

Optimization of Microbial Metabolite Production for Enhanced Plastic Degradation

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ABSTRACT

Plastics, particularly polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), are extensively utilized yet remain in the environment due to their prolonged degradation rates. The global challenge of plastic waste accumulation has necessitated the exploration of effective and sustainable solutions. A promising approach involves utilizing microbial strains that can degrade plastic into less harmful compounds. These microbial strains are capable of metabolizing plastic components, thereby converting them into valuable metabolites. The optimization of metabolite production in these strains is essential for improving their biodegradation capabilities. This review examines strategies for optimizing metabolite production in microbial strains involved in plastic biodegradation, specifically highlighting *Gluconobacter oxydans*. The abstract of the paper summarizes the principal findings and importance of the research on the bioconversion of

ethylene glycol (EG) to glycolic acid (GA) utilizing the microorganism *Gluconobacter oxydans*. This document outlines the key points. Optimization of medium composition, enhancement of growth through specific additives, performance of bioreactors, implementation of an extended fed-batch strategy, and high process efficiency. The abstract provides a thorough overview of the research, highlighting the potential of *Gluconobacter oxydans* for glycolic acid production via optimized bioconversion processes. The main objective is to determine the optimal conditions for the bioconversion process by modifying operational variables, thus enhancing the efficiency and sustainability of GA production via microbial methods.

INTRODUCTION

Plastic pollution represents a significant global challenge. It is responsible for a broad range of environmental hazards and physiological toxicity. Plastic litter exerts significant physical effects on the environment. Nano plastics exhibit significant chemical effects and are associated with health hazards. Microplastic occupies a position within this spectrum. The degradation of plastic presents significant challenges owing to its durability and resistance to natural decomposition processes. Several methods have been developed or are under investigation to enhance the degradation of plastic. Mechanical recycling involves the collection, sorting, and melting of plastics to create new products. Chemical recycling includes processes such as depolymerization, pyrolysis, and gasification. Biodegradation processes include microbial degradation, enzymatic degradation, and composting for biodegradable plastics. The process of photodegradation, specifically through UV radiation. Thermal degradation, specifically through incineration and hydrothermal liquefaction. Additional advanced innovative technologies encompass Oxo-Biodegradable Plastics, supercritical Water, and Bioplastics. Each method presents advantages and disadvantages, and frequently, an integrated approach is necessary for the effective management of plastic waste. The primary objective is to establish processes that are environmentally sustainable and

economically feasible. (Sulajärvi et al., 2019) (Santos et al, 2024) The microbial biodegradation process can completely degrade plastic monomers and is sustainable; however, it is not yet applicable at the industrial level, unlike other methods that may not fully degrade plastic or may generate toxic byproducts. Microorganisms capable of degrading plastic monomers include *Ideonella sakaiensis*, *Pseudomonas putida*, *Aspergillus niger*, *Rhodococcus ruber*, and *Gluconobacter oxydans*, among others. Ethylene glycol (EG) can be synthesized from plastic waste via glycolysis, a chemical recycling technique specifically applicable to polyethylene terephthalate (PET) plastics. Glycolic acid (GA), a small molecule belonging to the alpha-hydroxy acid (AHA) group, is extensively utilized in diverse industries such as food, pharmaceuticals, textiles, and manufacturing. GA is conventionally synthesized from petrochemical sources via the catalytic reaction of formaldehyde with synthesis gas. There is increasing interest in sustainable production methods, particularly the use of microorganisms to convert ethylene glycol (EG), which can be derived from bioethanol, glycerol, or cellulosic biomass. (Santos et al, 2024)(Sulajärvi et al., 2019) Studies indicate that specific yeasts and acetic bacteria, including *Gluconobacter oxydans*, are capable of oxidizing EG to generate GA. Synthetic metabolic pathways utilizing genetically modified microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* are being investigated for the efficient production of GA from simpler carbon sources, including glucose. (Zhang et al., 2022) A study aimed to optimize the bioconversion process of EG to GA utilizing *Gluconobacter oxydans*. The study examined critical operational variables such as bioreactor aeration, process temperature, pH, and cell concentration to enhance the efficiency, productivity, and yield of GA. (Zhou et al., 2021)

