

A Short Bibliometric Analysis of Detoxification Processes in Butanol Production from Biomass Hydrolysates Via Fermentation

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The fermentative production of products from biomass is hindered by microbial inhibitory compounds generated during thermochemical pretreatment. To address this challenge, various detoxification strategies have been implemented either before or during fermentation. An overview of the most explored strategies can help identify promising directions for further investigation. This study thus examines research on detoxification techniques used in fermentation processes, specifically focusing on butanol production from biomass hydrolysates. A bibliometric analysis was conducted using the Web of Science (WoS) database alongside the Bibliometrix tool (RStudio) and the Biblioshiny app for statistical and network analyses, as well as bibliometric mapping. A search using the terms "fermentation" and "detoxification" resulted in 2,307 documents (1960 to 2024). The search was refined by adding the terms "hydrolysate" or "hemicellulosic hydrolysate," and finally "butanol." Brazil was the most cited country when "detoxification" was associated with "hemicellulosic hydrolysate." Butanol production was predominantly associated with Acetone-Butanol-Ethanol (ABE) fermentation by *Clostridium* species. Highly cited articles utilized hydrolysates derived from various biomass sources, including sugarcane bagasse, corn stover, and wood. Among the most cited articles (excluding reviews), the predominant detoxification techniques are overliming with $\text{Ca}(\text{OH})_2$, activated charcoal, and resins (or membranes). All these techniques were applied before fermentation, suggesting a potential gap in research on *in-situ* detoxification strategies.

1. Introduction

Using residual biomass as a renewable source to produce different bio-based compounds usually obtained by the petrochemical route is an important aspect of biorefineries. Biomass can have distinct origins, such as forestry and agriculture residuals and food waste (Raj *et al.*, 2022). Agriculture residuals are lignocellulosic materials, which are mainly composed of three main structures: cellulose [17 – 50 %], hemicellulose [13 – 33 %], and lignin [9 – 35 %]. From the cellulosic (C6) and hemicellulosic (C5) fractions, fermentable sugars can be obtained using different hydrolysis strategies (Candido *et al.*, 2020; Gyan *et al.*, 2024).

Biomass hydrolysates containing sugar can be used to produce second-generation alcohols such as ethanol and butanol. However, the traditional yeast *Saccharomyces cerevisiae* can only metabolize hexoses. An alternative is to use other microorganisms that can also consume C5 sugars as substrate, such as *Clostridium* species. These bacteria can convert both C6 and C5 sugars into butanol by the Acetone-Butanol-Ethanol (ABE) fermentation (Nogueira *et al.*, 2021). C5 sugars, such as xylose, are the main substrate in hemicellulosic hydrolysates, often obtained by dilute acid pretreatment (DA), and the second most relevant sugar in hydrolysates of cellulose and hemicellulose. However, DA also generates microbial inhibitory compounds, such as furan derivatives (furfural), organic acids (acetic acid), and phenolic derivatives (p-coumaric acid), which can reduce or inhibit microbial growth (Candido *et al.*, 2020; Xu *et al.*, 2023).

Due to the challenges posed by lignocellulose-derived microbial inhibitory compounds in the production of second-generation alcohols, significant research has focused on understanding their impact on fermentation

processes and developing strategies to mitigate these effects (Gao *et al.*, 2016; Raj *et al.*, 2022; Gyan *et al.*, 2024). This study provides a concise bibliometric analysis and an overview of advances in detoxification strategies for butanol production from hydrolysates derived from various biomass sources. It highlights key journals, examines country-specific scientific output, and identifies emerging trends in the field.

2. Methodology

Data for the bibliometric analysis was collected from the Web of Science™ (WoS) database (Clarivate™), applying different combinations of topic words and logic operation terms (Table 1) to refine the search window. The search was also limited by the type of documents (only research, review, and conference proceeding articles were considered). The bibliometric analysis was conducted using the Bibliometrix tool (RStudio v.4.3.2) and the Biblioshiny app (Aria and Cuccurullo, 2017) for statistical and network analyses and bibliometric mapping.

