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Biodegradation and Utilization of the Pesticides Glyphosate and Carbofuran by Two Yeast Strains

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Abstract: The widespread use of synthetic pesticides in agricultural practice is associated with the risk of environmental pollution, damage to non-target organisms, and harm to the health of consumers due to the presence of pesticides in the resulting products. Metabolically active microbial species play a significant role in eliminating harmful chemicals from the environment. The two yeast strains used in this study are *Trichosporon cutaneum* R57 and *Candida tropicalis* V1. Both strains showed growth and development in the presence of the pesticides glyphosate and carbofuran used as the sole carbon sources. The gas-chromatographic analysis performed showed that *C. tropicalis* V1 reached 76% of 0.3 g/L glyphosate biodegradation in 192 h. At the same time, the amount of glyphosate decreased by about 58% in the culture of *T. cutaneum* R57. During the glyphosate biodegradation process, the presence of the intermediate metabolites methylglycine and glycine was revealed. Strain *T. cutaneum* R57 demonstrated nearly total biodegradation of 0.3 g/L carbofuran in 192 h of cultivation. Strain *C. tropicalis* V1 showed a slow decrease (23.4%) of the same amount of carbofuran during 192 h. Pyruvic acid and carbofuran-7-phenol have been found to be intermediate metabolites in the breakdown of carbofuran. This report provides new information on the potential of yeasts to participate in environmental pesticide cleanup processes.

Keywords: biodegradation; pesticides; *Trichosporon cutaneum*; *Candida tropicalis*; carbofuran-7-phenol; methylglycine; glycine

Citation: Stoyanova, K.; Gerginova, M.; Peneva, N.; Dincheva, I.; Alexieva, Z. Biodegradation and Utilization of the Pesticides Glyphosate and Carbofuran by Two Yeast Strains. *Processes* **2023**, *11*, 3343. https://doi.org/10.3390/pr11123343

Academic Editor: Davide Dionisi

Received: 13 November 2023
Revised: 27 November 2023
Accepted: 29 November 2023
Published: 30 November 2023

1. Introduction

The application of the principles of the circular economy requires the fullest possible return of the resources invested in production. The correct and innovative management of natural resources and the environment as a whole depends on the creation of clean production technologies, renewable energy sources, and the reduction of waste polluting air, water, and soils. The majority of chemical pollutants found in the environment are of industrial origin and are widely used in various spheres of production activity, including agriculture.

Various national and supranational documents and strategies aimed at improving the state of the environment have been adopted [1–4]. One of these documents is the Stockholm Convention on Persistent Organic Pollutants (POPs). The main objective of the Convention is to eliminate 22 of these pollutants that accumulate in the natural environment and to replace them with harmless alternative substances. One of the properties that the Convention has set is the exemption of accumulated pesticides in ecosystems [5].

Pesticides are preparations that are used for plant protection, mainly against insects but also against nematodes, rodents, fungi, and weeds. The pesticides are beneficial for the agricultural sector because they are toxic to the target pests and control their number. Their use is essential for good crop production and without it the loss would be significant. However, along with the target organisms, pesticides also affect non-target organisms, even

if their effect is less serious. Pesticides used in agricultural practice are washed away with the rain and carried with the winds over great distances. They also leach into the soil and end up in the groundwater. Thus, many of the used plant protection products end up in the environment and act as pollutants.

Abiotic disintegration of pesticides takes place under the action of solar radiation (photolysis, volatilization), interaction with oxygen (oxidation), and other processes [6]. The ability to transform, degrade, and remove harmful pollutants from water and soil, such as undoubtedly the majority of pesticides used, with the help of biological agents, leads to the recovery and new use of cleaned areas and volumes and is defined by the term bioremediation [7,8].

Pesticides have very different chemical compositions and structures. They are designed to suppress a variety of target pests. Among the most often used and controversial pesticides are glyphosate and carbofuran, which belong to different groups in terms of structure and functional impact.

Glyphosate (N-(phosphonomethyl) glycine—NPG) is probably the most widely used herbicide at our time. It has a pronounced effect on practically any plant object. Glyphosate is also the subject of much controversy regarding the harmful effects it causes on human and animal health. The issue of the ban on its use has been discussed more than once at meetings of the European Parliament and the European Commission of the European Union. The compound belongs to the organophosphorus pesticide group. The high solubility of glyphosate in water is well known. It has been shown that highly soluble pesticides can quickly penetrate the soil and be carried away from the target treated regions [9].

In general, several methodologies, such as biological and physical methods, as well as advanced oxidation processes, have been successfully employed for glyphosate elimination [10,11]

The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) classifies glyphosate as possibly carcinogenic to humans. The Food and Agriculture Organization (FAO) considers glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) as potentially toxic due to the potential for their derivatives to accumulate in elements of the food chain [12,13].

