

Proceeding Paper

Clostridium pasteurianum Bioprocessing: Pioneering Circular Bioeconomy Advancements through Sustainable Resource Utilization [†]

Radu Tamaian

ICSI Analytics, National Institute for Research and Development for Cryogenic and Isotopic Technologies—ICSI Rm. Vâlcea, 4th Uzinei Street, Râmnicu Vâlcea, VL, Romania; radu.tamaian@icsi.ro

[†] Presented at the 2nd International Electronic Conference on Microbiology, 1–15 December 2023; Available online: <https://ecm2023.sciforum.net>.

Abstract: The transition towards a circular bioeconomy represents a paradigm shift in sustainable resource management, aiming to synergize biological processes, waste valorization, and renewable product synthesis. A key contender in this transformative landscape is the versatile anaerobic bacterium *Clostridium pasteurianum*. With its unique metabolic capabilities, *C. pasteurianum* holds substantial promise as a cornerstone of bioprocessing strategies within the circular bioeconomy framework. As we navigate the path towards a sustainable and regenerative future, continued research and innovation are imperative to unlock the full potential of *C. pasteurianum*.

Keywords: ABE fermentation; bioprocessing; biorefinery; circular bioeconomy; environmental impact; waste management; PBE fermentation

1. Introduction

The transition towards a circular bioeconomy marks a profound departure from the traditional linear economic model [1,2]. In the conventional “take-make-dispose” linear economy, resources are extracted, processed, used, and finally discarded as waste. This linear model has long been associated with overconsumption, resource depletion, environmental degradation, and an unsustainable approach to economic development. Waste generation is one of the repercussions of this typical linearly productive method. Moreover, economic growth has expedited the way that primary resources are extracted, produced, used, and disposed of, which has increased the amount of trash produced [3–5]. In contrast, the circular bioeconomy introduces a fundamental paradigm shift in how we conceptualize and manage resources.

The concept of the circular bioeconomy represents a paradigm shift in how modern society views and manages resources. It underscores the need to break away from the linear “take-make-dispose” model and instead foster a regenerative system where resources are used efficiently, waste is minimized, and environmental impact is curtailed. At the heart of this paradigm lies the aspiration to create a closed-loop system where biological processes are harnessed to their fullest potential and the value of resources is continually preserved and enhanced. The circular bioeconomy is founded on several key principles: resource efficiency, biological processes, waste valorization, renewable product synthesis, closed-loop systems, and environmental stewardship [1,2,6–8].

One of the core tenets of the circular bioeconomy is the seamless integration of biological processes with waste valorization and renewable product synthesis. This synergy is pivotal in achieving the overarching goals of sustainability and resource efficiency [1,9]. Biological processes/systems, such as the bacterium *Clostridium pasteurianum* (full scientific name: *Clostridium pasteurianum* Winogradsky, 1895), are key agents in this synergy.

Citation: Tamaian, R. *Clostridium pasteurianum* Bioprocessing: Pioneering Circular Bioeconomy Advancements through Sustainable Resource Utilization. *Biol. Life Sci. Forum* **2023**, *31*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor(s): Name

Published: date



Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

C. pasteurianum possesses enhanced abilities to convert organic materials into valuable products with high efficiency and minimal environmental impact.

This paper aims to shed light on the multifaceted potential of *C. pasteurianum* within the circular bioeconomy framework. By delving into its metabolic capabilities, its proficiency in acetone-butanol-ethanol (ABE) fermentation (acetone, butanol, and ethanol are produced in the ratios of 3:6:1), and its compatibility with biorefinery strategies, this review seeks to elucidate how *C. pasteurianum* can advance the circular bioeconomy's goals of waste minimization, resource efficiency, and reduced environmental impact. Furthermore, it acknowledges the challenges that lie ahead in harnessing *C. pasteurianum*'s full potential and underscores the importance of continued research and innovation in this burgeoning field.