Table 1: Combinations of terms explored in the WoS database

Scenario	Topics	Total of documents
1	"fermentation" AND "detoxification"	2302
2	"fermentation" AND "detoxification" AND "hydrolysate"	818
3	"fermentation" AND "detoxification" AND "hemicellulosic hydrolysate"	108
4	"fermentation" AND "detoxification" AND "hydrolysate" AND "butanol" ^a	101
5	"fermentation" AND "detoxification" AND "hydrolysate" AND "ABE" ^b	73
6	"fermentation" AND "detoxification" AND "hemicellulosic hydrolysate" AND "butanol" ^a	5

a – "butanol" OR "biobutanol" OR "bio-butanol" OR "bio butanol" OR "n-butanol"
 b – "ABE" OR "acetone-butanol-ethanol" OR "acetone-butanol-ethanol (ABE)"

3. Results and Discussions

3.1 General information

Among the groups generated by the combinations, the majority of the documents are research articles (90 – 95 %), and the review articles are the minority group (1.4 – 7.1 %). The exception was scenario 6, in which all five documents were research articles. Interestingly, the analysis of the publications through the years (Figure 1) shows time marks for the association between the terms and research fields that are emerging.

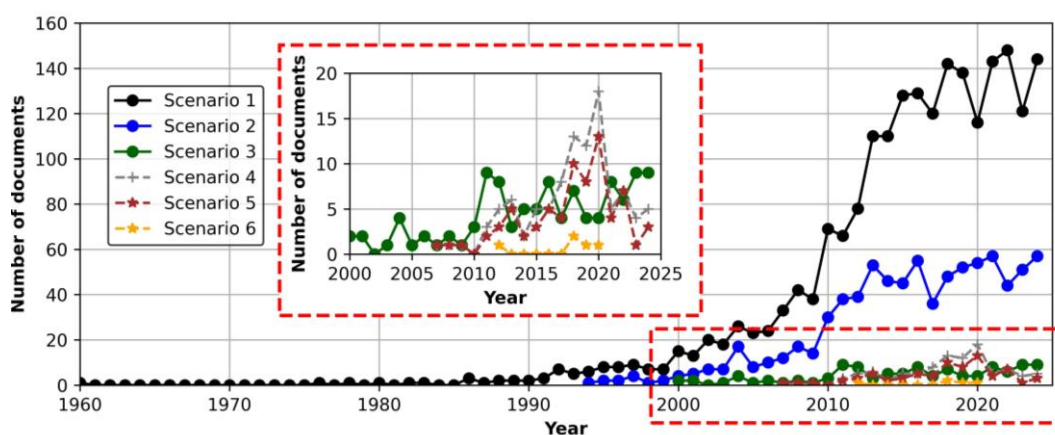


Figure 1: Annual growth of publications for each combination of terms used in the bibliometric analysis.

3.2 Main journals

Considering the combinations of terms explored in the WoS database, the journal "Bioresource Technology" emerged as the most relevant source, publishing at least twice as many articles as the second-ranked journal. In scenarios 1 to 3, the top five sources are mostly the same journals. However, when the terms "butanol" or "ABE" were added to the search (scenarios 4 to 6), the journals "Renewable Energy" and "Biotechnology for Fuels" emerged as second and third in the ranking, respectively.

3.3 Country-specific scientific output

Countries were compared for productivity and citation impact of publications. China and the United States are the leading countries in terms of productivity across all scenarios, except in those related to hemicellulosic hydrolysate (scenario 3), where Brazil and India are the most prolific producers of articles. Brazil ranks among the top five in scenarios 1, 2, and 4, but falls to sixth place in scenario 5. When comparing countries based on citation levels, Sweden ranks first in scenario 1 and third in scenario 2, while China and the United States are the most prominent in the majority of cases. Brazil and India keep their leading positions in scenario 3. Brazil also ranked in the top four for scenarios 1 and 2 but placed sixth in scenario 4 and ninth in scenario 5. Other countries that appear in the rankings for productivity and citation level are Spain, Canada, and Finland, mainly when the terms “butanol” or “ABE” are added. These comparisons highlight Brazil’s significant role in research on the fermentation of hydrolysates. Furthermore, as its publications primarily focus on ethanol production, this expertise could be applied to butanol production in the future.

