American Journal of Biochemistry and Biotechnology

Original Research Paper

Maximizing Fungal Growth in State-of-the-Art Tray Solid-State Fermenter: Aeration Strategies Optimized for Enhanced Performance

[1,2]Musaalbakri Abdul Manan and [2]Colin Webb

[1]Enzyme and Fermentation Technology Programme, Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
[2]School of Chemical Engineering and Analytical Science, The University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom

Article history
Received: 18-04-2023
Revised: 13-07-2023
Accepted: 20-07-2023

Corresponding Author:
Musaalbakri Abdul Manan
Enzyme and Fermentation Technology Programme, Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
Email: bakri@mardi.gov.my

Abstract: This study introduced a tray fermenter, featuring eight layers of squared perforated trays, specifically designed for solid-state fermentation applications. It is named the multi-layer squared tray solid-state fermenter. The fermenter employs two aeration strategies with varying air flow rates to optimize fungal growth. Firstly, sterilized moistened air at 8 L/min flows from the bottom through the perforated trays. Secondly, a flow rate of 1 L/min delivers sterilized moistened air directly onto the substrate surface. Real-time monitoring of fungal growth is enabled by an oxygen/carbon dioxide gas analyzer and a thermocouple, tracking oxygen consumption, carbon dioxide production and metabolic heat generation. *Aspergillus awamori* and *Aspergillus oryzae* thrive on wheat bran substrate within this fermenter. Carbon dioxide evolution, indicative of fungal activity, is modeled using a Gompertz model, showing strong agreement and fitting well with experimental data. The respiratory quotient, calculated from carbon dioxide evolution and oxygen uptake rates, confirms fungal activity levels. Additionally, the correlation between fungal metabolic heat generation and carbon dioxide evolution offers complementary insights into growth dynamics. Aeration strategies notably affected fungal productivity. Both *A. awamori* and *A. oryzae* showed higher spore production with moistened air at 1 L/min. *A. awamori* exhibits strong enzyme production, particularly glucoamylase, xylanase and cellulase, while *A. oryzae*, excelled in protease production under similar conditions. Continuous monitoring of environmental variables ensures precise control of fermentation conditions. These initial findings highlight the potential of the multi-layer squared tray solid-state fermenter, with its forced moistened aeration, as a promising instrument for solid-state fermentation processes.

Keywords: Fermenter Design, Tray Solid-State Fermenter, Aeration Strategies, Gompertz Model, Fungal Growth

Introduction

Solid-state fermentation offers a highly favorable approach for advancing various bioprocesses and products, boasting advantages such as enhanced volumetric productivity, elevated product concentrations, reduced effluent generation and the utilization of simple fermentation equipment or fermenter systems. Since the turn of the millennium, solid-state fermentation has garnered substantial interest for its potential to drive industrial bioprocess expansion. This surge in attention can be attributed to several factors, including its diminished energy demands, correlation with heightened product yields, mitigated catabolic repression, cost-effectiveness and diminished wastewater production coupled with reduced susceptibility to bacterial contamination (Costa *et al*., 2018). Furthermore, solid-state fermentation aligns with environmentally conscious practices by utilizing solid residues sourced from agricultural and food industries as carbon substrates, further underlining its eco-friendly credentials.

As technology continues to evolve and modernize, solid-state fermentation is poised to be closely associated with the design of solid-state fermenters, marking crucial progress and innovation in biotechnological processes and bioprocess engineering. Numerous configurations

have been developed specifically for solid-state fermentation applications, including syringe-type fermenter (Costa *et al*., 2021), semi-continuous plug-flow systems (Wang *et al*., 2021), packed-bed (Mitchell *et al*., 2023; Pakaweerachat *et al*., 2023; Finkler *et al*., 2021a; Maiga *et al*., 2021; Casciatori *et al*., 2016), packed-bed column fermenter (Sosa-Martinez *et al*., 2024), tray (Dallastra *et al*., 2023; Sentis-More *et al*., 2023; Nascimento *et al*., 2021), trickle bed (Shahryani *et al*., 2019), multipurpose fixed-bed (Sabrini *et al*., 2019), rotating drum (Dabaghi *et al*., 2021; Mahmoodi *et al*., 2019), column-tray (Hector *et al*., 2012), novel deep-bed cubical (Brijwani *et al*., 2011), multi-layer packed-bed (Mitchell *et al*., 2006), column (Jang and Yang, 2008), fluidised-bed (Foong *et al*., 2009), hexahedral modular (da Cunha *et al*., 2009), solid state fermentation reactor (Ano *et al*., 2009), horizontal rotary drum (Alam *et al*., 2009), continuous counter-current reactor (Varzakas *et al*., 2008), scrapped-drum (Nagel *et al*., 2001), zymotis packed-bed (Mitchell *et al*., 2000), gas-solid spouted-bed fermenter (Silva and Yang, 1998), horizontal solid state stirred tank reactor (Berovic and Ostroversnik, 1997), vertical cylindrical shaped (Bandelier *et al*., 1997), cylindrical (Gutierrez-Rojas *et al*., 1996) fermenters. Advancements have been achieved in refining laboratory-scale processes, yet there remains a need for intensified research on fermenter designs and the scaling-up of processes to facilitate the utilization of microorganisms (Torres-Leon *et al*., 2021)