2. *Clostridium pasteurianum*: A Microbial Architect of Circular Bioeconomy

The genus *Clostridium* encompasses a diverse group of anaerobe bacteria with unique metabolic capabilities that hold significant potential within the circular bioeconomy. Among the many species within this genus, *C. pasteurianum* stands out as an exemplar due to its preeminent attributes and suitability for sustainable resource utilization. One of its most remarkable features is its ability to ferment an impressive array of feedstocks, ranging from lignocellulosic biomass to agricultural residues, and organic waste. Alongside ABE fermentation, *C. pasteurianum* exhibits a non-biphasic metabolism, alternatively producing 1,3-propanediol-butanol-ethanol (PBE fermentation). This metabolic flexibility grants *C. pasteurianum* the capacity to transform otherwise underutilized and often discarded resources into valuable biofuels and bio-based chemicals. *C. pasteurianum* has the ability to convert carbohydrates (e.g., glycerol, glucose and another hexoses, and biopolymers in the form of cellulose fibers and starches) to acetates, butanol, ethanol, and 1,3-propanediol (1,3-PDO), while releasing gaseous compounds (molecular hydrogen and carbon dioxide) [10–13]. The metabolism of this anaerobe bacterium can be switched from alcohols and reduced acids to volatile fatty acids (VFAs; for example, acetic, propionic, and butyric acids) in order to maximize the yield of molecular hydrogen from reduced fermentation end-products (e.g., butanol, ethanol, and lactate) [14–17]. The fermentation end-products of *C. pasteurianum* depend on the growth conditions (Table 1).

Table 1. The fermentation end-products of various *C. pasteurianum* strains.

Feedstock (Pretreatment) [Carbon Source]	Strain	Growth Conditions	Main Product and Additional Fermentation Products	Reference
Rice straw (enzymatic hydrolysis) [glucose, xylose ^{RS} , arabinose ^{RS}]	MTCC 116	shake flasks (150 mL); anaerobic chamber; rota- tion: 192 rpm; tempera- ture: 37 ± 2 °C	Total H₂ production: 2580 mL/L (from 54.18 g/L sugars in 144 h). Maximum H₂ production rate: 23.96 mL/L/h (recorded in 96 h). SM: butyrate, acetate, formate, succinate. NgA: ethanol, propionate. 30 °C = the highest yield of 1,3- PDO (0.60 mol/mol substrate from 10 g/L substrate).	[18]
Biodiesel-derived crude glycerol (no pretreatment) [glycerol]	MTCC 116, im- mobilized on silica	custom-built Erlenmeyer flasks (250 mL); anaero- bic chamber; rotation: 200 rpm; temperature: 30/37/45 °C	37 °C = the most suitable temper- ature for maximum yield of buta- nol (0.28 mol/mol substrate from 25 g/L substrate) and ethanol (0.27 mol/mol substrate from 10 g/L substrate).	[19]

Biodiesel-derived crude glycerol (no pretreatment) [glycerol 01, respectively glycerol 02]	DSM 525, entrapped into PVA particles	stirred bioreactor (1.3 L tank with 1.0 L of production medium); rotation: 200 rpm; temperature: 34 °C; 68 RB	45 °C = not suitable for fermentation.	
			Initial glycerol concentration: 50.51 ± 2.77 g/L	
			Residual glycerol concentration: 10.46 ± 1.74 g/L	
			Fermentation time: 2.69 ± 0.05 h	
			Produced butanol : 8.38 ± 0.86 g/L	[20]
			Butanol productivity ^{6.3X} : 3.12 ± 0.33 g/L/h	
			Butanol yield: 0.21 ± 0.02 g/g	
			Produced 1,3-PDO: 3.22 ± 0.30 g/L	

^{RS} Residual sugars: *C. pasteurianum* was unable to efficiently use those sugars as compared to glucose. **Bolded and underlined products**: main products of the fermentation process; SM: secondary metabolites; NgA: negligible amounts; PVA: polyvinyl alcohol; glycerol 01 and 02: crude glycerol samples (89.55%, respectively 52.66% glycerol content); 68 RB: 68 repeated batches (results are showed for the last 56th–68th batches as the process duration gradually decreased until the 56th batch and was quite stable for the rest of them); ^{6.3X} 6.3 times more butanol was produced from glycerol by PVA-entrapped cells than by free cells; butanol yield is calculated as final butanol concentration divided by concentration of utilized glycerol.