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is an insecticide belonging to the group of carbamate pesticides. It is among the most toxic chemical compounds used to protect a number of agricultural plants from harmful insects. Numerous studies have been conducted on the detrimental effects of carbofuran on the neurological system and some organs of both animals and humans [14]. Currently, Sri Lanka, Canada, and the European Union prohibit the use of this insecticide. In the US, it is categorized as an extremely hazardous substance [15]. Despite this, carbofuran continues to be one of the most commonly used carbamate insecticides globally. Because of its high solubility, it is easily distributed in soil, surface, and subsurface water, and consequently in plant and animal species. Although carbofuran undergoes abiotic disintegration, the main route for its removal is biodegradation.

The exceptional role of microbial populations in the biotransformation of various substances in nature has generally been known for a long time. The number of microbial strains and microbial communities capable of degrading the vast variety of toxic xenobiotics is recognized and reported, whether they are found naturally in certain natural settings or are the result of scientific creation [16,17]. Most results have been reported on the biodegradative capacity of a number of prokaryotes. Among the most numerous biodestructors are representatives of the genera *Pseudomonas*, *Alcaligenes*, *Rhodococcus*, and others [18–24]. The biodegraded or transformed pesticide can be utilized by the degrading microorganisms as a source of carbon, nitrogen, phosphorus, or other necessary elements in their growth and development [25,26].

The other intensively studied microorganisms are fungi, which are known for their resistance to adverse living conditions, including toxic environments, and their rich enzyme apparatus that contributes to their survival. The degradative abilities of fungi are also

manifested in relation to a number of widely used pesticides, different in their chemical composition and structure [27–30].

Phanerochaete chrysosporium, a typical representative of white rot fungi, is characterized by the presence of lignolytic enzyme complexes, cytochrome P450 monooxygenases, and other mechanisms that contribute to the destruction of pesticide structures [31]. Strains of *Trametes versicolor* are also capable of removing a number of different pesticides [32,33]. More and more different fungi, with different taxonomies and typical habitats capable of degrading different types of pesticides are reported [34].

One of the most studied biodestructors of glyphosate are representatives of fungi as *Aspergillus niger*, *Aspergillus oryzae* A-F02, *Penicillium chrysogenum*, *Trichoderma harzianum*, and *Fusarium*, among others [9,35–38].

Efforts to find suitable bioremediation microbes capable of removing carbofuran from contaminated soils and waters are quite active. Studies with prokaryotic objects are numerous, while studies with fungi are significantly fewer [39]. Strains of *Aspergillus niger*, *Fusarium graminareum*, *Mucor ramannianus*, and *Trametes versicolor* have been studied and shown potential to degrade this insecticide [40–42].

Yeasts play a significant role in the development of bioremediation technology for the polluted environment. Thay have established themselves as an active participant in the remediation of xenobiotic-contaminated waters and soils [43]. Numerous strains belonging to the genera *Candida*, *Pichia*, *Rhodotorula*, *Trichosporon*, *Yarrowia*, and others have been described, successfully degrading aliphatic and aromatic hydrocarbons, crude oil, and low-density polyethylene [43–46]. Yeasts have long been known and applied to remove and transform metal ions and various dyes from textile industry wastewater.

However, studies on the potential of yeasts to degrade and assimilate pesticides are relatively few. The capacity of *Clavispora lusitaniae* to degrade the dinitroaniline herbicide pendimethalin is known [47]. The degree of biodegradation of diazinon by a strain of *Candida pseudolambica* was significant [48]. Interesting are the studies on the effect of *Saccharomyces* and non-*Saccharomyces* yeasts involved in the alcoholic fermentation process to reduce the present pesticide residues of fungicides such as Pyrimethanil, Cyprodinil, Procymidone, Fludioxinil, Quinoxyfen, Pyrimethanil, and Cyprodinil [49]. The use of the phenol hydroxylase enzyme isolated from a strain of Trichosporon cutaneum in the biodegradation of the insecticide endosulfan has also been described [50]. There are fewer studies on the biodegradation of the pesticides carbofuran and glyphosate. A strain of *Pichia anomala* has long been known as an efficient carbofuran degrader [51].

The need to find suitable microorganisms with a high potential to degrade widely applied pesticides is a current issue. The aim of the present study is to investigate the possibilities of the yeasts *Trichosporon cutaneum* and *Candida tropicalis* for effective biodegradation of the pesticides glyphosate and carbofuran and to determine the presence of intermediate and residual metabolic products in the process of their biodegradation.

