

Growth Kinetics of Grey Oyster Mushroom (*Pleurotus pulmonarius*) Spawn Culture Revival from Spray Drying Application

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ABSTRACT: Spray drying represents a promising approach for preserving fungal mycelium liquid cultures, with the assurance of successful revival from powder form being a critical factor for its application. Although liquid culture of grey oyster mushroom (*Pleurotus pulmonarius*) has been well established, its potential for drying and subsequent revival remains underexplored, thereby limiting its use in long-term storage. In this study, the effect of various spray dryer inlet temperatures (80, 90, 100, 110, 120, 130, 140, and 150 °C) on the revival and growth kinetics of *P. pulmonarius* powder cultures was evaluated using maltodextrin and Arabic gum (10%) as protective agents. Growth kinetics were modelled using the Contois model, which revealed that powder prepared at 80 °C with maltodextrin exhibited the highest μ_{\max} value (6.0205), indicating superior revival and growth capacity. With Arabic gum, the highest μ_{\max} (5.7604) was observed at 90 °C. At higher inlet temperatures, revival and growth were still observed with both protectants, albeit at reduced rates. Metabolic activity, reflected by glucose consumption and citric acid production, further confirmed successful revival, with citric acid reaching the highest level of 4.958 ppm. Overall, this study demonstrates that spray-dried powder cultures of *P. pulmonarius* retain viability and metabolic activity, highlighting their potential for long-term preservation.

ABSTRAK: Teknik pengeringan sembur merupakan satu inovasi berguna untuk kultur cecair miselium kulat, dan jaminan bahawa bentuk serbuk kultur tersebut boleh dihidupkan semula merupakan faktor penting dalam pengeringan. Walaupun kultur cecair miselium cendawan tiram kelabu telah berjaya dibangunkan, potensinya untuk dikeringkan dan seterusnya dihidupkan semula masih kurang diterokai, sekaligus menhadkan aplikasinya dalam penyimpanan jangka panjang. Kesan suhu masuk pengering sembur yang berbeza-beza (80, 90, 100, 110, 120, 130, 140 dan 150°C) terhadap kebolehan hidup semula dan kinetik pertumbuhan kultur serbuk tiram kelabu menggunakan 10% maltodekstrin dan gam Arab sebagai agen pelindung telah dikaji. Model Cointois telah digunakan dan nilai yang diperolehi daripada persamaan tersebut menunjukkan bahawa kultur serbuk yang disediakan pada 80°C dengan perlindungan maltodekstrin mempunyai nilai μ_{\max} tertinggi iaitu 6.0205 dan menunjukkan kebolehan hidup semula serta pertumbuhan yang paling baik. Dengan penggunaan gam Arab, nilai μ_{\max} tertinggi (5.7604) dicapai pada suhu 90°C. Bagi kedua-dua agen pelindung, walaupun pada suhu yang lebih tinggi, kultur serbuk masih menunjukkan kebolehan untuk hidup semula dan berkembang tetapi pada kadar yang lebih rendah. Dari aspek metabolisme, penggunaan glukosa dan penghasilan asid sitrik yang tertinggi iaitu 4.958

ppm menunjukkan bahawa kultur serbuk mempunyai aktiviti metabolik dalam tempoh hidup semula. Kajian ini membuktikan bahawa kultur serbuk *P. pulmonarius* mampu dibangunkan dan berpotensi untuk dipelihara dalam tempoh yang lebih lama.

KEYWORDS: *spray drying; mushroom spawn; powder culture; growth kinetics; Contois model*

1. INTRODUCTION

Grey oyster mushroom (*Pleurotus pulmonarius*) is the most popular mushroom in Malaysia and the second most widely cultivated mushroom globally. It belongs to the Pleurotaceae family and thrives on lignocellulosic materials rich in carbon, such as decaying wood and leaves. This fungus possesses enzymatic capabilities that enable it to degrade cellulose, thereby extracting carbon as an energy source for metabolic activities. *Pleurotus* spp. are extensively cultivated in Asia, America, and Europe due to several favourable factors, including low-cost production methods, high yield potential, environmentally sustainable agricultural processes, short harvest intervals, and minimal land requirements [1].

