]. Organic waste, including plant residues, can be effectively utilized in biofuels like biogas, reducing greenhouse gas emissions and generating sustainable energy. Typically composed of cellulose, hemicellulose, lignin, and other compounds, plant residues vary in composition depending on their source. However, they generally consist of approximately 40 to 50% cellulose, 15 to 25% hemicelluloses, 20 to 25% lignin, and 5 to 10% other compounds.[3]. Cellulose is an essential substance that can be used in the production of various functional products, such as biogas and bioethanol. This happens due to its ability to release glucose through chemical and biological degradation processes, which are facilitated by enzymes known as cellulolytics[4].

Plant cell walls have developed to resist natural chemical and physical processes over the years. Biorefineries use pre-treatments to access essential sugars and break down this structure[3]. It is important to avoid the formation of inhibitors during pre-treatments, as they can harm the fermentation stage. A helpful approach is enzymatic pre-treatment, which offers selectivity and reduces the formation of inhibitors and undesirable residues, making it a practical option for this purpose[5]. Pre-treatments are essential for effective biogas production. Understanding global pre-treatment methods is crucial. The Technology Roadmap methodology enables comprehensive analysis of different pre-treatment techniques, aiding in identifying optimal strategies for biogas production.

Based on the premises established previously, this work aims to develop a specific Technology Roadmap, aiming to compare different patented enzymatic pre-treatment methods on fruits and vegetable waste for biogas production.

2 MATERIAL & METHODS

In the present study, the Technology Roadmap (TRM) methodology[6] was used to compare several patents with enzymatic pre-treatments used to produce fruit and vegetable waste biogas. The research was divided into two phases: a short-term one involving patents already submitted and accepted and a medium-term one covering patents submitted and still in the analysis process. This search methodology was carried out in April 2024, through the *Google Patents* database, using the following search terms: "Pretreatment", "Enzyme", "Biogas" and "Fruit waste". After completing the search, the results were exported to an *Excel* spreadsheet to identify patents related to the study theme. Subsequently, the relevant patents were reviewed and compared for double checking in the *Espacenet* database and the *World Intellectual Property Organization* (WIPO). After this, they were classified in a table based on their characteristics to help the TRM construction.

3 RESULTS & DISCUSSION

The first prospection identified 35 patents in total. Then, after being carefully checked and analyzed through reading and comparison with other databases, the final 6 patents found were organized in a table sheet (Table 1) with the majority of

characteristics displayed to help the TRM technique, it was possible to develop a graphical representation (Figure 1). Only one patent (16%) from 2007 was already granted. The other technologies started to be published in 2011, with the most recent in 2022, as an indicator that this technology has been developing over the last 13 years and is still trending. Each patent showed unique aspects that differed and stood out among them; they were fundamentally related to the substrate used in each. Different materials need different enzymes to do the desirable specific reaction, in this case, within the available variety of cellulolytic, hemicellulolytic, ligninolytic, and amylolytic types of enzymes since the waste used is from vegetables and fruits. Still, one company nominated its "special enzyme" under confidentiality. Of these, 5 out of 6 (83%) needed a thermal adjustment to operate, and this is intrinsically associated with enzymatic application due to their optimal temperature to reach their full catalytical potential[7]. The scenario remains similar when the pH adjustment is also studied, 4 out of 6 (67%) need some chemical compound to raise the pH to a more basic or neutral condition, possibly related to the enzymes' kinetic and biochemical parameters necessary to be active and functional.

Table 1 Enzyme pretreatment patents

Code, County, Applicant, and Year	Title	Status per Country	Enzymes	Chemical Pre-treatment	Physical Pre-treatment	Substrate
WO2011092136 A1 – Denmark – Novozymes – 2011 –	Biogas production process with enzymatic pre-treatment	**Abandoned** (CA, US); **IP Right Cessation** (BR); **Withdrawn** (EP); **Pending** (CN); **Application Filling** (WO)	Amylolytic, lipolytic, proteolytic, cellulolytic enzymes, oxidoreductase, and a plant cell-wall degrading enzyme.	NaOH, Na_2CO_3, $NaHCO_3$, $Ca(OH)_2$, lime hydrate, ammonia and/or KOH	Microwave and/or ultrasonic irradiation. Temperature ranges from 20°C to 70°C	Fruits and vegetables, and also paper waste
WO2012093041 A1 – Denmark – Novozymes – 2011 –	Process for Producing Biogas from Pectin and Lignocellulose Containing Material	**Application Discontinuation** (BR); **Abandoned** (US); **Withdrawn** (EP); **Pending** (CN); **Application Filling** (WO)	Amylolytic, lipolytic, proteolytic, cellulolytic enzymes, oxidoreductase, and a plant cell-wall degrading enzyme.	NaOH, Na_2CO_3, $NaHCO_3$, $Ca(OH)_2$, lime hydrate, ammonia, and/or KOH. pH from 7 to 12, but preferably around 8,5	Temperature ranges from 20°C to 70°C, preferably 30-60 °C, and more preferably 40-50 °C.	Fruits and vegetables, with pectin emphasis, and paper waste
CN108546729A – China – Hainan Chengmai Shenzhou Vehicle Biogas Co Ltd – 2018–	A kind method of anaerobic fermentation production biogas	**Pending** (CN)	Hemicellulase, cellulase, and lignoenzyme	-	Temperature ranges from 24°C to 35°C. Mixer	Fruits and vegetables, with emphasis on wine waste and also excrement slag
CN106554224A – China – Huainan Run Ji Ecological Agriculture Co Ltd – 2016 –	A high-efficiency fermenting type *Hericium erinaceus (Bull. Ex Fr.)* Pers. compost and preparation method thereof	**Pending** (CN)	Cellulose Enzyme bacterium	$(NH_4)_2SO_4$, NH_4NO_3, carbamide, $CaCl_2$	Temperature ranges from 30°C to 35°C. Mixer	Mushroom (*Hericium erinaceus*), fruits and vegetables, herbivore excrement
CN114642255A – China – Xi'an Yinneng Biotechnology Co lt – 2022 –	Complete recycling process for kitchen waste sewage	**Pending** (CN)	"Special enzyme"	Salt addition	Crushing, mixing, and filtration. Temperature range of 35 to 40℃	Fruits and vegetables
IES20060090A2 or ES2393930T3 – Ireland – National University of Ireland Galway – 2007 –	*Talaromyces emersonii* enzyme systems	**IP Right Cessation** (IE, BR); **Granted** (SP, DK, EP, MX, JP, AU); **Published** (SL, PT, AP, PO); **Application Filled** (CY); **Abandoned** (US, CA); **Pending** (CN); **Application Filling** (WO).	cellobiohydrolase I or a cellobiohydrolase II or a mixture thereof, a 5-glucosidase 1, a xylanase, and an endo-3-(l,3)4-glucanase	Suitable buffers include 50mM ammonium acetate, pH 45-60 or 50mM sodium phosphate, pH 7.0- 8.0	Temperature range of 30°C to 90°C. With optimum conditions at 55°C	Plants or materials derived from plants