In summary, the data in Table 1 demonstrates the versatility of *C. pasteurianum* in utilizing various feedstocks (rice straw, crude glycerol) to produce a range of valuable gaseous (hydrogen) and liquid biofuels (butanol and ethanol) or organic chemicals (1,3-PDO). It is noteworthy that the generation of butanol and ethanol is simultaneously declining as 1,3-PDO is produced. The bacterium's ability to adapt to different growth conditions and efficiently convert these feedstocks underscores its potential in the circular bioeconomy, where waste materials and renewable resources can be harnessed for sustainable resource utilization and biofuel production.

3. Challenges and Future Directions

As we explore the potential of *Clostridium pasteurianum* within the circular bioeconomy, several challenges and opportunities emerge, paving the way for future research and innovation.

A promising avenue for advancing *C. pasteurianum*'s capabilities lies in genetic engineering. By manipulating its metabolic pathways and optimizing enzyme expression, researchers can potentially enhance the bacterium's substrate utilization efficiency [21], product yields [21,22], and tolerance to inhibitory compounds [23]. Genetic modifications can also be employed to tailor *C. pasteurianum* for specific feedstocks or target products, thereby increasing its versatility in different circular bioeconomy applications [24,25].

The transition from laboratory-scale experiments to industrial applications presents a substantial challenge. Scaling up *C. pasteurianum* fermentation processes to meet commercial demands requires careful consideration of bioreactor design, process control, and optimization of parameters such as pH, temperature, and nutrient supply. Addressing these challenges is crucial for achieving cost-effective and efficient large-scale production.

The concept of integrated biorefineries, where multiple valuable products are generated from the same feedstock, can be further explored. *C. pasteurianum*'s ability to produce both ABE and PBE fermentation products makes it an attractive candidate for the development of sustainable and multifunctional biorefinery systems.

In conclusion, while *Clostridium pasteurianum* offers immense potential within the circular bioeconomy, addressing challenges related to genetic engineering, scale-up, regulatory compliance, and safety is imperative for its successful industrial adoption. Additionally, leveraging the complementary PBE fermentation pathway and exploring novel

feedstocks can unlock new possibilities for sustainable resource utilization and product diversification. These future directions hold the promise of a more sustainable and regenerative bioeconomy driven by the metabolic prowess of *C. pasteurianum*.

Funding: This research was funded by the Romanian Ministry of Research, Innovation and Digitization through NUCLEU Program, Contract no. 20N/05.01.2023, Project PN 23 15 04 01: “The cascade valorisation of agro-industrial waste of plant biomass type in bioproducts with added value in the circular bioeconomy system”.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: R.T is responsible for keeping and giving access to the data for the entire in silico work.