3.4 Trending topics

Trending topics were first identified by assessing authors’ keywords that appeared in the publications found in the search scenarios (Table 2). In scenarios 1 to 3, the keywords indicate that detoxification and fermentation processes are primarily associated with ethanol and xylitol production, suggesting that these products are trends in research on the fermentation of biomass hydrolysates. When the terms “butanol” or “ABE” were included in the search (scenarios 4 and 5), the resulting top five keywords were the same. The term “ABE fermentation” notably ranked second in both scenarios, suggesting that most studies focus on n-butanol rather than isobutanol, which is primarily produced at the commercial level by genetically modified yeast (Lakshmi et al., 2021). This differentiation is presumably reinforced by adding the genus and species name of the microorganism (note that solventogenic *Clostridia* are xylose-fermenting microorganisms) to the keywords list.

Table 2: Most frequently used terms as authors’ keywords in scenarios of the bibliometric analysis.

Word (occurrence)					
#	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
1	detoxification (406)	detoxification (188)	xylitol (43)	butanol (37)	butanol (32)
2	fermentation (333)	ethanol (143)	detoxification (29)	ABE fermentation (30)	ABE fermentation (30)
3	ethanol (296)	fermentation (133)	hemicellulosic hydrolysate (28)	<i>Clostridium</i> (15)	<i>Clostridium</i> (12)
4	lignocellulose (106)	xylitol (82)	fermentation (16)	detoxification (15)	detoxification (12)
5	pretreatment (105)	inhibitors (63)	ethanol (15)	<i>Clostridium acetobutylicum</i> (13)	<i>Clostridium acetobutylicum</i> (10)

To further confirm the trending topics, a co-occurrence network analysis was performed considering scenarios 2 and 4 (Figure 2). In scenario 2 (Figure 2A), butanol production is a secondary topic (represented by green nodes), while the primary emphasis is on the production of xylitol and ethanol. Additionally, detoxification is the most prominent node, highlighting significant efforts made to tackle the challenge of converting biomass hydrolysates into these products. In scenario 4 (Figure 2B), on the other hand, detoxification appears as a secondary topic, with a node size similar to terms not used in the search such as gas stripping and liquid-liquid extraction — both *in-situ* product recovery techniques. This suggests that research on the fermentative production of butanol from biomass hydrolysates may have focused more on minimizing butanol inhibition than on advancing existing methods to detoxify microbial inhibitors derived from lignocellulose.