In aerobic solid-state fermentation processes, utilizing any type of fermenter, numerous factors exert important influences on the overall performance. These factors encompass the choice of appropriate microorganisms, solid medium, pre-treatment of the medium base, composition, which affects both inter-particle space and surface area, substrate heterogeneity, water activity or moisture gradients, starter culture, Oxygen (O_2) supply (rate of consumption), Carbon Dioxide (CO_2) transfer (rate of evolution), temperature control, removal of metabolic heat, fermentation duration, maintenance of environmental uniformity within the solid-state fermentation system and fermenter configuration. Operational challenges may arise from issues such as inadequate O_2 supply, moisture levels and heat build-up in the fermenter and control systems, which warrant attention (Arora *et al*., 2018). Gervais and Molin (2003) suggested that the exchange of O_2 and CO_2 between solid and gas phases relies on factors like enhanced contact surface area through added aeration from forced sterile air, agitation and substrate moisture levels. Throughout the fermentation process, heat generation can directly impact microbial metabolic activities, potentially leading to effects such as moisture loss. Failure to control temperature and provide adequate aeration may disrupt the selectivity of biosynthesis processes, potentially resulting in product formation issues or even complete process failure. Effective heat removal strategies are essential to mitigate these risks (Mendez-Gonzalez *et al*., 2020). The interplay between moisture content, heat levels, CO_2 evolution and O_2 transfer rates is fundamental in aerobic processes (El Zein *et al*., 2015). Direct aeration serves as the primary mechanism for O_2 transfer, while simultaneously influencing moisture loss, heat generation and CO_2 removal. However, these effects are amplified in scenarios involving high biomass concentrations and the presence of microorganisms, particularly filamentous fungi, in solid-state fermentation, complicating its implementation. To mitigate these challenges, a viable strategy is the implementation of forced aeration. Forced aeration through the solid substrate controls water content and activity, removes volatile compounds and CO_2, dissipates fermentation heat and supplies O_2 for aerobic metabolism (Gervais and Molin, 2003). This approach enables real-time analysis of CO_2 and O_2 concentrations in the exhaust gas, aligning with and regulating the fermentation process dynamics (Mendez-Gonzalez *et al*., 2020). Furthermore, exhaust gas analysis has been leveraged as an indirect method for estimating biomass growth (Torres-Mancera *et al*., 2018). A fundamental principle in fermenter design is to consolidate multiple essential operations within a single vessel. Serving as the nucleus of the biological process, the fermenter fulfills four key functions: Containment of the solid substrate, providing a habitat for the cultivated cultures, protection from desirable microorganisms and controlling factors and surroundings to enhance both productivity and yield (Nadal-Rey *et al*., 2020).

The paramount consideration in microbial fermentation lies in ensuring sterilization. Sterility is indispensable, particularly in submerged fermentation, where the abundance of water creates an environment ripe for various contaminants to potentially outpace the desired organisms. Solid-state fermentation, often involving fast-growing microorganisms like fungi, presents a unique scenario where a vigorous starter culture introduced to a solid substrate can effectively outcompete unfavorable contaminants. Consequently, while strict aseptic conditions may not be imperative in solid-state fermentation, meticulous attention to cleanliness remains crucial for optimal operation. Sindhu *et al*. (2017) emphasize the imperative nature of sterilization in effectively managing bio-contaminants. This assertion finds further validation in the findings of Arora *et al*. (2018), who underscore the necessity for solid-state fermentation fermenters to be hermetically sealed, thereby thwarting the ingress of undesired microorganisms or detrimental environmental agents. A steam-based sterilization system was implemented in the pilot-scale tray fermenter introduced by Dallastra *et al*. (2023) In addition to sterilization, meticulous attention must be paid to

controlling and optimizing factors such as temperature, O_2, CO_2 and heat removal, as highlighted by Zhong (2011). The design of solid-state fermenters necessitates meticulous consideration of diverse design and operational parameters. Particular attention must be given to aeration transfer to guarantee the efficient circulation of O_2 in the gaseous state, efficient removal of CO_2 and heat and accurate temperature control. Additionally, implementing an efficient cooling system is vital for optimal performance. (Arora *et al*., 2018; Mendez-Gonzalez *et al*., 2020; Zhong, 2011). In solid-state fermentation, there can be challenges with inefficient O_2 transfer in certain designs, leading to uneven distribution throughout the solid substrate. This can impede the homogeneous oxygenation of every particle within the substrate. Additionally, maintaining precise control and measurement of temperature and moisture level in the solid medium is paramount, as deviations in these factors can further exacerbate the situation. Therefore, it is essential to tightly regulate and monitor these parameters simultaneously (Rodriguez-Fernandez *et al*., 2011). Fermenters designed with a constructive approach exhibit elevated rates of biological reactions, encompassing microbial growth performance, substrate utilization, product synthesis and the synthesis of by-products (Arora *et al*., 2018; Zhong, 2011; Ashok *et al*., 2017). Therefore, gaining insights into which criteria yield optimal performance is crucial for the constructive design of fermenters. Dallastra *et al.* (2023) introduced a pilot-scale tray fermenter designed for process efficiency, integrating upstream operations and fermentation in a single piece of equipment, featuring a dispersion system for spore suspension, nutrient solution and cooling water above the fermentative medium. Moreover, enhancing fermenter design necessitates a deeper comprehension of microbial biological systems and the underlying processes in solid-state fermentation (Torres-Leon *et al*., 2021; Deive and Sanroman, 2017).

The fermenter functions as the environment where microbial growth and activity take place, facilitating essential biological reactions. In solid-state fermentations, tray fermenters stand out as the most straightforward. Trays are commonly arranged in stacks with suitable spacing between each layer. These arrangements are housed within a chamber where controlled conditions are maintained to ensure optimal temperature, humidity and conditions conducive to the fermentation process. Temperature regulation is typically attained by manipulating air circulation, which might involve adjusting its humidity level, ranging from dry to moist and temperature, ranging from cold to warm, as necessary.

The research took place at a facility outfitted with a fermenter system specifically engineered to generate humidified air. Within this setting, two separate configurations were applied in the newly developed laboratory-scaled tray solid-state fermenter named the multi-layer squared tray solid-state fermenter. The introduction of humidified air played a crucial role in supplying water and O_2, as well as aiding in heat dissipation, all of which contributed to improving fungal growth. Throughout the fermentation process, the fermenter's performance was assessed by monitoring key parameters such as final moisture content, O_2 transfer (will be measured as Oxygen Uptake Rate (OUR)), CO_2 production (will be measured as Carbon dioxide Evolution Rate (CER)), temperature fluctuations, as well as spores and enzymes production. As the analysis of O_2 and CO_2 levels in exhaust gas has become a widely accepted standard measurement technique in bioprocessing, it was similarly employed in this study to preserve the sterile environment. The results will be extensively reviewed and assessed in accordance with the study designs proposed for the two fungal strains under investigation.

