

## LITERATURE MACRO ANALYSIS OF POLYHYDROXYALKANOATES PRODUCTION FROM WASTE USING SLUDGE AS INOCULUM

Milena M. H. Coelho<sup>1</sup>, Tatiana F. Ferreira<sup>2</sup> & Priscilla F. F. Amaral<sup>1,3\*</sup>

<sup>1</sup> Programa de Pós-Graduação em Engenharia de Processos Químicos e Bioquímicos, Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

<sup>2</sup> Department of Organic Processes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

<sup>3</sup> Department of Biochemical Engineering, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

\* Corresponding author's email address: pamaral@eq.ufrj.br

### ABSTRACT

Polyhydroxyalkanoates (PHA) are biopolymers produced by microbial synthesis under stressful conditions, with great potential to substitute fossil-based plastics. Using waste as feedstock and activated sludge (AS) as inoculum is one of the most cost-effective forms of PHA production. Since this is a developing bioprocess, technological prospecting is a valuable tool for the decision-making process in the research and development field. This work aimed to carry out a literature macro analysis of PHA production from waste using AS as inoculum. A search was conducted to select the most appropriate keywords in Scopus and Web of Science databases. After refining the documents, 160 relevant articles were selected and analyzed according to their year, type of institution, and country. The macro analysis showed that most articles on this subject were published in 2021 (29). Italy published 25.0% of the articles, followed by China (23.8%). Most of the published articles were developed by universities (69.38%), and Universidade NOVA de Lisboa (Portugal) was the leader in publishing (22), with Sapienza Università di Roma (Italy) right behind (18). The collected information helps to understand the world's current PHA production scenario and supports a deeper technological prospecting.

**Keywords:** Polyhydroxyalkanoates 1. Activated sludge 2. Macro analysis 3. Scientific articles 4. Technological prospecting 5.

## 1 INTRODUCTION

Plastic pollution has already caused irreparable environmental damage, in addition to the depletion of non-renewable energy sources for its production, characterizing one of the most significant challenges of this century. Polyhydroxyalkanoates (PHA) are an eco-friendly biopolymer alternative that can help to manage part of this challenge because of their high biocompatibility and similar mechanical properties to fossil-based plastics. PHA can be produced by microbial synthesis as bacterial intracellular granules under carbon and nitrogen imbalance environmental conditions<sup>1,2</sup>. However, PHA production using synthetic substrates (refined carbohydrates or volatile fatty acids) as the carbon source with pure strains as inoculum (aseptic conditions) is not economically competitive with fossil-based polymers. Therefore, the commercial price of PHA is very high (\$ 4000 to 15000/ton), while fossil-based plastics are much cheaper (\$ 1000 to 1500/ton)<sup>2,3</sup>.

Using activated sludge (AS), which is a mixed microbial culture abundantly found in sewage treatment plants, in addition to using waste as feedstock to the bioprocess, is feasible to make PHA production more competitive, with a significant cost reduction for upstream processing (up to 50%). Furthermore, the AS is well-adapted to using complex carbon sources as feedstock, including agroindustrial wastes and byproducts. Various substrates, such as cheese whey, waste oils, oil mill effluents, and lignocellulosic biomass, have been extensively studied to produce PHA using AS as inoculum in the last years<sup>2,4</sup>.

In this context, technological prospecting is one of the most viable tools for identifying promising technologies, using systematic bibliographic analysis to guide the decision-making process in the research and development (R&D) field. In the initial stage of technological prospecting, it is essential to carry out a macro analysis of the scientific literature to map general data on an emerging technology, such as PHA production<sup>5,6</sup>, to then develop a deeper technological prospecting. Based on that, this work aimed to conduct a macro analysis of the literature from the last ten years regarding the PHA production process from waste as feedstock using AS as the bioprocess inoculum.

## 2 METHODS

**Systematic search of articles:** To define the most appropriate keywords, an iterative search was carried out with a combination of keywords in the Scopus and Web of Science databases, filtering those documents in which combinations appeared only in the title, abstract and keywords, which resulted in the following strings: "polyhydroxyalkanoate" OR "polyhydroxyalkanoates" OR "polyhydroxybutyrate" OR "polyhydroxybutyrates" OR "poly(3-hydroxybutyrate)" OR "poly-β-hydroxybutyrate" AND "sludge" OR "mixed microbial culture" OR "mixed culture" AND "production" OR "bioproduction" OR "biosynthesis". The search period was limited to the last ten years (2014-2024), and only research articles were included.