LITERATURE REVIEW

Glycolic acid (GA) is widely used across various industries, including food, textiles, pharmaceuticals, and manufacturing. It serves multiple roles, such as a dyeing agent in food, a skincare component in pharmaceuticals, and a tanner in

textiles. Additionally, it is involved in producing disposable packaging, emulsion polymers, and industrial cleaners, highlighting its versatility and importance in industrial applications.

Traditionally, GA is produced from petrochemical resources through chemical transformations involving toxic formaldehyde. However, there is a growing interest in bioproduction methods that utilize microorganisms, which offer a more sustainable alternative. The bioconversion of ethylene glycol (EG) to GA is particularly promising, as it can be derived from renewable sources like bioethanol and glycerol, making the process more environmentally friendly.

Various microorganisms, including naturally occurring yeasts and acetic bacteria, have been studied for their ability to oxidize EG to produce GA. This includes both wild-type strains and genetically modified organisms, which can utilize simpler carbon sources like glucose for enhanced production. The research indicates that optimizing microbial pathways can lead to more efficient GA production.

The study employs response surface methodology to optimize production levels while minimizing costs. This approach is crucial for enhancing the efficiency of the bioconversion process, focusing on variables such as aeration, temperature, pH, and cell concentration. The optimization of growth media, particularly the use of yeast extract and peptone, has shown significant improvements in cell growth and bioconversion yields.

The research highlights the effectiveness of different bioreactor configurations, such as stirred tank and bubble column bioreactors, in achieving high concentrations of GA. The extended fed-batch strategy demonstrated remarkable results, achieving a GA concentration of 94.2 g/L, showcasing the potential for industrial-scale applications. (Santos et al., 2024) (Zhou et al., 2021) (Jem & Tan, 2020)

The findings emphasize the importance of developing sustainable and economically viable methods for GA production. The study addresses technical challenges in scaling up the bioconversion process from laboratory to industrial

levels, aiming to create a more sustainable production pathway that incorporates renewable resources into the existing petrochemical framework. This literature survey underscores the significance of optimizing bioconversion processes and the potential of using microorganisms for sustainable glycolic acid production, paving the way for future research and industrial applications. (Santos et al, 2024)

The paper provides a comprehensive overview of the production methods for glycolic acid (GA) and ethylene glycol (EG), focusing on biotechnological approaches. GA is a versatile compound used in various industries, including textiles, food, pharmaceuticals, and cleaning agents. Its market has been growing, due to increased demand in cosmetic products and cleaning agents. Traditionally, GA is produced chemically from petrochemical resources, primarily through carbonylation of formaldehyde. However, biotechnological methods using microorganisms like *Alcaligenes* sp. and *Gluconobacter oxydans* have shown promise, with significant yields reported. EG is predominantly produced through the hydration of ethylene oxide. The paper highlights the potential for biotechnological production from renewable resources, although current methods are still reliant on chemical processes.

The paper discusses various metabolic engineering strategies employed to enhance the production of GA and EG using genetically modified microorganisms such as *E. coli* and yeast. These strategies have led to improved yields from sugars, although challenges remain in achieving commercially viable production levels. The paper identifies several challenges in the biotechnological production of GA and EG, including low production titers and the need for efficient metabolic pathways. It emphasizes the importance of balancing enzyme activities and metabolic fluxes to improve yields. The authors suggest that future research should focus on developing metabolic routes that utilize various cheap and renewable feedstocks, including agricultural waste. They also highlight the need for pathways that can produce both GA and EG at high yields. This literature survey encapsulates the current state of research on the biotechnological production of GA