The successful cultivation of grey oyster mushroom (GOM) depends on various factors, such as spawn quality, growing conditions, and substrate selection. Spawn, which serves as the seed material for mushroom cultivation, plays a critical role in determining production efficiency. There are two primary types of spawn: solid and liquid, both of which can be produced using tissue culture or spore print techniques [2]. With increasing global demand for mushrooms, spawn technology has evolved from solid to liquid, and, more recently, from liquid to powder, to enhance production efficiency and improve long-term quality preservation. However, both solid and liquid cultures present challenges, including high susceptibility to contamination, limited storage stability, increased packaging costs, and complex logistics. Their production also carries risks such as contamination, large spatial requirements, and extended incubation periods [3].

Spray drying has emerged as an innovative technique for protecting biological materials, extending shelf life, and regulating the controlled release of components under specific conditions [4][5]. Several studies have investigated its effectiveness in converting microbial cultures into powder form. For instance, *Trichoderma* spp. have been successfully spray-dried to reduce spoilage risks and extend shelf life [6]. In that study, sucrose, molasses, and glycerol were tested as protectants, with 2% sucrose demonstrating the highest survival rate. Braga et al. [7] further investigated different protectants and drying temperatures for *Trichoderma* spp. finding that maltodextrin provided a 92.89% survival rate at 90°C, although survival declined at higher temperatures. Similarly, Ishak et al. [8] studied *Trichoderma asperellum*, using Arabic gum and maltodextrin as protective agents. Their results showed survival rates of 68.2% at 170°C, 15.9% at 160°C, and 0.2% at 150°C. In contrast, spray drying of *P. pulmonarius* at 80°C produced the lowest powder culture yield but resulted in the highest mycelial dry weight of 1.68 g upon revival [9].

Encapsulation technology, or protective techniques, has also been explored to improve microbial viability, particularly in biological control applications. Maruyama et al. (2020) encapsulated *Trichoderma harzianum* in polymeric microparticles, enhancing its antifungal and enzymatic activities while preserving soil microbiota. The encapsulated fungi exhibited increased chitinolytic and cellulolytic activity, thereby improving control over *Sclerotinia sclerotiorum*. Chandralekha et al. [10] investigated yeast viability post-spray drying by

incorporating various carrier materials, including maltodextrin, corn starch, Arabic gum, and acacia gum. Their study concluded that corn starch and maltodextrin yielded optimal powder output (59% w/w) and cell survival (80.5%), with corn starch being the most effective carrier. More recently, Suryabhan et al. [11] examined yeast microencapsulation using maltodextrin combined with sucrose or sorbitol, finding that survival rates increased by 35–45% with the addition of either sugar.

The Contois model has been widely applied to study microbial growth kinetics. Numerous studies have employed this model to evaluate the specific growth rates (μ) of various microorganisms, including *Lactobacillus* strains and *Shewanella baltica*, providing valuable data for industrial process design and scale-up [12][13]. Its robustness has also been demonstrated in studies of microalgae, supporting the model's applicability across a broad range of microbial species [14].

Therefore, the present study aims to evaluate the revival and growth capabilities of grey oyster mushroom powder cultures using the Contois kinetic model, quantifying key parameters such as maximum specific growth rate (μ_{\max}), half-saturation constant (K_s), and doubling time. These growth characteristics were further validated by measuring metabolic activity via glucose consumption and citric acid production in the revived powder cultures.

2. METHODOLOGY

2.1. Preparation of inoculum for mycelium liquid culture

Mature fruiting bodies of grey oyster mushroom (GOM) with stipe lengths of approximately 9–11 cm were harvested. The inner gill tissue was aseptically cut into 0.5 cm × 0.5 cm sections and inoculated onto prepared Potato Dextrose Agar (PDA) in sterilized Petri dishes. The cultures were incubated at 28°C in the dark (Hipoint, Taiwan) until white mycelium fully colonized the PDA surface [15].