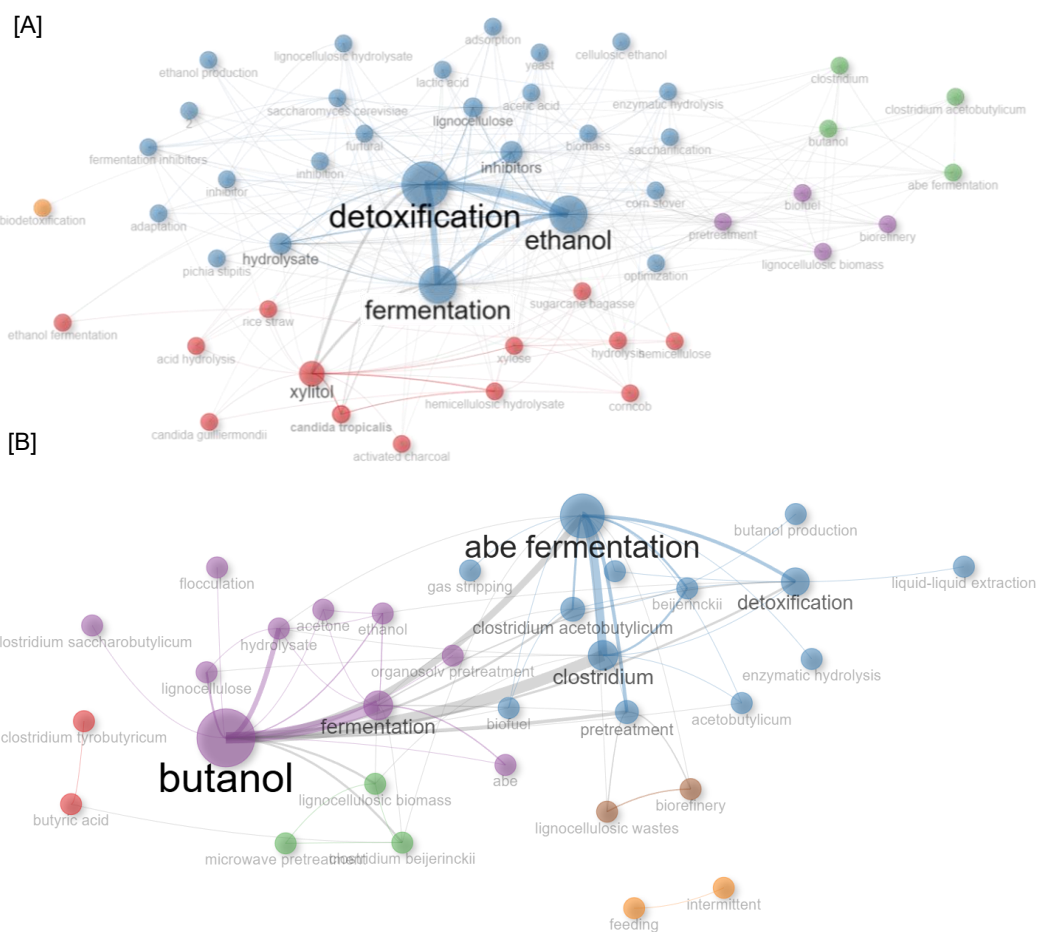


Figure 2: Authors' keywords co-occurrence ([A] – scenario 2; [B] – scenario 4). The size of each node is proportional to the number of occurrences.

3.5 Trends in detoxification strategies

Articles found in scenario 4 were examined to find major trends in detoxification strategies applied to butanol production from biomass hydrolysates. Among the top 25 most cited articles (with the top 10 presented in Table 3), several general observations can be made. First, the most common types of biomass are derivatives from crops, featured in 17 articles, and wood, found in 5 articles. For the preparation of hydrolysates, 16 studies employed pretreatment in combination with enzymatic hydrolysis while the remaining studies used pretreatment alone. Among the pretreatment techniques, dilute acid (DA) and liquid hot water (LHW), whether used individually or in combination, are the most prevalent (Table 3). The microorganisms used in the fermentation were *Clostridium* species, with *C. beijerinckii* and *C. acetobutylicum* being the most commonly utilized. Each of these species is found in 11 articles. Regarding the detoxification methods, eight studies do not employ any detoxification strategies. In the remaining 17 studies, the prevalent methods for removing microbial inhibitory compounds from hydrolysates are overliming (typically using $\text{Ca}(\text{OH})_2$), followed by activated charcoal (at concentrations ranging from 2 to 7 % w/v), synthetic resins, and membranes. These are methods extensively used in the fermentative production of ethanol from biomass hydrolysates (Nogueira et al., 2021). In almost all 25 studies, detoxification was performed prior to fermentation, adding an extra step in the production of second-generation butanol. The only exception was Sun *et al.* (2020) (ranked 19th), who added 1 g of biochar during fermentation to remove inhibitors from switchgrass hydrolysate. Since most of the detoxification techniques have been applied before fermentation, this implies a potential gap in research regarding *in-situ* detoxification strategies oriented to solventogenic *Clostridium* fermentation. Such strategies could enhance the fermentative production of butanol from biomass while eliminating one process step for the preparation of fermentation broths containing hemicellulosic hydrolysates.

Table 3: List of most cited publications on biomass hydrolysate production, detoxification, and fermentation strategies identified in scenario 4.