2. Materials and Methods

2.1. Chemicals

Carbofuran (98%) and Glyphosate (98%), two pesticide formulations used in this study, were purchased from Sigma Aldrich in St. Louis, MO, United States. Pyruvic acid, glycine, and methylglycine (sarcosin) have an analytical standard for synthesis with purity above 98% and were purchased from was supplied by the same company. Carbofuran-7-phenol (purity 98.9%) was delivered by the Laboratory of the Government Chemist (LGC Standards Ltd., Tedddington, UK).

Yeast extract, peptone, and glucose were purchased from Merck-Schuchardt, Hohenbrunn, Germany.

Agar-agar polysaccharide was supplied by Carl Roth GmbH, Karlsruhe, Germany.

2.2. Strains

Two yeast strains used in this study are *Trichosporon cutaneum* R57 and *Candida tropicalis* V1. The yeast strain *T. cutaneum* R57 was isolated from a breeding apparate, and it was further deposited in the National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC) N 2414, Sofia, Bulgaria. Strain *Candida tropicalis* V1 was initially isolated from samples taken from domestic sewage entering the wastewater treatment plant (WWTP) of Sofia, located in Kubratovo village, Bulgaria. It was selected on carbonless nutrient medium (YNB w/o AA) in the presence of 0.3 g/L phenol.

2.3. Growth Media and Culture Conditions

The rich nutrient medium Yeast Extract Peptone Dextrose (YEPD) contains per 1 L: 10 g yeast extract, 20 g peptone, and 10 g glucose. YEP medium contains the same content but without glucose. The carbonless medium, Yeast Nitrogen Base without Amino Acids (YNB w/o AA), was bought from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Agar-agar polysaccharide was used to solidify the media. The two investigated strains were stored on a solid YNB w/o AA with 1% glucose (15 g/L agar) at 4 °C.

The inoculum for culturing the strains in all experiments was obtained by cultivating the corresponding strain in a liquid YEPD medium for 48 h at 30 °C on a shaker with a speed of rotation of 150 rpm. The cultural medium YNB w/o AA was initially inoculated with 1 mL of the inoculum, and then it was further corrected if necessary, so the optical density (OD) of the culture medium was between 0.150 and 0.170 at the 610 nm wavelength. The optical density of the inoculum at the moment of inoculation was about 1.9.

The process of cultivating for the pesticide biodegradation was performed in 200 mL cone flasks with 20 mL of the YNB w/o AA medium added to them on a rotary shaker (150 rpm).

Spectrophotometric analysis at a wavelength of 610 nm was used to calculate the OD of the cell suspension. The Jenway® 6305 UV/Visible Spectrophotometer, Cole-Parmer Instrument Co. Europe, St Neots, UK, was used for spectrophotometry in this investigation.

2.4. Molecular Identification

The isolate V1 was cultivated in a rich YEPD medium for 24 h at 30 °C. After that, the cultural media was centrifuged for 15 min at 4000 rotations per minute. The precipitated biomass was further washed with a 5 mL saline solution of 0.9% to remove certain contaminants and nutritional materials and centrifuged one more time under the same conditions. The strain's genomic DNA was obtained using the Lio et al. (2000) technique for preparing DNA for Polymerase Chain Reaction (PCR) [52]. Nano Drop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to confirm the amount and purity of the acquired DNA.

Amplification of the 18S rRNA genes was used to identify the isolated yeast strain.

The primers used for this purpose are: PFf AGGGATGTATTTATTAGATAAAAAATCAA and Pfr CGCAGTAGTTAGTCTTCAGTAAATC [53]. The working solution of the primers was prepared (10 pmol), and they were used for gene amplification in a 25 µL reaction volume. The puReTaq Ready-To-Go PCR Beads (GE Health Care, Chicago, IL, USA) were used to conduct the PCR assays. The yeast DNA template concentration ranged from 30 to 50 ng.

The mixture was put into a thermal cycler for PCR-amplification, which was heated to 95 °C for a 5-min initial denaturation temperature, followed by 35 cycles of 95 °C for 30 s, 54 °C for 30 s of annealing, 72 °C for 45 s, and 72 °C for 1 min of the final extension step, before being held at 4 °C indefinitely. The PCR products were sequenced by Macrogen Company (Seoul, Republic of Korea). The National Centre for Biotechnology Information (NCBI) (Bethesda, MD, USA) database's resulting sequences were examined using the az (BLAST 2.14.1) search engine.

2.5. Biodegradation of Carbofuran and Glyphosate

The degradation abilities of strains towards carbofuran and glyphosate were examined in YNB w/o AA medium, pH 6.0, which is adjusted by adding 5 M NaOH until it reaches the required value.

In the present study, in the cultivation of both test strains, the initial concentration of carbofuran or glyphosate in the culture medium was 0.3 g/L. A sample (1 mL) was taken every 24 h during the 192 h cultivation process. Samples were centrifuged for five minutes at 10,000 rpm. A pesticide concentration study was conducted on the resulting supernatant.