2.2. Preparation of stress media and induced sporulation

A 1 L stress medium was prepared by mixing 5 g of D-glucose, 130 mg of monopotassium phosphate, and 3.5 g of soluble starch in distilled water. A saline solution was also prepared by dissolving 0.85 g of NaCl and 1% (v/v) Tween® 80 in 100 mL of distilled water. Both media were sterilized at 121°C and 101.5 kPa for 15 minutes.

Hyphal fragments were harvested from the fully colonized PDA cultures. Prior to collection, 10 mL of sterile saline was added to the Petri dish, and the mycelial surface was vigorously rubbed with a sterile loop to dislodge the hyphae. Then, 10 mL of the resulting saline–hyphae suspension was inoculated into the sterile stress medium and incubated in the dark at 25°C without agitation. After 14 days of incubation, the liquid culture, also referred to as the stress culture, was ready for spray drying to produce powder culture.

2.3. Production of powder culture using a spray dryer

After 14 days of incubation, the liquid culture, containing an average of 3.298 g/L of mycelial dry weight, was subjected to spray drying. Before drying, sterile aqueous solutions of 10% (w/v) maltodextrin and 10% (w/v) Arabic gum were prepared and added separately to the liquid cultures as protective carriers.

Spray drying was performed using a laboratory-scale spray dryer (BUCHI B-290, Switzerland) equipped with a pneumatic nozzle. The dryer was operated under the following

conditions: 100% aspiration rate, feed rate of 3 mL/min, nozzle cleaning 5 times per minute, and airflow of 30 m³/h. Drying was conducted at various inlet temperatures (80°C, 90°C, 100°C, 110°C, 120°C, 130°C, 140°C, and 150°C).

A Schott bottle containing 1L of the liquid culture was placed on a magnetic stirrer to ensure uniform mixing during drying. The bottle opening was sealed with parafilm and aluminum foil to prevent contamination. The spray dryer's feed tube was inserted into the Schott bottle, enabling continuous suction and atomization of the liquid culture into powder. Upon completion, the resulting powder cultures were collected, weighed, and transferred into WHEATON® centrifuge tubes. These were sealed in plastic bags and stored at 4°C for further analysis.

2.4. Determination of powder culture growth kinetics

To determine growth kinetics, 5 g of each prepared powder culture was inoculated into 25 mL of brown sugar, rice bran, malt, and yeast extract (BRMY) containing medium [16], prepared with varying glucose concentrations of 5%, 10%, and 15% (w/v). Mycelial dry weight was measured at 5-day intervals over 15 days. Growth kinetics were modelled using the Contois equation (Eq. 1):

$$\mu = \frac{\mu_{max} \times S}{K_s + S} \quad (1)$$

where μ is the specific growth rate of the mycelium, μ_{max} is the maximum specific growth rate of the mycelium in the substrate, S is the concentration of the limiting nutrient, and K_s is the saturation constant.

2.5. Analysis of glucose and citric acid for metabolism study

A 10 g sample of powder culture prepared at 80°C was inoculated into BRMY medium and incubated for 14 days. A culture sample (1–2.5 mL) was then mixed with 25 mL of HPLC-grade water and centrifuged at 16,000 rpm for 10 minutes. The supernatant was filtered using a 0.2 μ m nylon membrane filter.

Glucose and citric acid concentrations were analyzed using a High-Performance Liquid Chromatography (HPLC) system equipped with a Supelcosil LC-NH₂ reverse-phase column (25 cm \times 4.6 mm, 5 μ m) and a refractive index detector. Isocratic elution was performed using two mobile phase compositions: CH₃CN:H₂O at 85:15 (v/v) and 65:35 (v/v), both at a flow rate of 1.5 mL/min. The sample injection volume was 20 μ L, and analysis was carried out at 35°C. Standard curves for glucose (1–10% w/v) and citric acid (1–5% w/v) were prepared using HPLC-grade water. All samples and standards were analyzed in triplicate.