#	Document	TC	TC/year	Biomass	Hydrolysate production	Microorganism	Detoxification method
1	Ezeji <i>et al.</i> (2007)	446	24.78	Corn fiber	DA	<i>C. beijerinckii</i> BA10	Overliming (Ca(OH) ₂)
2	Ezeji <i>et al.</i> (2008)	218	12.82	Distillers dried grains and solubles (DDGS)	LHW and DA + Enzymatic	<i>C. beijerinckii</i> BA101, <i>C. acetobutylicum</i> (260, 824), <i>C. saccharobutylicum</i> 262, and <i>C. butylicum</i> 592	Overliming (Ca(OH) ₂)
3	Lu <i>et al.</i> (2012)	182	14.00	Cassava bagasse	LHW + Enzymatic	<i>C. acetobutylicum</i> JB200	-
4	Wang <i>et al.</i> (2011)	129	9.21	Chipped corn stover	SE and AP + Enzymatic	<i>C. acetobutylicum</i> ATCC 824	Activated charcoal (7.5 %)
5	Lu <i>et al.</i> (2013)	107	8.92	wood pulp (maple, beech, and birch trees)	DA	<i>C. beijerinckii</i> CC101 (adaptive mutant)	Ion exchange resins, overliming (Ca(OH) ₂), Activated charcoal (2 %)
6	Cai <i>et al.</i> (2013)	92	7.67	Sweet sorghum bagasse (SSB)	DA	<i>C. acetobutylicum</i> ABE1201	PDMS
7	Zhang <i>et al.</i> (2018)	85	12.14	Hybrid poplar (Populus)	DA	<i>C. saccharobutylicum</i> BAA-117	Overliming (Ca(OH) ₂), Activated charcoal (5 %)
8	Sun <i>et al.</i> (2012)	76	5.85	Sugar maple (Acer saccharum) wood	LHW and DA	<i>C. acetobutylicum</i> ATCC824	Membrane (nano)filtration + overliming (Ca(OH) ₂)
9	Lopez-Linares <i>et al.</i> (2019)	76	12.67	Brewer's spent grain (BSG)	MAH + Enzymatic	<i>C. beijerinckii</i> DSM 6422	Activated charcoal (2 %)
10	Gottumukkala <i>et al.</i> (2013)	73	6.08	Rice straw	DA+ Enzymatic	<i>C. sporogenes</i> BE01	Filtration with Amberlite and anionic resin

TC - Total of citation; LHW - Liquid Hot Water pretreatment; DA - Dilute Acid pretreatment; SE - Steam Explosion pretreatment; AP - Alkaline Peroxide pretreatment; MAH - Microwave-assisted hydrothermal pretreatment; PDMS - Pervaporation membrane composed of polydimethylsiloxane.

4. Conclusions

The most commonly employed detoxification strategies among the top-cited articles for butanol production from biomass hydrolysate were overliming (using Ca(OH)₂) and activated charcoal (at concentrations of 2 – 7 % w/v). The hydrolysates are primarily derived from crop or wood biomass, employing pretreatments such as dilute acid or liquid hot water, with or without enzymatic hydrolysis. In the fermentation, various species of *Clostridium* were utilized, with *C. beijerinckii* and *C. acetobutylicum* being the most commonly used. The country-specific bibliometric analysis of productivity and citation impact revealed that China and the United States play a significant role in scientific production. Brazil is emerging as a significant player and has the potential to leverage

its expertise in cellulosic ethanol production for ABE fermentation. Butanol production accounts for approximately 15% of scientific production related to the detoxification of biomass hydrolysates, with most detoxification processes occurring prior to fermentation. Therefore, future studies should also investigate the further development of *in-situ* detoxification methods for lignocellulose-derived microbial inhibitors during fermentation.