2.6. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The concentrations of the pesticides in each sample taken of the cell-free suspension, as well as the presence of intermediate metabolites, were determined by gas chromatography–mass spectrometric analysis. To determine the degradation of glyphosate and carbofuran, 0.5 mL samples were taken at 0, 48, 72, 120, 144, and 192 h. The pesticides were extracted three times with dichloromethane (1:1) in a supersonic purifier, after which the combined organic phases were evaporated. The extract, along with the dichloromethane, was concentrated in a vacuum concentrator RVC 2-25 CD plus (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

The GC–MS analysis was carried out in Hewlett Packard 7890 apparatus connected to MSD 5975 equipment (Hewlett Packard, Palo Alto, CA, USA) in EI mode at 70 eV. The HP-5 MS (30 m cc 0.25 mm × 0.25 μm) column was used. Each sample was injected in undivided mode with a quantity of 1 μm. Helium was used as a gas carrier with a flow speed of 1.0 mL/min. The temperature schedule was as follows: 50 °C for 0.4 min; then, for 1 min, a further temperature decrease to 25 °C; then, for 1.5 min, a temperature increase to 195 °C; next, for less than a minute, a second temperature drop to 8 °C; and finally, a temperature increase to 265 °C. The third drop to 20 °C/min and a further temperature rise to 315 °C for 1.25 min were applied. The mass spectra were recorded in two scans within a range of 50–550 m/z. The temperatures of the ion source and the interface were regulated, respectively, to 250 and 280 °C.

2.7. Statistical Analysis

Three separate experiments were conducted to gather the qualitative data from the growth and degradation experiments. They were processed on MS Excel and are represented as the mean ± the standard deviation.

3. Results

3.1. Molecular Identification

Molecular identification was performed by PCR analysis and DNA sequencing of the 18S rRNA gene from isolate V1. Molecular identification indicated that the strain belonged to the *Candida tropicalis* species. The obtained sequence (652 bp) was registered in the NCBI database with Acc. No. OR482135.1.

3.2. Biodegradation of Glyphosate

The tolerance of *Candida tropicalis* V1 and *Trichosporon cutaneum* R57 strains to glyphosate was tested by cultivation in YEPD medium containing glyphosate at concentrations of 0.1 to 0.4 g/L. It was found that in the glyphosate-containing, nutrient-rich YEPD medium, both tested strains were found to show good growth. In the next experimental stage, the ability of both strains to grow and develop in a mineral medium including only glyphosate as a carbon source was investigated. Their ability to grow in a carbon-free medium (YNB without AA) containing up to 0.3 g/L glyphosate was established, which showed their potential to degrade and assimilate this compound as a sole carbon source (Figure 1).

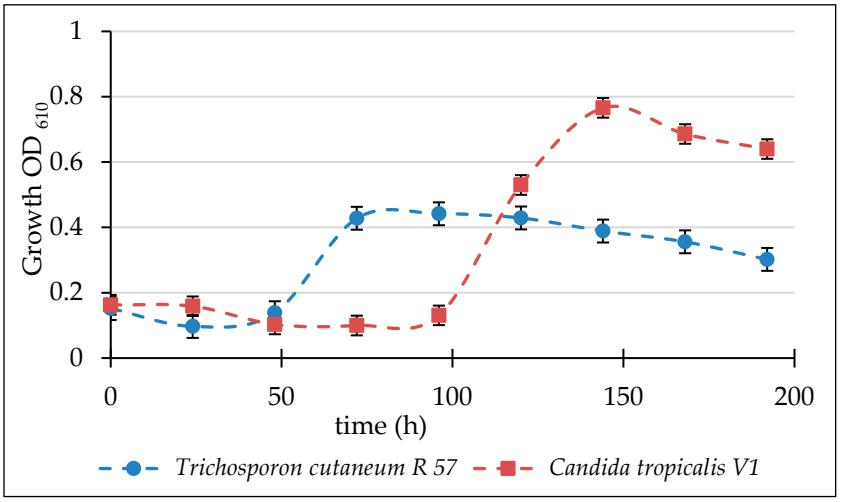


Figure 1. Growth curves of *Trichosporon cutaneum* strain R57 and *Candida tropicalis* strain V1. Nutrient medium—YNB w/o AA with 0.3 g/L glyphosate, included as a sole carbon source.

The degradation process of glyphosate was followed within 192 h of cultivation in a medium YNB w/o AA. The samples taken from the culture medium during the growth of the strains were subjected to gas chromatographic analysis. Both strains degrade glyphosate, but the advantage of the *C. tropicalis* V1 strain is evident. It degraded 76% of the initial amount of this carbon substrate within the investigated time of 192 h (Figures 2 and S1).