3. RESULTS AND DISCUSSIONS

3.1. Contois model profile at various temperatures

Figures 1 and 2 present graphs based on the Contois model at various temperatures. In Figure 1, for maltodextrin, the graph at 80°C (T80) exhibits a significantly steeper slope, with a corresponding intercept ($1/\mu_{max}$) of 0.1661, indicating more favourable growth kinetics. At 90°C (T90) and higher temperatures, the slopes are relatively similar, with intercept values ($1/\mu_{max}$) ranging from 0.2047 to 0.2841. In Figure 2, for Arabic gum, the T90 plot shows the steepest slope (0.1736), whereas the other plots are more gradual, with intercept values ranging

from 0.2574 to 0.9209. These positive, linear upward trends indicate that the dried culture powder retains the ability to revive and grow after thermal treatment.

Notably, temperature treatments from 80°C to 150°C did not destroy the mycelial cultures. Instead, they retained viability and regrew upon subculture in BRMY medium. Moreover, increasing glucose concentration in the medium further enhanced mycelial growth. The use of protectants, specifically maltodextrin and gum Arabic, resulted in slightly different trends across the temperature profiles. These results could not be achieved without the use of protectants, as glucose-containing solutions became sticky during spray drying, leading to poor powder formation and no viable outcomes in the control (no protectant) samples [17].

These findings suggest that temperatures between 90°C and 150°C imposed some thermal stress on the mycelium, affecting growth performance. However, cultures dried at 80°C experienced less thermal damage, effectively preserving cellular vigour and enabling robust regrowth. All positive linear trends support the conclusion that the culture powder retained revival capability after drying. Importantly, these results highlight the notable heat tolerance of grey oyster mushroom (GOM) cultures and their potential for long-term preservation through spray drying. Both protectants demonstrated that GOM mycelium could tolerate drying temperatures up to 150°C, although lower temperatures (e.g., 80°C) are preferable for maintaining cell integrity and future growth potential. Similarly, spray-drying *Lactiseibacillus rhamnosus* GG at 85°C resulted in successful revival after 13 weeks of storage, with minimal cellular damage and preserved viability during drying [18].

Several studies have identified 80°C as the lowest feasible spray-drying temperature that still yields desirable powder properties. This experiment was designed to balance optimal powder formation with maximum preservation of cellular viability. Therefore, the 80°C-90°C range is optimal for drying, producing viable powder while ensuring good survival and revival characteristics. These findings underscore the heat resilience of GOM mycelia and spores, making them excellent candidates for thermal preservation through spray drying.