Acknowledgments: Research work was conducted under the NUCLEU Program, Contract no. 20N/2023, Project PN 23 15 04 01: “The cascade valorisation of agro-industrial waste of plant biomass type in bioproducts with added value in the circular bioeconomy system”. Administrative and technical support was given by Ministry of Research, Innovation and Digitization through Program 1—Development of the national research and development system, Subprogram 1.2—Institutional performance—Projects for financing excellence in R&D, Contract no. 19PFE/2021.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Handa, A.; Rajamani, P. Waste Management and Environment. In *Basic Biotechniques for Bioprocess and Bioentrepreneurship*; Bhatt, A.K., Bhatia, R.K., Bhalla, T.C., Eds.; Academic Press: Cambridge, MA, USA, 2023; pp. 391–413, ISBN 978-0-12-816109-8.
2. Velenturf, A.P.M.; Purnell, P.; Macaskie, L.E.; Mayes, W.M.; Sapsford, D.J. A New Perspective on a Global Circular Economy. In *Resource Recovery from Wastes: Towards a Circular Economy*; Green Chemistry; The Royal Society of Chemistry: 2019; pp. 3–22 ISBN, 978-1-78801-381-9.
3. Cerqueira, P.A.; Soukiazis, E.; Proença, S. Assessing the Linkages between Recycling, Renewable Energy and Sustainable Development: Evidence from the OECD Countries. *Environ. Dev. Sustain.* **2021**, *23*, 9766–9791. <https://doi.org/10.1007/s10668-020-00780-4>.
4. Namliş, K.-G.; Komilis, D. Influence of Four Socioeconomic Indices and the Impact of Economic Crisis on Solid Waste Generation in Europe. *Waste Manag.* **2019**, *89*, 190–200. <https://doi.org/10.1016/j.wasman.2019.04.012>.
5. Minoglou, M.; Komilis, D. Describing Health Care Waste Generation Rates Using Regression Modeling and Principal Component Analysis. *Waste Manag.* **2018**, *78*, 811–818. <https://doi.org/10.1016/j.wasman.2018.06.053>.
6. Gunasekaran, M.; Kumar, G.; Karthikeyan, O.P.; Varjani, S. Lignocellulosic Biomass as an Optimistic Feedstock for the Production of Biofuels as Valuable Energy Source: Techno-Economic Analysis, Environmental Impact Analysis, Breakthrough and Perspectives. *Environ. Technol. Innov.* **2021**, *24*, 102080. <https://doi.org/10.1016/j.eti.2021.102080>.
7. Cheng, S.Y.; Tan, X.; Show, P.L.; Rambabu, K.; Banat, F.; Veeramuthu, A.; Lau, B.F.; Ng, E.P.; Ling, T.C. Incorporating Biowaste into Circular Bioeconomy: A Critical Review of Current Trend and Scaling up Feasibility. *Environ. Technol. Innov.* **2020**, *19*, 101034. <https://doi.org/10.1016/j.eti.2020.101034>.
8. Mak, T.M.W.; Xiong, X.; Tsang, D.C.W.; Yu, I.K.M.; Poon, C.S. Sustainable Food Waste Management towards Circular Bioeconomy: Policy Review, Limitations and Opportunities. *Bioresour. Technol.* **2020**, *297*, 122497. <https://doi.org/10.1016/j.biortech.2019.122497>.
9. Gupta, A.; Nagrath, G. Biobusiness Opportunities. In *Basic Biotechniques for Bioprocess and Bioentrepreneurship*; Bhatt, A.K., Bhatia, R.K., Bhalla, T.C., Eds.; Academic Press: Cambridge, MA, USA, 2023; pp. 417–425, ISBN 978-0-12-816109-8.
10. Sabra, W.; Wang, W.; Surandram, S.; Groeger, C.; Zeng, A.-P. Fermentation of Mixed Substrates by *Clostridium Pasteurianum* and Its Physiological, Metabolic and Proteomic Characterizations. *Microb. Cell Factories* **2016**, *15*, 114. <https://doi.org/10.1186/s12934-016-0497-4>.
11. Munch, G.; Mittler, J.; Rehmann, L. Increased Selectivity for Butanol in *Clostridium Pasteurianum* Fermentations via Butyric Acid Addition or Dual Feedstock Strategy. *Fermentation* **2020**, *6*, 67. <https://doi.org/10.3390/fermentation6030067>.
12. Sarchami, T.; Johnson, E.; Rehmann, L. Optimization of Fermentation Condition Favoring Butanol Production from Glycerol by *Clostridium Pasteurianum* DSM 525. *Bioresour. Technol.* **2016**, *208*, 73–80. <https://doi.org/10.1016/j.biortech.2016.02.062>.
13. Munch, G.; Schulte, A.; Mann, M.; Dinger, R.; Regestein, L.; Rehmann, L.; Büchs, J. Online Measurement of CO₂ and Total Gas Production in Parallel Anaerobic Shake Flask Cultivations. *Biochem. Eng. J.* **2020**, *153*, 107418. <https://doi.org/10.1016/j.bej.2019.107418>.
14. Dabrock, B.; Bahl, H.; Gottschalk, G. Parameters Affecting Solvent Production by *Clostridium Pasteurianum*. *Appl. Environ. Microbiol.* **1992**, *58*, 1233–1239.