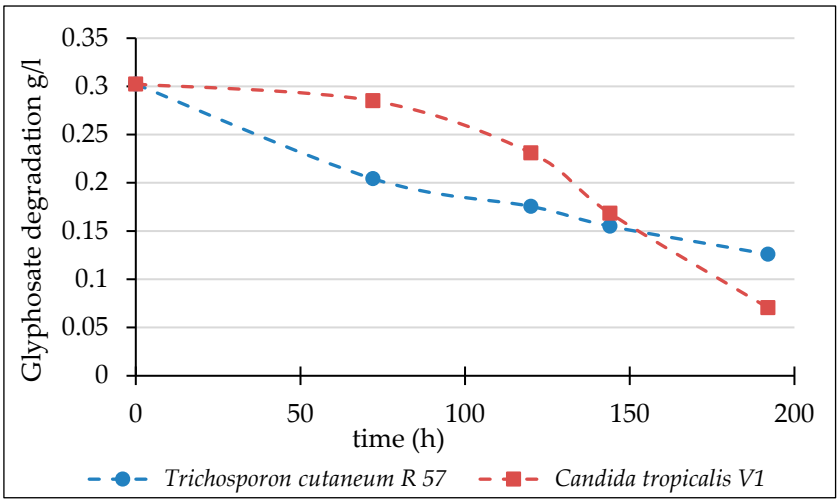


Figure 2. Biodegradation of 0.3 g/L glyphosate by *Trichosporon cutaneum* strain R57 and *Candida tropicalis* V1 strain in a YNB w/o AA medium, as a sole carbon source.

GC–MS analysis revealed the process of glyphosate degradation in dynamics. The gas chromatographic analyses established in probes taken at different points of cultivation are shown in Figure S1.

Analyses of the strain *C. tropicalis* V1 showed the presence of methylglycine in samples taken at 120 and 144 h of cultivation. zezw. Despite the observed slower decrease in glyphosate concentration, GC–MS analysis of *T. cutaneum* strain R57 samples showed a constant presence of methylglycine from 72 to 192 h of cultivation (Table 1).

Table 1. Mass spectral analysis of metabolites found in the biodegradation of glyphosate by *Trichosporon cutaneum* R57 and *Candida tropicalis* V1.

Pesticide	Degradation Products	m/z of Fragment Ions (Relative Abundanse, %)
Glyphosate	Methylglycine	89(6), 30(100), 29(3), 28(9), 15(3)
	Glycine	75(4), 44(6),30(100), 29(5), 28(8)
Carbofuran	Carbofuran-7-phenol	164(100), 149(83), 131(32), 123(29), 122(28), 121(21), 103(17),77(14)
	Pyruvic acid	88(5), 45(13), 44(18), 43(100), 42(9)

3.3. Biodegradation of Carbofuran

The tolerance of strains *T. cutaneum* R57 and *C. tropicalis* V1 to the carbofuran presence was studied in a manner similar to the trials for cultivation with glyphosate. It was found that both strains grew normally in the nutrient-rich medium YEPD containing carbofuran. A considerable growth slowdown was noticed when the carbofuran content exceeded 0.3 g/L in the carbon-free medium YNB w/o AA. The growth curves obtained as a result of the strains' cultivation in that medium containing 0.3 g/L carbofuran are shown in Figure 3. The faster growth of strain *T. cutaneum* R57 stands out.

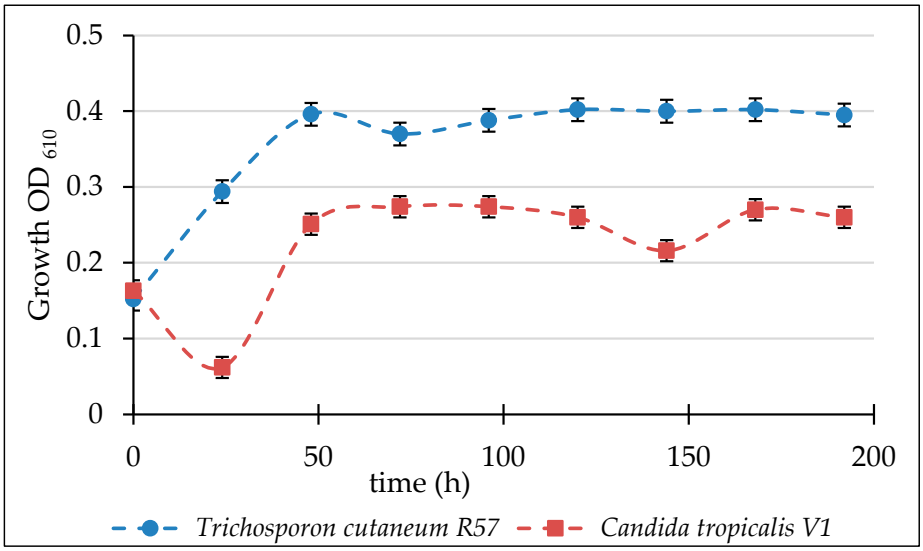


Figure 3. Growth curves of *Trichosporon cutaneum* strain R57 and *Candida tropicalis* strain V1. Nutrient medium—YNB w/o AA with 0.3 g/L carbofurane, included as a sole carbon source.