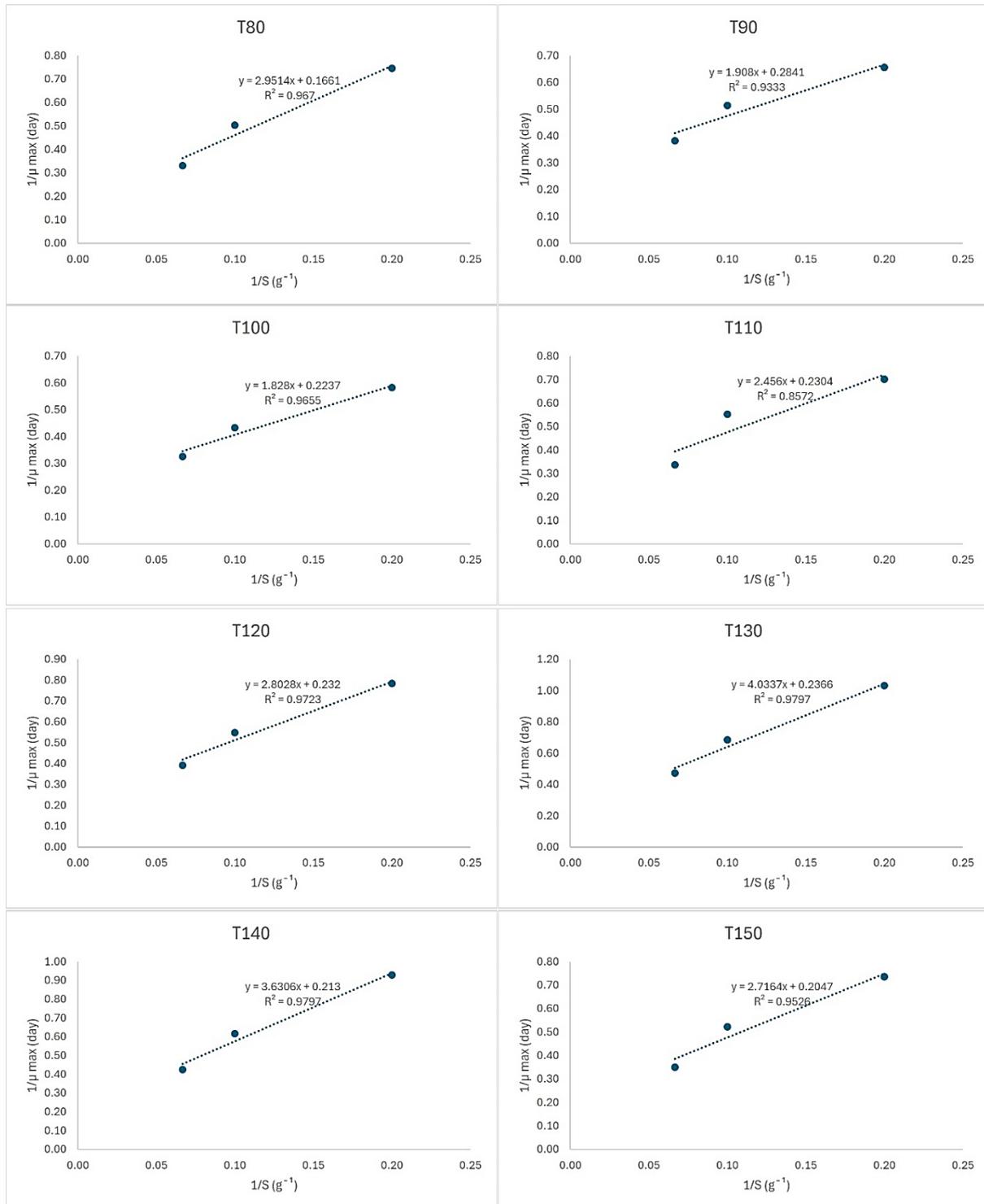


Figure 1. Linearized plot of the Contois model with maltodextrin as protectant

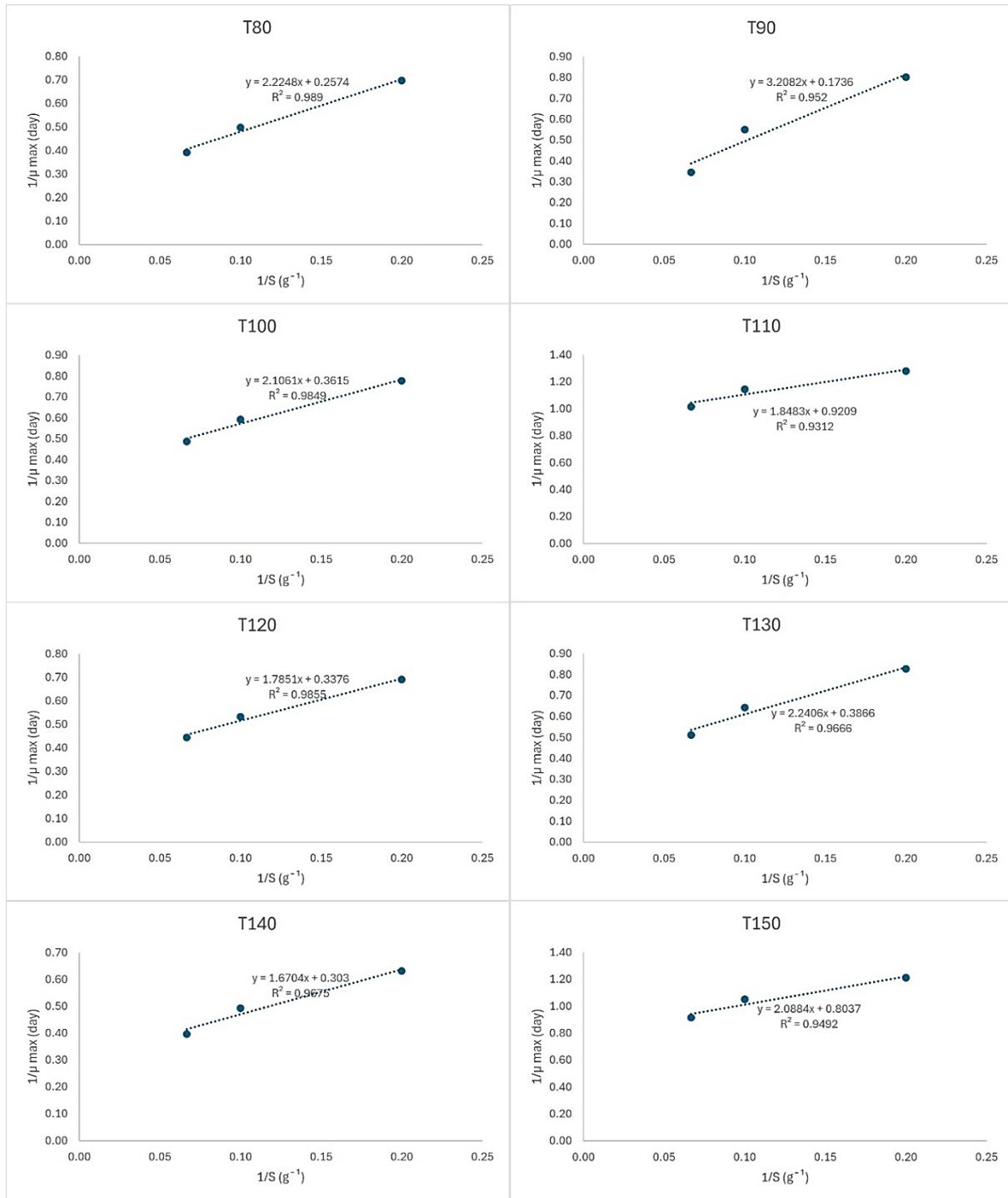


Figure 2. Linearized plot of the Contois model with Arabic gum as protectant

3.2. Effect of temperature on μ_{max} and K_s

Tables 1 and 2 present values derived from the plots of $1/\mu_{max}$ (day) and $1/S$ (g^{-1}), corresponding to μ_{max} , K_s and doubling time for cultures protected with maltodextrin and Arabic gum. These results provide insight into the growth kinetics of dried GOM mycelial cultures across varying spray-drying temperatures. For maltodextrin, the highest μ_{max} value (6.0205) was observed at 80°C, indicating optimal growth performance under this condition (Table 1). Above this temperature, μ_{max} values declined consistently with increasing drying temperatures. This trend suggests increasing thermal stress on the cells, leading to reduced growth capability. Interestingly, higher μ_{max} values were often accompanied by higher K_s values, indicating a trade-off between growth rate and substrate affinity. For Arabic gum, the peak μ_{max} (5.7604) was achieved at 90°C, followed by a noticeable decline at higher temperatures (Table 2). This demonstrates that Arabic gum offered some protective benefit, but its effectiveness diminished beyond 90°C. In terms of doubling time, cultures with maltodextrin had a shorter doubling time (0.1151 days) than those with Arabic gum (0.1203 days), indicating faster growth with maltodextrin as a protectant. These kinetic parameters affirm that both protectants contributed to preserving culture viability, although maltodextrin was slightly more effective. The cultures were well encapsulated as shown by scanning electron micrograph (SEM) in an earlier study by Zakaria et al. [19], whose SEM analysis showed that cultures dried between 80°C and 120°C retained a generally spherical particle shape, despite the presence of wrinkles and surface indentations. Such structural integrity supports the hypothesis that cells were well encapsulated during drying, thereby enhancing protection and revival capacity.