Materials and Methods

Fungal and Spore Development Culture

The fungal strains of *Aspergillus awamori* and *Aspergillus oryzae* were employed in this study. The strains originated from the School of Chemical Engineering and Analytical Science, University of Manchester. These strains were selected based on their suitability for the research objective. The fungal strains were cultivated on solid spore cultivation substrate in universal bottles or petri dishes, each with a working volume of 10.0 mL. This substrate was composed of 5% (w/v) whole wheat flour and 2% (w/v) agar. Following activation in sterilized media, the fungal strains were cultured at 32°C for 7 days. Subsequently, the fungal strains were stored at 4°C for future use. Regular sub-culturing was conducted at two-month intervals throughout the study to maintain strain viability and stability.

Preparation and Inoculation Technique for Fungal Spores

The fungal spores were carefully washed, by gently agitating them with 10.0 mL of sterile 0.1% (v/v) Tween 80 solution. Subsequently, from this spore suspension, 0.5 mL was transferred onto the surface of 100.0 mL of the same sporulation substrate contained within a 500.0 mL Erlenmeyer flask, followed by a 7-days incubation period at 32°C. Upon completion of the incubation, 50.0 mL of sterile 0.1% (v/v) tween 80 solution and several sterile glass beads (4.0 mm diameter) were added to the flask. Gentle shaking facilitated the suspension of spores, which were then collected in a separate container to obtain a spore suspension. The concentration of the spore

suspension was determined utilizing a hemocytometer. Subsequently, the spore suspensions underwent dilution until achieving a visually uniform distribution without overlapping. The entire volume within the enclosure was considered a representative sample. The concentration of spores per milliliter and the total spore count were calculated utilizing Eq. 1:

$$Number\ of\ \frac{spores}{mL} = \left[average\ number\ of\ \frac{spores}{grid}\right] \times dilution\ factor \times 10^4 \quad (1)$$

The total spore count was calculated by multiplying the spore concentration per milliliter by the initial volume of the spore stock suspension sampled. To ensure consistent inoculation across experiments, the required volume of suspension was calculated to achieve a target concentration of approximately 1.0×10^6 spores per gram of solid substrate.

Utilizing Wheat Bran as a Solid Substrate in Bioprocessing

The wheat bran employed in this research was sourced from the Cargill wheat processing plant in Manchester, United Kingdom and stored in a sealed container at 4°C to maintain freshness. This wheat bran served as the solid medium for cultivating both fungal strains without undergoing any further treatment.

Procedures for Solid Substrate Preparation and Inoculum Transfer

A uniform protocol was established for preparing the inoculum, ensuring even spore distribution in the solid substrates before fermentation. Wheat bran (20.0 g) was placed in individual flasks and sterilized at 121°C for 15 min. After cooling, the substrates were inoculated with spores of *A. awamori* and *A. oryzae* and moistened with sterile distilled water to reach 65% initial moisture content. Approximately $1.0\text{-}1.2\times10^6$ spores per gram of substrate were evenly mixed into each flask. The mixture was then distributed into trays and incubated at 32°C for 72 h before sample collection for analysis.

Design and Development of a Tray Solid-State Fermenter

The fermenter chamber consists of eight identical square trays stacked on top of each other, each with perforations at the base for aeration. It measures 14.0×14.0×73.5 cm (length × width × height) when assembled (Fig. 1). The main body is made of aluminum for durability, with Perspex material used for the back wall and door. The transparent material of the enclosing structure allows for easy observation of the chamber's contents.

Within a single structure, the tray measures 12.0×12.0×2.0 cm (length × width × height) (Fig. 2). Crafted from Perspex material, the tray features a perforated base with apertures measuring 150.0 µm in size, facilitating operations, sterilization and efficient heat conduction.

The fermenter chamber was designed as a module to fit into an autoclave for sterilization before operation. Once sterilized, it was filled with sterile inoculated substrate in a laminar flow cabinet. Each tray holds about 20.0 g of substrate with a height of 1.5 cm. The substrate is introduced through the door, which is then sealed to prevent contamination. A schematic diagram of the experimental setup is shown in Fig. 3.

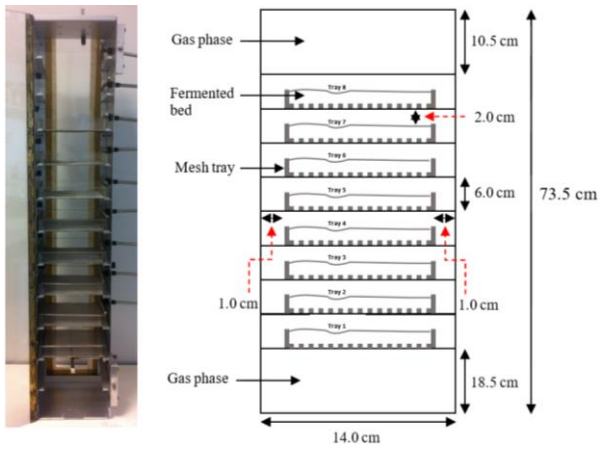


Fig. 1: A side-view schematic of a tray solid-state fermenter, highlighting the positioning of the individual trays

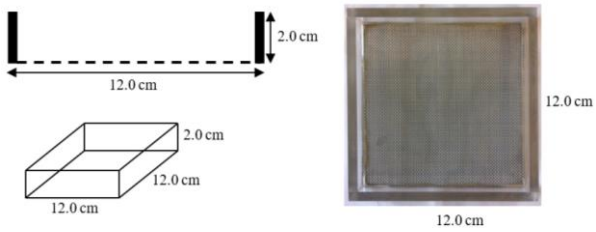


Fig. 2: A square mass tray designed to fit within the tray solid-state fermenter

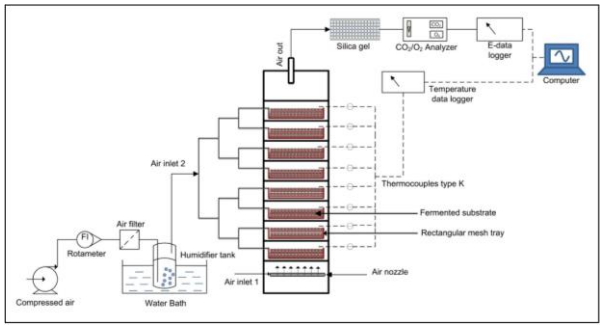


Fig. 3: A schematic diagram depicting the experimental setup of a tray solid-state fermenter

Design, Execution of Fermenter Setup and Fermentation Trials

Both the fermenter and solid substrate were autoclaved individually before use. The sterilized substrate was inoculated with spores and transferred into the fermenter under sterile conditions. Two different air flow rates were chosen for two distinct sparging setups. In the first setup (Exp 1), sterilized moistened air at 8 L/min was directed into the bottom of the fermenter and forced through the tray. In the second setup (Exp 2), sterilized moistened air at 1 L/min was applied directly onto the substrate surface. These specific air arrangements are detailed in Table 1.