Data from the carbofuran biodegradation assay within 192 h showed trends consistent with the growth of the strains. Practically complete biodegradation and assimilation of carbofuran by strain *T. cutaneum* R57 were established. Parallel to the observed slower growth, a very slow decrease of 23.4% in the concentration of carbofuran was observed during the cultivation of strain *Candida tropicalis* V1 (Figure 4).

The visualization of the extent of carbofuran biodegradation at various stages in the growth of a strain of *T. cutaneum* R 57 is demonstrated in Figure S2.

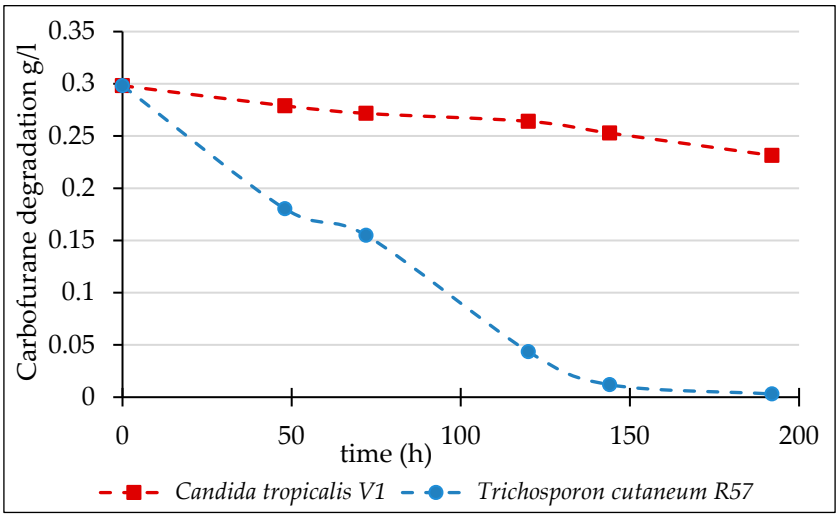


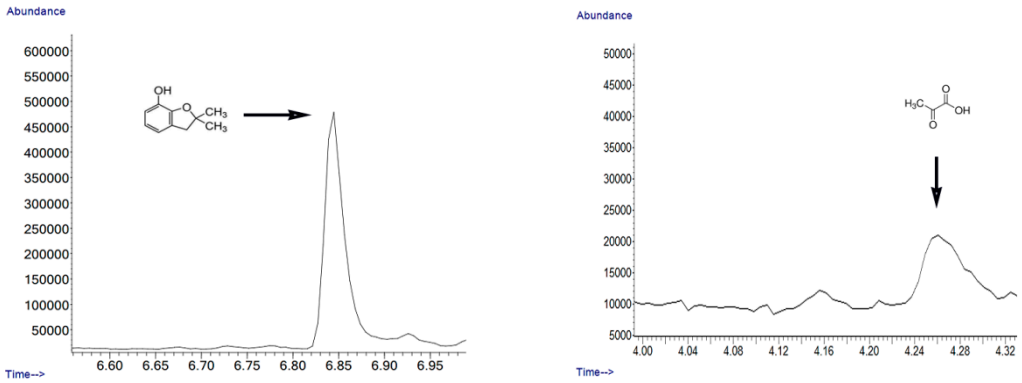
Figure 4. Biodegradation of 0.3 g/L carbofurane by *Trichosporon cutaneum* strain R57 and *Candida tropicalis* strain V1 in a YNB w/o AA medium, as a sole carbon source.

3.4. Intermediate Metabolic Compounds

During the gas chromatographic analysis, some intermediate metabolites characteristic of the biodegradation of both pesticides were recognized.

Methylglycine and glycine were identified in the degradation process of glyphosate. Analyses of the strain *C. tropicalis* V1 showed the presence of methylglycine in samples taken at 120 and 144 h of cultivation. In the 192 h sample, when three-quarters of the amount of available glyphosate was depleted, the presence of the next methylglycine metabolite, namely, glycine, was detected (Figure S3). Despite the observed slower decrease in glyphosate concentration, GC–MS analysis of *T. cutaneum* strain R57 samples showed a constant presence of methylglycine from 72 to 192 h of cultivation (Table 1 and Figure S4). Chromatograms of methylglycine and glycine obtained at 192 h of cultivation show the observed differences between the two yeast strains tested for glyphosate biodegradation (Figures S3 and S4).