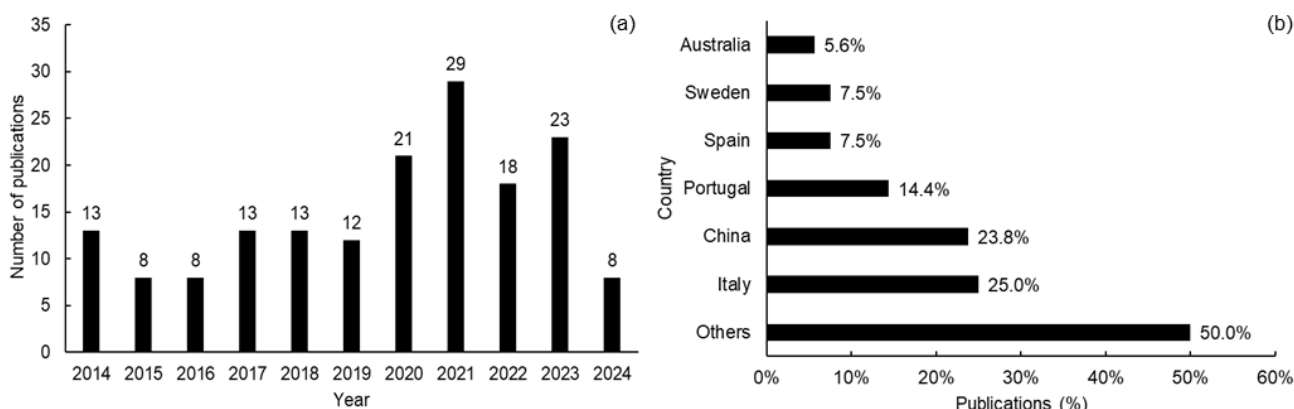
**Relevance analysis and macro processing data:** After removing duplicates and review or conference papers that were still left, the search returned 703 documents. A relevance analysis was carried out considering two main criteria: 1) the PHA must be produced by a mixed microbial culture (activated sludge), and 2) PHA must be produced using waste as feedstock. As a result,

the analysis returned 160 relevant articles. From a macro perspective, these documents were analyzed according to their year of publication, the type of institution that published them (university, research center, company), and the country where they are<sup>5,6</sup>.

### 3 RESULTS & DISCUSSION

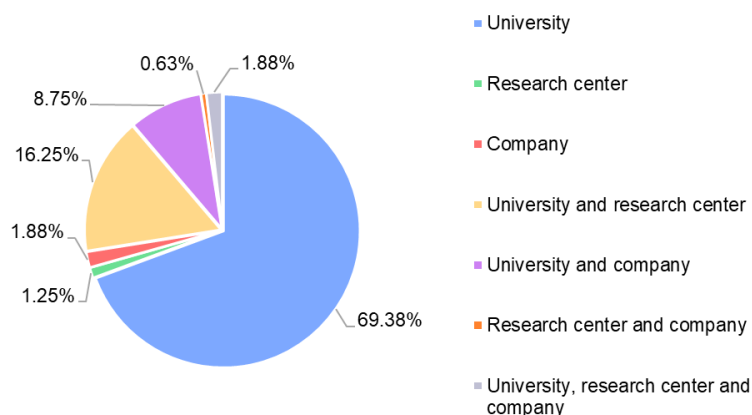
Figure 1 (a) shows that the number of scientific articles related to PHA production from waste with AS has been increasing during the selected period, with a peak in 2021, with 29 articles published. According to PlasticsEurope<sup>7</sup>, after stagnation in 2020 due to the Covid-19 pandemic, global plastics production increased from 375.5 Mt in 2020 to 390.7 Mt in 2021, showing a favorable scenario in that year. Bioplastics production also increased in this period, from 3.9 Mt in 2020 to 5.9 Mt in 2021. This is probably why research on this topic increased so much in 2021, as there was an active search for cheaper and cleaner plastic production techniques, especially to meet the high demand during the pandemic.