and EG, outlining both the advancements made and the challenges that lie ahead in this field. (Sulajärvi et al., 2019). Glycolic acid (GA) is recognized for its diverse applications, particularly in cosmetics, packaging, and medical fields due to its properties as a bifunctional α -hydroxy acid. It is used in products for exfoliation and moisturizing, and in its polymeric form, polyglycolic acid (PGA), it serves as an effective packaging material and drug delivery substrate. Traditional methods for GA production involve chemical reactions that raise concerns about petroleum resource depletion and by-product formation. This has led to increased interest in microbial production methods utilizing renewable resources, particularly through engineered microorganisms that modify genes involved in the glyoxylate bypass. Recent studies have focused on constructing novel pathways for GA production in *E. coli*. These include the Dahms pathway and the glyoxylate bypass, which have been optimized to enhance GA yields. The research highlights the importance of overexpressing specific genes, such as lactaldehyde dehydrogenase (AldA) and glyoxylate reductase (YcdW), to improve production efficiency. The literature indicates that successful GA production in *E. coli* often involves gene knockouts and overexpression strategies. For instance, the deletion of the glycolate dehydrogenase gene (glcD) and the construction of strains like X17ld have been shown to significantly enhance GA production from D-xylose.

The regulation of the glyoxylate bypass is complex, involving transcriptional and protein-level controls. The aceBAK operon, which is crucial for the glyoxylate bypass, is activated under specific conditions, such as the presence of acetate or fatty acids. This regulation is sensitive to anaerobic conditions, which can impact GA production. The paper reports that the engineered *E. coli* strain GA19 achieved a notable production level of 4.57 g/L GA with a yield of 0.46 g/g from D-xylose, marking a significant advancement in microbial GA production through the Dahms pathway. This literature survey underscores the ongoing efforts to optimize microbial pathways for GA production, highlighting the importance of genetic engineering and metabolic regulation in achieving higher yields. (Cabulong et al., 2018). Prior

research indicates that incorporating organic nitrogen sources and reducing peptone concentrations can markedly enhance bacterial growth. The current study substantiates this finding by identifying yeast extract and peptone as the most effective nitrogen sources for *Gluconobacter oxydans*. The combination of mannitol with YE and PEP results in increased yields. Mannitol and glycerol demonstrate potential for industrial applications. (Santos et al, 2024) (Zahid et al., 2019) (Li et al., 2021).

CONCLUSION

The assays conducted for optimizing the composition of growth media demonstrated favorable outcomes, resulting in enhanced bacterial biomass and improved bioconversion yields of ethylene glycol (EG) to glycolic acid (GA). This suggests that the appropriate media composition is essential for improving microbial productivity. Thereseearchrevealedthatvariablesincludingcellconcentration, aeration, and medium pH have a substantial impact on glycolic acid production in bioreactors. Managing these parameters is crucial for optimizing process yields, as they have a direct impact on the enzymes involved in glycolic acid synthesis. The bioconversion of ethylene glycol into glycolic acid by *Gluconobacter oxydans* indicates a promising avenue for industrial production. The findings indicate that this organic acid, currently in high demand, has the potential for sustainable production. However, there are notable challenges associated with scaling the process from bench to industrial levels. The research identifies specific technical limitations that impede the transition from laboratory-scale to industrial-scale production. This work enhances the development of a more sustainable and cost-effective approach to glycolic acid production, which is essential for its commercial success. The biotransformation process is associated with the petrochemical industry, utilizing ethylene glycol, a substrate predominantly sourced from petrochemical origins. The integration of renewability into the production process plays a crucial role in diminishing dependence on fossil feedstocks. The study emphasized that, although earlier research attained higher product concentrations, it failed to

consider the residual ethylene glycol present at the conclusion of the process. This oversight highlights the critical role of complete substrate utilization in enhancing operational efficiency and sustainability. The findings collectively highlight the efficacy of the optimization strategies utilized in the study and their possible implications for the sustainable production of glycolic acid.

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