List of country abbreviations: AP: ARIPO; AU: Australia; BR: Brazil; CA: Canada; CN: China; CL; CY: Cyprus; DK: Denmark; EP: Europe; IE: Ireland; JP: Japan; MX: Mexico; PO: Poland; PT: Portugal; SL: Slovenia; SP: Spain; US: United States of America; WO: Worldwide.

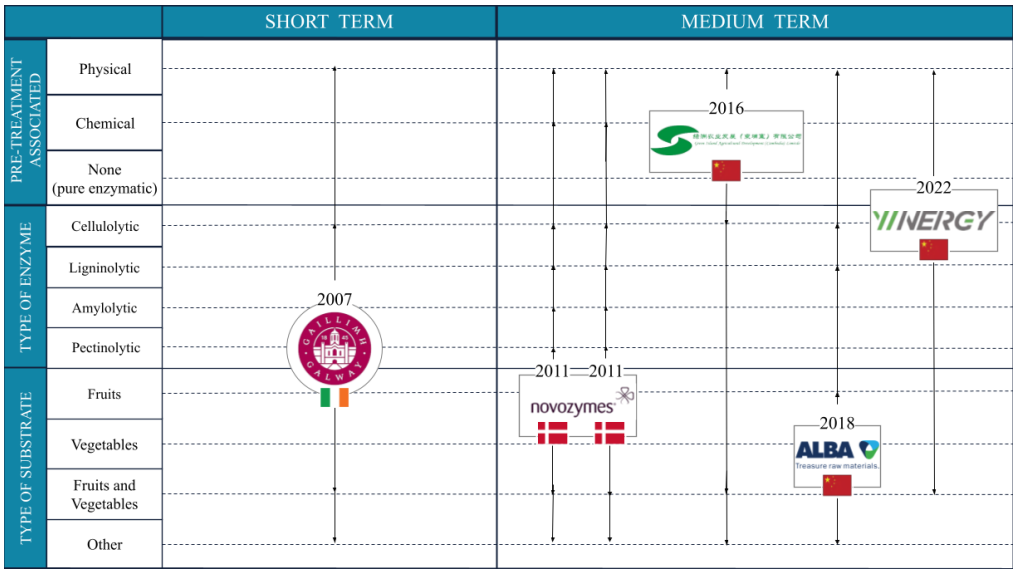

Figure 1 Technological Roadmap on enzymatic pre-treatment for fruit waste aiming biogas production.

As for the substrate, a wide variety of possible waste was observed that could be used for biogas production by an enzymatic pathway. The chosen vegetable and fruit waste possibly differ among patients due to location, availability, and impact on the enzyme's choice, as different vegetables and fruits have nonidentical biochemical compositions. These patents indicate an alternate association with paper industry waste (33%) or excrement (33%). Throughout this analysis, it is possible to identify combinations available to work on waste usage for biogas production and future development in this area as new applications with the same enzymes on variable waste.

The players responsible for submitting patents are from Europe (50%) and China (50%). The noteworthy player was Novozymes from Denmark, with 2 out of 6 (33%). The National University of Ireland Galway was the only institute of research owner of one patent (16%). Remarkably, the European players submitted patents in many other countries, as displayed in Table 1, representing their interest in and competitive aspect of implementing their novel technology. Unfortunately, different countries have different laws and high costs to maintain a patent, and this might be the reason it is so difficult to develop and implement a patent qualified to work under divergent legislation, recognized as the many longstanding and undesirable statuses, for instance, the Irish patent. However, Chinese patents have chosen to stay in their national territory, even though it is the only country with 3 (50%) of the most recent and emerging technology, almost certainly attributable to China's climate priorities and green energy production[8].

4 CONCLUSION

This study made it possible to successfully understand marketing tendencies in biogas production associated with enzymatic pre-treatments. Almost all of them are associated with chemical or physical pre-treatments due to the necessity of enzymes to maintain their activity. China is ahead globally on this emerging subject, as a warning for Brazil and other countries to work and compete. There are still 5 patents waiting to be granted, and possibly some research associated with future innovations with this theme. The variety of enzymes and substrates indicates the potential for development as there are still many different types of waste and enzymes to be tested, meaning a long path for future studies and findings in this area.

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