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References

- Aria, M., Cuccurullo, C., 2017, Bibliometrix: An R-tool for comprehensive science mapping analysis, *Journal of Informetrics*, 11, 959–975.
- Cai, D., Zhang, T., Zheng, J., Chang, Z., Wang, Z., Qin, P., Tan, T., 2013, Biobutanol from sweet sorghum bagasse hydrolysate by a hybrid pervaporation process, *Bioresource Technology*, 145, 97–102.
- Candido, J. P., Claro, E. M. T., de Paula, C. B. C., Shimizu, F. L., de Oliveria Leite, D. A. N., Brienzo, M., de Angelis, D. de F., 2020, Detoxification of sugarcane bagasse hydrolysate with different adsorbents to improve the fermentative process, *World Journal of Microbiology and Biotechnology*, 36.
- Ezeji, T., Blaschek, H. P., 2008, Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic *Clostridia*, *Bioresource Technology*, 99, 5232–5242.
- Ezeji, T., Qureshi, N., Blaschek, H. P., 2007, Butanol production from agricultural residues: Impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation, *Biotechnology and Bioengineering*, 97(6), 1460–1469.
- Gottumukkala, L. D., Parameswaran, B., Valappil, S. K., Mathiyazhakan, K., Pandey, A., Sukumaran, R. K., 2013, Biobutanol production from rice straw by a non acetone producing *Clostridium sporogenes* BE01. *Bioresource Technology*, 145, 182–187.
- Gyan, K., Afedzi, A. E. K., Tanypramphan, P., Parakulsuksatid, P., 2024, A review of the advances in detoxification strategies of lignocellulosic hydrolysate for bio-based succinic acid production, *Biocatalysis and Agricultural Biotechnology*, 60.
- Lakshmi, N. M., Binod, P., Sindhu, R., Awasthi, M. K., Pandey, A., 2021. Microbial engineering for the production of isobutanol: current status and future directions. *Bioengineered*, 12, 12308-12321.
- López-Linares, J. C., García-Cubero, M. T., Lucas, S., González-Benito, G., Coca, M., 2019, Microwave assisted hydrothermal as greener pretreatment of brewer's spent grains for biobutanol production, *Chemical Engineering Journal*, 368, 1045–1055.
- Lu, C., Dong, J., Yang, S.-T., 2013, Butanol production from wood pulping hydrolysate in an integrated fermentation–gas stripping process, *Bioresource Technology*, 143, 467–475.
- Lu, C., Zhao, J., Yang, S.-T., Wei, D., 2012, Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping, *Bioresource Technology*, 104, 380–387.
- Nogueira, C. da C., Padilha, C. E. de A., Dantas, J. M. de M., Medeiros, F. G. M. de, Guilherme, A. de A., Souza, D. F. de S., dos Santos, E. S., 2021, *In-situ* detoxification strategies to boost bioalcohol production from lignocellulosic biomass. *Renewable Energy*, 180, 914–936.
- Raj, T., Chandrasekhar, K., Morya, R., Kumar Pandey, A., Jung, J. H., Kumar, D., Singhanian, R. R., Kim, S. H., 2022, Critical challenges and technological breakthroughs in food waste hydrolysis and detoxification for fuels and chemicals production, *Bioresource Technology*, 360.
- Sun, Z., Liu, S., 2012, Production of n-butanol from concentrated sugar maple hemicellulosic hydrolysate by *Clostridia acetobutylicum* ATCC824, *Biomass and Bioenergy*, 39, 39–47.
- Wang, L., Chen, H., 2011, Increased fermentability of enzymatically hydrolyzed steam-exploded corn stover for butanol production by removal of fermentation inhibitors, *Process Biochemistry*, 46, 604–607.
- Xu, C., Ding, C., Zhou, X., Xu, Y., Gu, X., 2023, *In-situ* detoxification and enhanced oxygen mass transfer for C5 sugar acid production from corncob hemicellulose hydrolysates using activated carbon particles, *Industrial Crops and Products*, 197.
- Zhang, Y., Xia, C., Lu, M., Tu, M., 2018, Effect of overliming and activated carbon detoxification on inhibitors removal and butanol fermentation of poplar prehydrolysates, *Biotechnology for Biofuels*, 11, 178.