Dry air from the JUN-AIR compressor (USA) was humidified before entering the system. The airflow rate was controlled using a rotameter and different rates were used to improve oxygen access. The air was sterilized with a 0.45 µm cellulose acetate membrane filter before entering the humidifier chamber with sterilized distilled water. The system included an additional compartment in Exp 1 for uniform air distribution to each tray. Thermocouples and an O_2/CO_2 analyzer were used to monitor and control environmental conditions.

Quantifying Metabolic Activity in Solid-State Fermentation

Real-Time Temperature Monitoring

A type K thermocouple from Pico Lab Technology (UK) was placed on the surface of the fermented substrate in each tray to monitor bed temperature continuously. Data were displayed and recorded on a computer for analysis.

Real-Time Respiratory Gases Monitoring

The system continuously monitored O_2 and CO_2 levels in the exhaust gas in real-time. To ensure accuracy, the humid exhaust air was dried using silica gel before entering an O_2/CO_2 analyzer (FerMac 368 Electrolab, UK) connected to a data logging system (Electrolab eLogger) for precise recording.

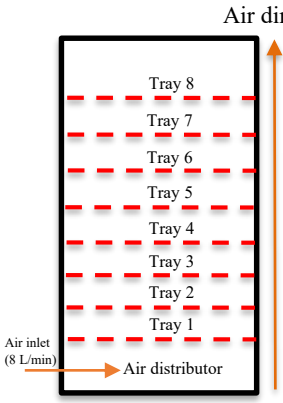
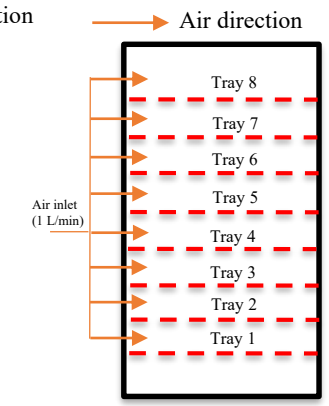
Determining Moisture Content at the End of Fermentation

Moisture content was analyzed using the oven method, where samples were heated at 95°C until reaching a constant weight. Initially, samples were weighed in pre-dried metallic dishes and then dried for 24 h. After cooling in desiccators, the final weight was measured to calculate moisture content using Eq. 2.

$$MC = \left[\frac{W_i - W_f}{W_i}\right] \times 100 \quad (2)$$

where, MC was the moisture content, %; W_i was the initial weight before drying, g; W_f was the final weight after drying, g.

Table 1: Comparative analysis of two air distribution strategies in tray solid-state fermenter

Experiment	
Exp 1	Exp 2
	

Counting Spores: A Measure of Fungal Proliferation

To collect spores, 2.0 g of fermented substrate with *A. awamori* and *A. oryzae* were mixed with 40.0 mL of 0.1% tween 80 solution in 250.0 mL flasks. The flasks were shaken at 100.0 rpm for 30 min at 30°C, then filtered using a stainless-steel sieve (45.0 µm aperture). Spore counting was done using a hemocytometer, following previously established protocols.

Extraction of Enzymes and Measurement of Activity

After 72 h of fermentation, samples were collected for enzyme analysis, including glucoamylase, protease, xylanase and cellulase. Each fermented sample (2.0 g, wet basis) was mixed with 40.0 mL of distilled water and shaken for 30 min at 250 rpm and 30°C. The solid suspensions were then centrifuged at 10,000 rpm for 10 min at 4°C and the clear supernatant was used for enzyme analysis following a standardized procedure established in a prior publication (Abdul Manan and Webb, 2016a).

Approaches to Mathematical Modelling

Measurement of Oxygen Uptake and Carbon Dioxide Evolution Rates

In this investigation, the O_2 Uptake Rate (OUR) and CO_2 Evolution Rate (CER) were determined utilizing the equations outlined by Sukatsch and Dziengel (1987):

$$OUR = \frac{F_1}{V_m \times Vo}\left(Xo_{2(in)} - \frac{1-\left(Xo_{2(in)} + Xo_{2(in)}\right)}{Xo_{2(out)} + Xo_{2(out)}} \times Xo_{2(out)}\right) \quad (3)$$

$$CER = \frac{F_1}{V_m \times V_0}\left(X_{CO_2(out)} \times \frac{1-\left(X_{O_2(in)}+X_{CO_2(in)}\right)}{1-\left(X_{O_2(out)}+X_{CO_2(out)}\right)} - X_{CO_2(in)}\right) \quad (4)$$

where, *OUR* and *CER* were the O_2 uptake rate and CO_2 evolution rate, respectively, mole/L. h; was the rate at which the gas enters the system, L/h; V_m was the molar volume of gases, 24.88 L/mole; V_o was the working volume of the solid phase, L; $Xo_{2(in)}$ and $Xo_{2(out)}$ were the proportion of O_2 molecules at the gas inlet and outlet, respectively; $Xo_{2(in)}$ and $Xo_{2(out)}$ were the proportion of CO_2 molecules at the gas inlet and outlet, respectively.

The development of this formula depends on the principles of gas equilibrium, particularly when CO_2 stands as the exclusive gaseous output resulting from the fermentation process (Sukatsch and Dziengel, 1987).

The Gompertz Curve: Modelling Fungal Growth

The Gompertz model, introduced by Benjamin Gompertz in 1825 and analyzed by Winsor (1932), features a sigmoid function similar to the logistic curve. It has been widely used in various fields, including biology and economics. Skiadas and Skiadas (2008) enhanced the model using systems theory principles. The model's differential equation form is represented as:

$$(ln\, x)' = -b\ ln\ x \tag{5}$$

where, x was the function with respect to time and b was the positive constant that signifies the rate at which the system grows.