Figure 1 (b) shows the distribution of publications according to the country where the study was performed. Thirty-four countries were involved in the publications on PHA production in the biorefinery concept. Italy was the leading country, responsible for 25.0% of the 160 articles analyzed. Following Italy, China published 23.8% of the articles, followed by Portugal, Spain, Sweden, and Australia. Italy is the headquarters of Novamont, an international leader in the bioplastics sector and in the development of biochemicals, which has four production sites and four research centers<sup>8</sup>. This fact possibly explains the leadership of this country in producing scientific articles, given that there is a massive investment in R&D on PHA. Furthermore, Italy is Europe's second-largest bioplastics producer, with 27% of the total output, falling behind Germany, which has 50.9% of the total bioplastics output in this continent<sup>9</sup>. On the other hand, in 2022, China was the world's leader in bioplastics production, responsible for 33% of the global output in that year. Therefore, considerable investments are expected in R&D on PHA<sup>10</sup>.



**Figure 1** (a) Number of publications related to PHA production from waste using AS as inoculum over the last ten years (2014-2024) according to Scopus and Web of Science databases. (b) The percentage of publications related to PHA production from waste using AS as inoculum over the last ten years (2014-2024), according to Scopus and Web of Science databases, shown by country.

Most of the published scientific articles were developed by universities (69.38%), followed by partnerships between universities and research centers (16.25%) and between universities and companies (8.75%), as shown in Figure 2.



**Figure 2** Percentage of the types of institutions and partnerships involved in scientific articles about PHA production from waste using AS as inoculum over the last ten years (2014-2024) according to Scopus and Web of Science databases.

Portugal published 23 articles, and Universidade NOVA de Lisboa is involved in 22 of them (Table 1), confirming the university's leading role in research on PHA in this country. Sapienza Università di Roma is the leader in publications in Italy, being involved in 18 articles, followed by Università Ca' Foscari Venezia (11) and Università degli studi di Verona (8), which, together, represent 92.5% of articles published in the country. In China, many universities spread across the country publish in smaller quantities, highlighting the Chinese Academy of Tropical Agricultural Science (7) and Tsinghua University (7).

**Table 1** Top 10 institutions of PHA production from waste using AS as inoculum scientific articles over the last ten years (2014-2024) according to Scopus and Web of Science databases.

Institution	Type of institution	Number of publications	Country
Universidade NOVA de Lisboa	University	22	Portugal
Sapienza Università di Roma	University	18	Italy
Università Ca' Foscari Venezia	University	11	Italy
Tianjin University of Science & Technology	University	10	China
Veolia Water Technologies AnoxKaldnes	Company	9	Sweden
The University of Queensland	University	8	Australia
Università degli studi di Verona	University	8	Italy
Chinese Academy of Tropical Agricultural Science	University	7	China
Tsinghua University	University	7	China
Universidade de Santiago de Compostela	University	6	Spain
Others	-	217	-

Veolia Water Technologies is the water division of the French company Veolia Environment and the world's largest supplier of water services<sup>10</sup>. In the last 20 years, Veolia and its Swedish subsidiary AnoxKaldnes have been working on R&D at the different steps of the wastewater-to-bioplastic chain using AS, with a pilot installation at the Aquiris wastewater plant in Brussels, Belgium<sup>11</sup>. Veolia is the only company that stands out among the 10 top institutions, possibly due to these reasons.

## 4 CONCLUSION

The macro analysis from scientific literature showed that 2021 was the year with the highest number of articles on the subject (29), possibly due to the recovery of the plastics market after a period of pandemic-related stagnation. Italy published 25.0% of the scientific articles, followed by China (23.8%), the world's largest plastics producer, highlighting these countries as the leaders in R&D on PHA. Most of the published scientific articles were developed by universities (69.38%), and Universidade NOVA de Lisboa (Portugal) and Sapienza Università di Roma (Italy) published 22 and 18 articles, respectively, with the most significant scientific production between the affiliations. Veolia Water Technologies AnoxKaldnes was the only company in the top 10 institutions, highlighting the company's considerable investment in R&D on PHA. The collected information is essential to understanding the world's current scenario of PHA production, and they can provide support for the beginning of a deeper technological prospecting, which may help the decision-making process in the R&D field on PHA from waste using AS in the coming years.

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## ACKNOWLEDGEMENTS

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support to this work.