In this study, μ_{max} is the primary indicator of the culture powder's revival potential in BRMY medium. A higher μ_{max} value indicates greater specific growth capacity and thus better survival during the drying process. Importantly, even though μ_{max} decreased at higher temperatures, growth was still observed, suggesting that some portion of the mycelium retained viability despite the thermal stress. It is hypothesized that this surviving portion may consist of heat-resistant spores, particularly chlamydospores [20]. The heat resistance of these cultures appears to be influenced both by spore structure and the effectiveness of the protectant used. Compared with maltodextrin, Arabic gum was less effective, as evidenced by lower μ_{max} values, particularly at temperatures above 90°C, where mycelial vigour declined significantly [21].

Another key consideration is the relationship between μ_{max} and K_s , (the Contois saturation constant). Theoretically, K_s , reflects the affinity of cultures for the substrate here, glucose. A higher K_s , indicates lower substrate affinity, implying that the culture requires a higher glucose concentration to sustain growth. This suggests that revival cultures may initially require a longer adaptation phase and thus benefit from increased substrate availability. Conversely, when μ_{max} decreases, K_s , also tends to decrease, which indicates improved substrate affinity, possibly due to gradual adaptation by stressed cells, enhancing their efficiency in glucose uptake. This adaptive behaviour has been described by Jefferson et al. [22], who demonstrated a similar inverse relationship between μ_{max} and K_s , in *Potamosiphon* spp. cultures under phosphate limitation, using the Contois model. A similar trend has been observed in bacterial cultures. For example, Zahari et al. [23] reported that well-adapted bacterial cells exhibited lower K_s , values and higher division rates, confirming the connection between nutrient affinity and culture recovery. In the present study, the strong correlation between $1/\mu_{max}$ and $1/S$ ($R^2 > 96\%$ across most temperatures) further supports the suitability of the Contois model for describing the growth dynamics of spray-dried GOM cultures.

Table 1. Growth kinetics derived from the Contois model at various spray drying temperatures using maltodextrin as protectant.

Temperature(°C)	μ_{max}	K_s	Doubling time (t_d)	R^2
T80	6.0205	17.7688	0.1151	0.9670
T90	3.5199	6.7159	0.1969	0.9673
T100	4.4703	8.1717	0.1551	0.9655
T110	4.3403	10.6597	0.1597	0.8572
T120	4.3103	12.0810	0.1608	0.9988
T130	4.2265	17.0486	0.1640	0.9981
T140	4.6948	17.0451	0.1476	0.9797
T150	4.8852	13.2702	0.1419	0.9526

Table 2. Growth kinetics derived from the Contois model at various spray drying temperatures using Arabic gum as protectant.