The function of x is bounded within the range $0<x<1$, with $x = 1$ representing the entire population, serving as the probability density function of the growth process. Integrating Eq. 5 directly yields a solution to the Gompertz function (Skiadas and Skiadas, 2008):

$$x = exp(ln(x_o)\, exp(-bt)) \tag{6}$$

The integrated Gompertz model, utilized for kinetic data analysis, relates the product ($[CO_2]$) to time (t) based on a logistics-like equation:

$$[CO_2] = [CO_{2_{max}}]\, exp(-b\, exp[-kt]) \tag{7}$$

where, $[CO_{2max}]$ was the maximum concentration of CO_2, mole; b was the constant associated with the initial conditions (when $t = 0$, then $[CO_2] = [CO_{20}] = [CO_{2max}]$ exp $(-b)$, dimensionless; k was the specific rate of CO_2 evolution, 1/h; t was the duration of fermentation, h.

The constants $[CO_{2max}]$, b and k were estimated using a non-linear regression program. The value of k represents the time taken for $[CO_2]$ to reach $[CO_{2max}]$, with higher values indicating faster attainment. The value of b determines the shape of the sigmoidal curve, with larger values resulting in a slower initial growth phase and a faster approach to the asymptote. On the other hand, smaller b values lead to a rapid growth phase followed by a slower approach to the asymptote of $[CO_2]$ (Winsor, 1932).

Gas Equilibrium and Determination of Respiratory Quotient

The Respiratory Quotient (RQ) can be calculated from the exhaust gas composition, providing insights into the fermenter's physiology and performance. Real-time monitoring allows for online RQ calculation during fermentation:

$$RQ = \frac{CER}{OUR} \tag{8}$$

where, RQ was the respiratory quotient, dimensionless; CER was the CO_2 evolution rate, mole/L.h; OUR was the O_2 uptake rate, mole/L.h.

Results

Air Arrangement's Role in Determining Final Moisture Content During Fungal Development

Figure 4 illustrates moisture content profiles from the tray solid-state fermenter. In Exp 1[AA], *A. awamori* lost more moisture (51.95-59.82%) than in Exp 2[AA] (59.8-62.22%). For *A. oryzae*, final moisture content exceeded 65%, ranging from 66.04-67.84% in Exp 1[AO] and 66.71-69.89% in Exp 2[AO]. *A. oryzae* retained more water than *A. awamori*, indicating better water retention. This highlights *A. oryzae* superior water retention ability compared to *A. awamori*, consistent with earlier findings (Abdul Manan and Webb, 2016b). The system was tightly sealed to prevent air exchange inside and outside, yet it intentionally included significant gas spaces between trays. Direct sparging of air onto the fermented substrate surface in Exp 2[AA] and Exp 2[AO] offered benefits to trays positioned from 2-8, allowing them to access air from both the surface and the bottom of the perforated tray where air was introduced, while tray 1 only received aeration from the surface.

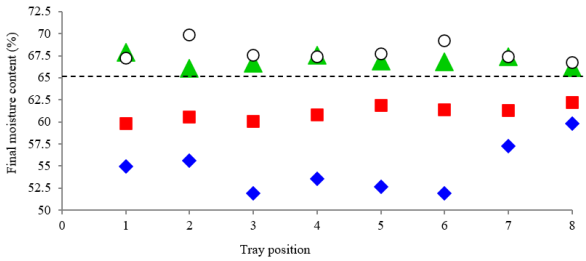


Fig. 4: The final moisture content at the end of the 72 h fermentation period was determined for *A. awamori* [AA] and *A. oryzae* [AO] cultivated in a tray solid-state fermenter using two different air arrangements. (◆) Exp 1[AA]; (■) Exp 2[AA]; (▲) Exp 1[AO] and (o) Exp 2[AO]. The dashed line represents the initial moisture content of 65%. Exp 1: 8 L/min; Exp 2: 1 L/min

Understanding the influence of essential elements on moisture content in solid-state fermentation is crucial. *A. awamori* and *A. oryzae* show sensitivity to air distribution within fermenter systems, with moistened air effectively controlling moisture levels. Airflow direction significantly affects moisture transport, with bottom-to-top airflow (Exp 1) negatively impacting *A. awamori* but benefiting *A. oryzae*, while surface blowing (Exp 2) decreases moisture content for *A. awamori* while increasing it for *A. oryzae*. Yet, it is essential to note that air arrangement is just one of several factors influencing cultural productivity. During the initial stages of fermentation, it is anticipated that temperature, O_2 levels and moisture content remain uniform throughout the solid-state fermentation system. However, as fermentation advances, O_2 is utilized, heat is generated and water evaporates, resulting in the development of gradients in temperature, moisture, gases, substrate and products within the fermented bed (Sabrini *et al*., 2019). He *et al*. (2019) highlighted the challenge of heterogeneous heat and mass transfer due to water loss in solid-state fermentation, emphasizing the need for more effective strategies to address this issue. Water plays various essential roles in solid-state fermentation, including regulating moisture content and water activity (Casciatori *et al*., 2016). This underscores the necessity for solid-state fermenter designs that prioritize efficient heat transfer to prevent excessive temperatures or high air demand.

Gompertz Curve Analysis and Gas Distribution Dynamics in Fungal Growth

During fermentation, CO_2 evolution profiles for both fungal species are presented in Fig. (5a), while the fitting of the Gompertz model to these data is illustrated in Fig. (5b), revealing insights into fungal growth dynamics with wheat bran. The analysis highlights the direct impact of air distribution on metabolic activity and CO_2 evolution, notably showing that supplying air onto the substrate surface (Exp 2) significantly enhances CO_2 evolution compared to Exp 1. In Exp 2[AA] and Exp 2[AO], the higher CO_2 evolution is likely due to increased O_2 concentration at the substrate surface, facilitated by a lower airflow rate of 1 L/min, ensuring adequate O_2 supply for fungal growth. On the other hand, in Exp 1, O_2 concentration may vary across trays, possibly due to pressure build-up from the higher airflow rate of 8 L/min. The kinetic constants obtained from the model, effectively describing the CO_2 evolution data with high R^2 coefficients >0.997, are presented in Table 2. Sparging air onto the surface of the substrate in Exp 2 significantly increased [CO_{2max}] more than 2-fold compared to Exp 1. This outcome was anticipated given the uniform distribution of eight trays with identical amounts of solid substrate in this system.