15. Berthomieu, R.; Pérez-Bernal, M.F.; Santa-Catalina, G.; Desmond-Le Quéméner, E.; Bernet, N.; Trably, E. Mechanisms Underlying *Clostridium Pasteurianum*'s Metabolic Shift When Grown with *Geobacter Sulfurreducens*. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 865–876. <https://doi.org/10.1007/s00253-021-11736-7>.
16. Arbter, P.; Sabra, W.; Utesch, T.; Hong, Y.; Zeng, A. Metabolomic and Kinetic Investigations on the Electricity-aided Production of Butanol by *Clostridium Pasteurianum* Strains. *Eng. Life Sci.* **2020**, *21*, 181–195. <https://doi.org/10.1002/elsc.202000035>.
17. Detman, A.; Laubitz, D.; Chojnacka, A.; Wiktorowska-Sowa, E.; Piotrowski, J.; Salamon, A.; Kaźmierczak, W.; Błaszczuk, M.K.; Barberan, A.; Chen, Y.; et al. Dynamics and Complexity of Dark Fermentation Microbial Communities Producing Hydrogen From Sugar Beet Molasses in Continuously Operating Packed Bed Reactors. *Front. Microbiol.* **2021**, *11*, 612344.
18. Srivastava, N.; Srivastava, M.; Kushwaha, D.; Gupta, V.K.; Manikanta, A.; Ramteke, P.W.; Mishra, P.K. Efficient Dark Fermentative Hydrogen Production from Enzyme Hydrolyzed Rice Straw by *Clostridium Pasteurianum* (MTCC116). *Bioresour. Technol.* **2017**, *238*, 552–558. <https://doi.org/10.1016/j.biortech.2017.04.077>.
19. Khanna, S.; Goyal, A.; Moholkar, V.S. Bioconversion of Biodiesel Derived Crude Glycerol by Immobilized *Clostridium Pasteurianum*: Effect of Temperature. *Int. J. Chem. Biol. Eng.* **2012**, *6*, 301–304.
20. Krasňan, V.; Plž, M.; Marr, A.C.; Markošová, K.; Rosenberg, M.; Rebroš, M. Intensified Crude Glycerol Conversion to Butanol by Immobilized *Clostridium Pasteurianum*. *Biochem. Eng. J.* **2018**, *134*, 114–119. <https://doi.org/10.1016/j.bej.2018.03.005>.
21. Sarma, S.; Ortega, D.; Minton, N.P.; Dubey, V.K.; Moholkar, V.S. Homologous Overexpression of Hydrogenase and Glycerol Dehydrogenase in *Clostridium Pasteurianum* to Enhance Hydrogen Production from Crude Glycerol. *Bioresour. Technol.* **2019**, *284*, 168–177. <https://doi.org/10.1016/j.biortech.2019.03.074>.
22. Schwarz, K.M.; Grosse-Honebrink, A.; Derecka, K.; Rotta, C.; Zhang, Y.; Minton, N.P. Towards Improved Butanol Production through Targeted Genetic Modification of *Clostridium Pasteurianum*. *Metab. Eng.* **2017**, *40*, 124–137. <https://doi.org/10.1016/j.ymben.2017.01.009>.
23. Gallardo, R.; Alves, M.; Rodrigues, L.R. Influence of Nutritional and Operational Parameters on the Production of Butanol or 1,3-Propanediol from Glycerol by a Mutant *Clostridium Pasteurianum*. *New Biotechnol.* **2017**, *34*, 59–67. <https://doi.org/10.1016/j.nbt.2016.03.002>.
24. Pyne, M.E.; Moo-Young, M.; Chung, D.A.; Chou, C.P. Expansion of the Genetic Toolkit for Metabolic Engineering of *Clostridium Pasteurianum*: Chromosomal Gene Disruption of the Endogenous CpaAI Restriction Enzyme. *Biotechnol. Biofuels* **2014**, *7*, 163. <https://doi.org/10.1186/s13068-014-0163-1>.
25. Grosse-Honebrink, A.; Schwarz, K.M.; Wang, H.; Minton, N.P.; Zhang, Y. Improving Gene Transfer in *Clostridium Pasteurianum* through the Isolation of Rare Hypertransformable Variants. *Anaerobe* **2017**, *48*, 203–205. <https://doi.org/10.1016/j.anaerobe.2017.09.001>.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.