The mass spectral analysis of metabolites found in the biodegradation of carbofuran revealed two intermediate metabolites: carbofuran-7-phenol and pyruvate (Table 1). The relatively rapid biodegradation of carbofuran by *T. cutaneum* strain R57 led to the appearance of carbofuran-7-phenol as early as the 48th hour of cultivation. At the 120th hour of cultivation, only the presence of pyruvate was detected, which is probably quickly included in the normal cell cycle metabolism (Figure 5).



(**a**) (**b**)

Figure 5. GC–MS analyses of intermediate metabolites during biodegradation of carbofuran from *Trichosporon cutaneum* R57: (**a**) carbofuran-7-phenol—48 h; (**b**) pyruvic acid—120 h.

Under the same cultivation conditions, in the culture medium sample of strain *C. tropicalis* V1, the appearance of carbofuran-7-phenol was recorded at the 72nd hour. The concentration of this compound remained unchanged until the 144th hour (Figure 6). After this hour, a slight decrease in its amount was observed, with its lowest value being recorded at the 192nd hour. This was accompanied by an insignificant increase in the biomass of the strain.

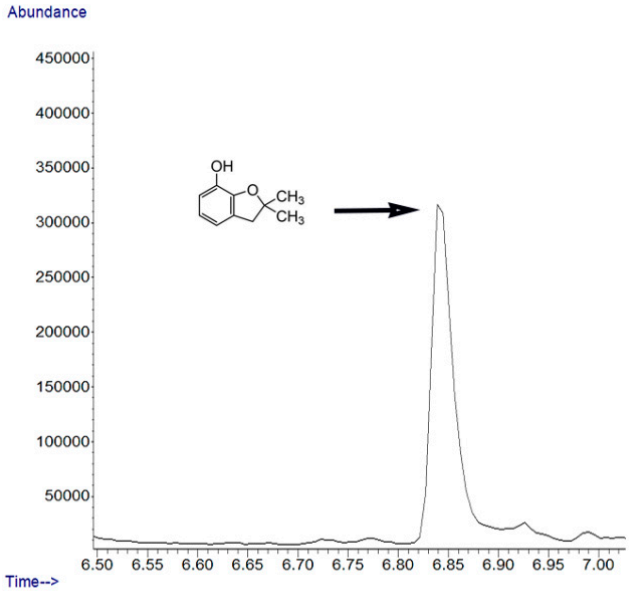


Figure 6. GC–MS analyses of intermediate metabolites during biodegradation of carbofuran from *Candida tropicalis* V1—carbofuran-7-phenol—192 h.

4. Discussion

Yeasts are among the most studied eukaryotic microorganisms due to their applicability in major areas of life and industry. They have an outstanding role in the food industry, pharmacy, and agriculture. Yeasts have established themselves as an important participant in environmental biotechnology. Yeasts are actively involved in environmental biotechnology and bioremediation.

The field of research related to the ability of yeast to degrade and remove pesticides from the environment has been very sparsely explored [46]. In the publications known to date in this area of scientific interest, some yeast strains have been presented, showing a clear potential to reduce the amount of pesticides in various environments.

The wide application, accompanied by the high toxicity of carbofuran, provokes the search for an effective and affordable way to remove it from the environment [54–56]. Glyphosate is also the subject of increased interest in terms of accumulating data on the harmful effects it causes on human and animal health [57]. Until now, the elaborations related to their removal with the participation of yeast have not received much attention.

There are fewer studies on the yeast degradation of the pesticides carbofuran and glyphosate [47–50].

Yarrowia lipolytica and *Candida krusei* yeasts, able to grow in a medium containing glyphosate at concentrations of 40 and 60 mg/L, were selected among those isolated from glyphosate-treated soil. Biotransformation of glyphosate was found within 20 days of cultivation. A more significant biodegradation exceeding 60% was reported for *Candida krusei* [58].

It is well known that representatives of the yeast species *Candida tropicalis* have been proven to assimilate different aromatic compounds, diesel oil, metalaxyl-acylalanine fungicide, and some others [59–61].

Trichosporon cutaneum is another representative of yeasts whose ability to degrade various compounds of xenobiotic nature is well known. It is proven that certain strains

of *T. cutaneum* degrade phenol and other aromatic compounds. Some of them appear as intermediate metabolites in the biodegradation of carbofuran, the pesticide endosulfan, as well as different aromatic compounds present in industrial wastewater [62–66].