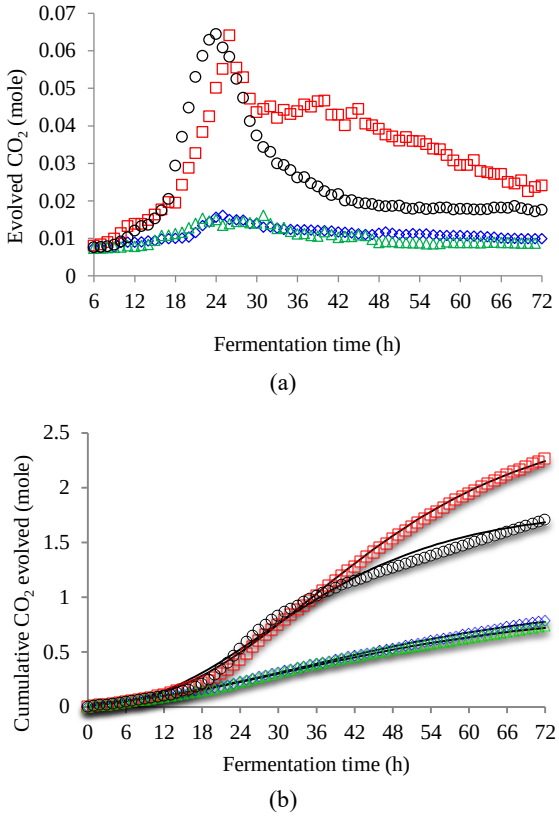


Fig. 5: The changes in cumulative CO_2 evolution over time were observed under different air flow rates and air arrangements during the growth of *A. awamori* (AO) and *A. Oryzae* (AO) on wheat bran in a tray solid-state fermenter. Experimental data are depicted using symbols. (◇) Exp1[AA]; (□) Exp 2[AA]; (△) Exp 1[AO] and (○) Exp 2[AO]. The Gompertz model is shown as a solid line. Exp 1: 8 L/min; Exp 2: 1 L/min

The *b* values for *A. awamori* and *A. oryzae* in Exp 1 were 3.59 and 3.48, respectively, while in Exp 2, they were 5.31 and 5.10, respectively. This suggests a rapid growth phase for both fungi in Exp 1, consistent with smaller *b* values leading to accelerated growth, as presented in Fig. (5a). Larger *b* values lead to a slower initial growth phase and a faster approach to the asymptote in the sigmoidal curve. The *k* values in Exp. 1 for *A. awamori* and *A. oryzae* were 0.038 and 0.039 1/h, respectively. On top of that, the *k* values in Exp. 2 for *A. awamori* and *A. oryzae* were 0.048 and 0.059 1/h, respectively. The value of *k* represents the time taken for [CO_2] to reach [CO_{2max}], with higher values indicating faster achievement. Fig. (5a), it is evident that Exp 2, characterized by higher *b* values, achieves [CO_{2max}], in a shorter time, approximately 24 h. Fig. 6 clearly showed that there was no obvious relationship between this parameter and [CO_{2max}] in all trials. Additionally, the parameter t_{max} aligns with the experimentally observed values for the maximum evolution of CO_2.

Table 2: Kinetic constants variation with different air flow rates using the Gompertz model

Experiment Flow rate – l/min)	*A. awamori*					*A. oryzae*				
	CO_{2max} (mole)	k (h^{-1})	b	t_{max} (h)	R^2 coefficient	CO_{2max} (mole)	k (1/h)	b	t_{max} (h)	R^2 coefficient
Exp 1 (8 L/min)	0.98	0.038	3.59	33.6	0.999	0.89	0.039	3.48	31.9	0.999
Exp 2 (1 L/min)	2.65	0.048	5.31	34.8	0.999	1.81	0.059	5.10	27.6	0.997

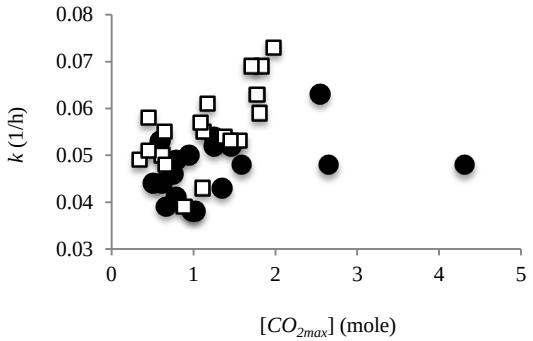


Fig. 6: The experimental correlation between [CO_{2max}] and the evolution rate (k) was examined using data from all experiments conducted with *A. awamori* (●) and *A. oryzae* (◘) on wheat bran

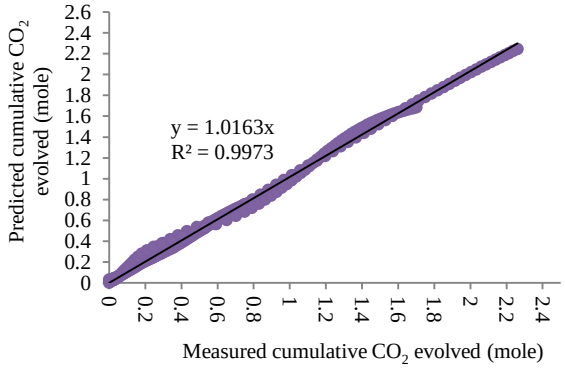


Fig. 7: Relationship between observed and forecasted data of *A. awamori* and *A. oryzae* across all trials conducted employing wheat bran in a tray solid-state fermenter

The Gompertz model was used to analyze the cumulative CO_2 evolution by *A. awamori* and *A. oryzae*, showing a strong agreement between experimental and predicted data (R^2>0.99) (Fig. 7). Fitting the growth models to raw data was significant, allowing for the interpretation of CO_2 evolution during solid-state fermentation, demonstrating an observable increase over time, as presented in the sigmoidal curve (Fig. 5a-b). The impact of two different arrangements of moistened air on the sigmoidal curve patterns was also observed. Across all experiments examining CO_2 evolution, parameters indicated that the total [CO_{2max}] depended on fungal species and air arrangement. The Gompertz model effectively described fungal growth in solid-state fermentation based on CO_2 evolution, enabling accurate prediction of the impact of moistened air arrangement. The study suggests the Gompertz model is suitable for depicting growth curves of both fungal strains, showing typical patterns with four distinct phases: A lag phase, an acceleration phase, a log phase and a deceleration phase, with no clear stationary phase or accelerated death phase observed.