Temperature(°C)	μ_{max}	K_s	Doubling time (t_d)	R^2
T80	3.8850	8.6434	0.1784	0.9890
T90	5.7604	18.4804	0.1203	0.9520
T100	2.7663	5.8260	0.2506	0.9849
T110	1.0859	2.0071	0.6383	0.9988
T120	2.9621	5.2876	0.2340	0.9876
T130	2.5867	5.7957	0.2680	0.9666
T140	3.3003	5.5129	0.2100	0.9930
T150	1.2442	2.5985	0.5571	0.9807

3.3. Evaluation of powder culture revival through glucose utilization and citric acid production

In powder spawn samples protected with maltodextrin, glucose was undetectable at all incubation time points (Days 5, 10, and 15), suggesting complete consumption of glucose during the early stages of incubation. In contrast, samples protected with Arabic gum exhibited a gradual reduction in glucose concentration, from 19.428 ppm on Day 5 to 8.612 ppm on Day 10, with no detectable glucose remaining by Day 15. The presence and subsequent reduction of citric acid further confirm metabolic activity in the revived powder spawn. In maltodextrin-treated samples, citric acid concentrations declined steadily from 4.958 ppm (Day 5) to 0.702 ppm (Day 15). In the Arabic gum group, citric acid levels were consistently lower, ranging from 3.804 ppm on Day 5 to 0.604 ppm on Day 15. The complete data on glucose and citric acid concentrations are presented in Table 3.

These findings indicate that the revived powder spawn metabolized available glucose, reflecting high metabolic activity and efficient substrate assimilation, particularly in maltodextrin-protected samples. The absence of residual glucose in these cultures suggests that the fungi resumed growth promptly upon rehydration, in line with the expected behaviour of viable fungal cultures post-revival [24]. In contrast, the delayed glucose utilization observed in Arabic gum-treated samples may reflect slower revival or metabolic activation. However, the eventual depletion of glucose by Day 15 still supports successful metabolic revival over time.

This trend highlights a pattern of early metabolic activity, characterized by high carbon uptake (as seen on Day 5), followed by a gradual decline in glucose and citric acid levels, likely due to substrate exhaustion or re-utilization of metabolic byproducts. Day 5 appears to represent an optimal period of active mycelial growth, after which metabolic activity declines. Several factors may influence citric acid production and accumulation, including possible re-utilization, dilution from increased biomass, or shifts in metabolic pathways [25]. Citric acid is a recognized metabolic byproduct of fungal growth, particularly in *Aspergillus* spp. and related filamentous fungi, and its presence serves as a reliable indicator of cellular respiration and biosynthetic activity. The lower citric acid concentrations observed in Arabic gum samples may indicate reduced or delayed metabolic activity, potentially due to differences in the physicochemical properties of Arabic gum. These may affect its protective efficiency during spray drying or influence the rehydration and revival performance of the powder spawn [26].

Table 3. HPLC analysis of glucose and citric acid concentrations during the incubation of revived powder spawn with different protectants.

Protectant	Incubation days	Glucose concentration [ppm]	Citric acid concentration [ppm]
Maltodextrin	5	Not detectable	4.958
	10	Not detectable	2.866
	15	Not detectable	0.702
Arabic Gum	5	19.428	3.804
	10	8.612	0.918
	15	Not detectable	0.604

4. CONCLUSION

This study demonstrates that spray drying is an effective and viable method for preserving liquid cultures of *Pleurotus pulmonarius* (grey oyster mushroom) in powder form, with the capacity to revive and sustain metabolic activity upon rehydration. The incorporation of protectants, specifically maltodextrin and Arabic gum, significantly enhanced the viability, revival, and growth performance of the powdered cultures across a range of inlet drying temperatures. Analysis using the Contois model revealed that the highest specific growth rates (μ_{max}) were achieved at 80°C with maltodextrin (6.0205) and 90°C with Arabic gum (5.7604), indicating these temperatures as optimal for post-drying culture revival. Although higher temperatures resulted in lower μ_{max} values, the cultures' ability to revive and grow suggests good thermal resilience of the mycelial cells in powder form. Further confirmation of metabolic activity was provided through glucose depletion and citric acid production, with citric acid concentrations reaching up to 4.958 ppm, underscoring the functional viability of the revived cultures. Overall, the findings support the potential application of spray-dried *P. pulmonarius* cultures for long-term storage, offering a practical and scalable solution for the mushroom industry, particularly for spawn distribution, preservation, and ready-to-use formulations.

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