In the present study, the yeast strains *C. tropicalis* V1 and *T. cutaneum* R57 reveal a serious potential for a complete biodegradation of two of the most widely used pesticides in our time. Each of the two strains showed its advantages in the biodegradation of glyphosate and carbofuran. *C. tropicalis* strain V1 showed significant capacity for complete glyphosate biodegradation, while *T. cutaneum* strain R57 was highly efficient in carbofuran biodegradation (Figures 2 and 4). It should be emphasized that the results shown were obtained under cultivation conditions where both strains used glyphosate and carbofuran as the sole carbon sources.

One of the most important questions in the biodegradation of toxic xenobiotics is whether metabolites with the same or higher toxicity do not appear and accumulate in the process of this degradation.

Putative catabolite schemes have been presented as a consequence of investigations monitoring the occurrence of intermediate metabolites in the microbial degradation process of carbofuran. Most such studies have been conducted on bacterial carbofuran degraders [39,67–69]. There are similar metabolic studies in some fungi [9,28]. The composition of the used cultivation media and the various sources of nitrogen, carbon, and phosphorus included in them have an impact on the detection of specific compounds. It has also been affected by other factors, such as cultivation time, degradation rate, etc. In most of the described metabolic pathways, the presence of carbofuran-7-phenol has been proven. This metabolite was also identified in our studies. In strain *C. tropicalis* V1, an accumulation of carbofuran-7-phenol was observed up to 192 h. In the *T. cutaneum* strain R57, this occurs during the exponential phase of growth, but in the stationary phase, the presence of pyruvate is also detected, which is indicative of complete biodegradation (Figure 5). In contrast to most bacteria, *T. cutaneum* demonstrates a capacity to catabolize carbofuran to carbofuran-7-phenol and further degrade this intermediate to pyruvate [26]. This is a reason to believe that carbofuran undergoes complete biodegradation into typically digestible compounds in the normal cellular metabolism.

Several different schemes have been published for the microbial catabolism of glyphosate. Each of them usually has an identical beginning, including the compounds AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), acetyl glyphosate, and sarcosine [70]. The differences are based on how these compounds may or may not be broken down to compounds natural to cellular anabolism. In our GC–MS analyses of intermediate metabolites during the biodegradation of glyphosate, methylglycine was identified in both tested strains. In strain *C. tropicalis* V1, which more actively degrades this pesticide, upon entering the stationary phase of growth, methylglycine is transformed into glycine. The amino acid glycine is among the elements of cellular metabolism common to all organisms.

5. Conclusions

This is the first report of *C. tropicalis* and *T. cutaneum* strains' use and biodegradation of glyphosate and carbofuran. The studies were conducted in a carbonless cultivation medium in which each of the two pesticides acted as the sole carbon source. The strain *C. tropicalis* V1 almost completely removed 0.3 g/L of glyphosate, while the *T. cutaneum* strain R57 highly effectively degraded 0.3 g/L carbofuran for about 8 days. Both strains utilize glyphosate via methylglycine, and carbofuran is used via the compound carbofuran-7-phenol.

In the backdrop of lacking information about the role and potential of yeasts to degrade and utilize pesticides such as glyphosate and carbofuran, we believe that the conducted research and obtained results are part of the process of enriching scientific knowledge in this field. It is possible to suggest using the studied yeast strains in the development of biotechnologies for the bioremediation and soil and water purification of pesticide-contaminated areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11123343/s1, Figure S1. Gas chromatographic analyses of glyphosate biodegradation by *Candida tropicalis* V1 at 0, 120, 144, and 192 h.; Figure S2. Gas chromatographic analyses of carbofuran biodegradation by *Trichosporon cutaneum* R57 at 0, 48 and 192 h.; Figure S3. GC–MS analyses of intermediate metabolites during biodegradation of glyphosate from *Candida tropicalis* V1 (a) methylglycine—144 h; (b) glycine—192 h.; Figure S4. GC–MS analyses of intermediate metabolites during biodegradation of glyphosate from *Trichosporon cutaneum* R57—Methylglycine 192 h.

Author Contributions: Conceptualization, Z.A.; methodology, M.G. and I.D.; software, M.G.; validation, I.D. and Z.A.; formal analysis, N.P.; investigation, K.S. and M.G.; data curation, M.G. and K.S.; writing—original draft preparation, Z.A.; writing—review and editing, Z.A.; visualization, M.G.; funding acquisition, Z.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Project BG05M2OP001-1.002-0019: Clean technologies for sustainable environment—water, waste, energy for circular economy (Clean&Circle), for development of a Centre of Competence, is financed by the Science and Education for Smart Growth Operational program (2014–2020), co-funded by the EU through the European Structural and Investment funds.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

Acknowledgments: This work was conducted according to the National Program "Young Scientists and Postdoctors-2" (NPMUPD-2) of the Ministry of Education and Science, Bulgaria. Special gratitude to the Bulgarian Academy of Science for including the team in the program. Thanks are due to O. Stoitsov, in English language, for a critical reading of the English text.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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