The Gompertz model shows promise in explaining diverse outcomes across different culture conditions, having been successfully applied in various studies: Anaerobic treatment of hazardous steel-mill waste (Ma *et al*., 2014), biomass growth analysis of *Streptomyces venezuelae* under ultrasonication influence (Naveena *et al*., 2012), microbial inactivation on food surfaces, anaerobic treatment of apple waste with swine manure (Kafle and Kim, 2013) and growth of *Pseudomonas* in raw pork under pallet packaging (Li *et al*., 2013). However, Augustine *et al*. (2015) noted limitations such as lack of symmetry restriction and shorter periods of fast growth in filamentous fungi studies. Employing the Gompertz model to depict growth dynamics in solid-state fermentation, as shown by Mitchell *et al*. (2004); Christen *et al*. (1997), proves valuable for monitoring product formation kinetics. Similarly, Soares *et al*. (2000) observed the growth of *Ceratocystis fimbriata* using the Gompertz model, while Erkmen (2008) employed the modified Gompertz model to characterize the growth of various microorganisms during the ripening and storage process of Turkish dry-fermented sausage. Researchers like Zhu *et al*. (2014) have used the Gompertz model to track fermentation progress for lipopeptide production by *Bacillus amyloliquefaciens* in solid-state fermentation, while Braissant *et al*. (2013) have suggested its potential use with microcalorimetric data. Augustine *et al*. (2015) also found the Gompertz model applicable in liquid media for bacterial and yeast growth, showcasing its versatility alongside other log models that share similar properties for representing growth dynamics.

Metabolic Assessment Throughout Fungal Growth

Assessing OUR and CER for Characterizing Fungal Growth

Examining parameters like OUR and CER offers quick and direct measures of microbial metabolism, aiding in estimating biomass growth in solid-state fermentation, as demonstrated by Fig. 8 for both *A. awamori* and *A. oryzae*. The OUR and CER curves depict

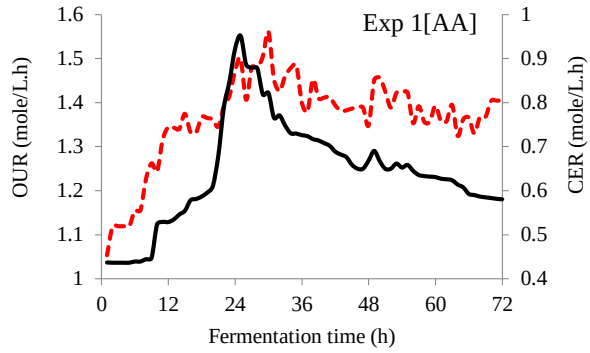
metabolic activity during fermentation, showing a relatively brief stationary growth phase lasting 1-2 h, with peak values of O_2 consumption, CO_2 and heat evolution reaching around 24-30 h of fungal fermentation. Subsequently, a gradual decline in heat, O_2 and CO_2 concentrations indicates slower fungal growth, with active spores continuing to consume O_2 and nutrients while producing CO_2 and heat at a reduced rate. In solid-state fermentation of *Rhizopus oligosporus* with rice bran, a similar pattern was noted: An initial rise in OUR and heat evolution around 24 h followed by a notable decline at 72 h (Ikasari and Mitchell, 1998). The relatively high OUR and CER observed in this study, particularly in the initial 24-30 h, may be due to the ample starch in wheat bran, fuelling fungal growth. However, fungal growth in solid-state fermentation is not continuous, as nutrient depletion and waste accumulation eventually slow growth, explaining the observed deceleration after peak activity.

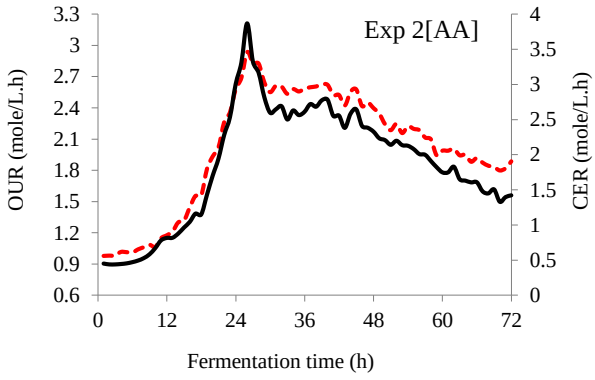
Describing Fungal Growth Through Heat Evolution Patterns

Temperature regulation is a critical consideration in the build-up of solid-state fermenters as they often encounter challenges related to the significant metabolic heat produced during fungal growth. Solid-state fermentation processes can lead to elevated temperatures that inhibit optimal growth conditions. This issue becomes more pronounced as the size of solid-state fermenters scales up, as conventional heat transfer mechanisms may become insufficient. To counteract this, forced air circulation is commonly employed to improve moisture-driven cooling of the fermenting base material and the complete environment. This approach underscores the importance of moisture management, making the introduction of forced aeration with saturated, moistened air essential for effectively addressing these thermal challenges.

Figure 9 illustrates the effect of two different arrangements of moistened air in a tray solid-state fermenter. Both experimental setups (Exp 1 and Exp 2) showed distinct temperature responses during the 72 h fermentation period, peaking around 27 h at temperatures of 37.03°C for Exp 2[AA] and 36.55°C for Exp 2[AO]. A consistent temperature increase was observed after 18 h across all experiments, peaking between 27 and 30 h before gradually decreasing. Exp 2, provided more favorable for fungal growth, maintaining temperatures below 40°C for both fungi, while Exp 1, facilitated heat dissipation primarily from bottom to top due to increased airflow. Exp 2 showed better growth due to optimal O_2 concentration. Unlike Exp 1, where the air was blown forcefully through the bed, Exp 2 gently circulated air above the bed, aiding in uniform mass and heat transfer. Perforations in the tray's base facilitated air flow, enhancing aeration and O_2 accumulation. The fermenter design, with a gap between trays, effectively sealed the environment while improving aeration and O_2 distribution.

In the current multi-layer tray fermenter system, maintaining optimal bed temperatures for fungal growth proved challenging, resulting in temperature variations during fermentation. However, it is noteworthy that substrate dehydration remained minimal across these studies. In this study, *A. awamori* might have encountered greater water loss compared to *A. oryzae*, highlighting the fungus's adaptability to fermentation conditions. Nevertheless, *A. awamori* consistently demonstrated robust growth across all experiments. Concurrently, the accumulation of inhibitory metabolites due to waste build-up within the system is a concern. Elevated temperatures often reach levels that significantly impede growth or even lead to microbial death (Nascimento *et al*., 2021; Mitchell *et al*., 2006; 2004; Ikasari and Mitchell, 1998). High temperatures can detrimentally affect fungal growth, as presented in the temperature profiles in Fig. 9, where temperatures exceeding 30°C were observed in all experiments.





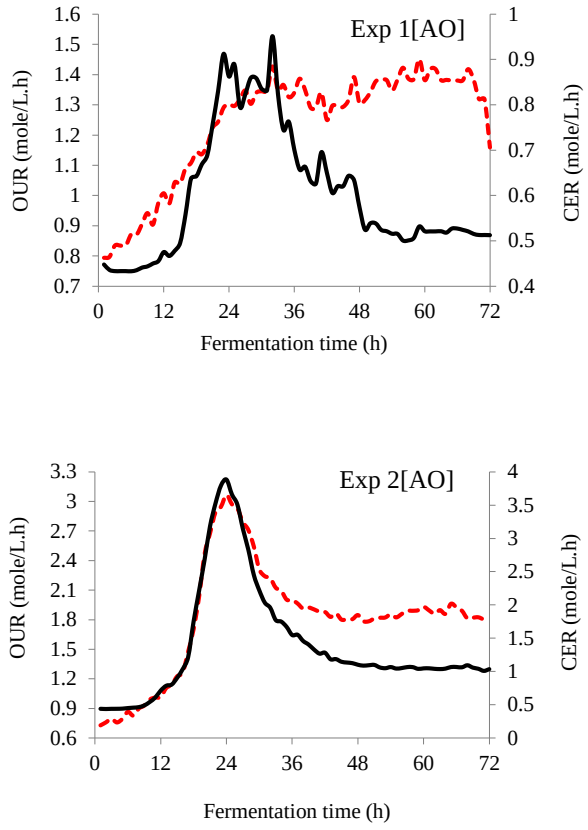


Fig. 8: Indirect assessment of the fungal growth rate of *A. awamori* [AA] and *A. oryzae* [AO] across four experiments concerning OUR (- - - -) and CER (———) under the influence of moistened air at flow rates of 8 L/min (Exp 1) and 1 L/min (Exp 2)

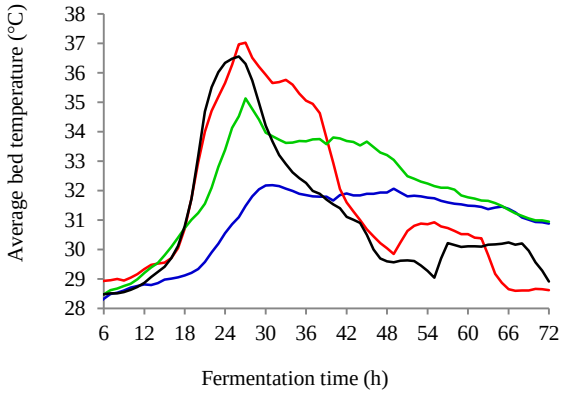


Fig. 9: The effect of distributing moistened air on heat evolution during the growth of *A. awamori* [AA] and *A. oryzae* [AO] on wheat bran conducted in a tray solid-state fermenter. (——) Exp1[AA]; (——) Exp 2[AA]; (——) Exp 1[AO] (———) Exp 2[AO]. Exp 1: 8 L/min; Exp 2: 1 L/min

The use of moistened airflow served as a cooling mechanism, ensuring sufficient moisture levels for fungal growth and metabolic processes within the fermentation substrate. Notably, the rate of proliferation appeared to be primarily influenced by the prevailing temperature, as indicated by the experimental findings. The temperature fluctuations observed during solid-state fermentation in tray and insulated packed-bed fermenters have been shown to impact the utilization of soybean oil and protease secretion by *Yarrowia lipolytica* (Nascimento *et al*., 2021). Another effective strategy involves intermittently trickling water into the bed fermenter, which aids in temperature control, prevents substrate drying and compaction and fosters enhanced mycelium growth and phytase production by *Aspergillus ficuum* (Shahryani *et al*., 2019). Vauris *et al*. (2022) have emphasized the significance of physicochemical substrate characterization prior to commencing solid-state fermentation, particularly in relation to heat transfer dynamics under forced aeration. They introduced a method involving the addition of calcium oxide to a moistened substrate to enhance reproducibility, enabling the investigation of heat dissipation independent of microbial heat generation influenced by strain development. Furthermore, temperature correlates with CO_2 evolution, reflecting the complete growth curve shows four distinct phases of growth. Yet, it should be noted that this profile does not fully capture the death phase. Finkler *et al*. (2021b) found a growth slowdown after the exponential phase, consistent with our observations, suggesting heat and mass transfer's role in non-growing conditions. Sentis-More *et al*., (2023) highlighted the essential role of temperature control in tray fermenters to optimize *Rhizopus oryzae* growth on oilseed meals during solid-state fermentation, recognizing significant microbial growth variations caused by temperature fluctuations, thereby impacting protein quality.

Respiratory Quotient and Its Implications for Fungal Growth

This study conducts into off-gas analysis within the context of a tray solid-state fermenter featuring two different air arrangements. Examining O_2 consumption rates and CO_2 evolution rates and calculating the RQ provides insights into fermentation dynamics. RQ profiles, shown in Fig. (10a), were derived for both fungal species in the air arrangements, with all RQ values 1 (Fig. 10b). In Exp 1, RQ values were 0.533 for *A. awamori* and 0.578 for *A. oryzae*, while in Exp 2, they rose to 0.947 and 0.868, respectively. Both fungal species exhibited RQ values approaching 1, suggesting advantageous fungal growth facilitated by adequate O_2 concentration from bottom tray perforations and moistened air introduction at 1 L/min (Exp 2) onto the fermented surface.

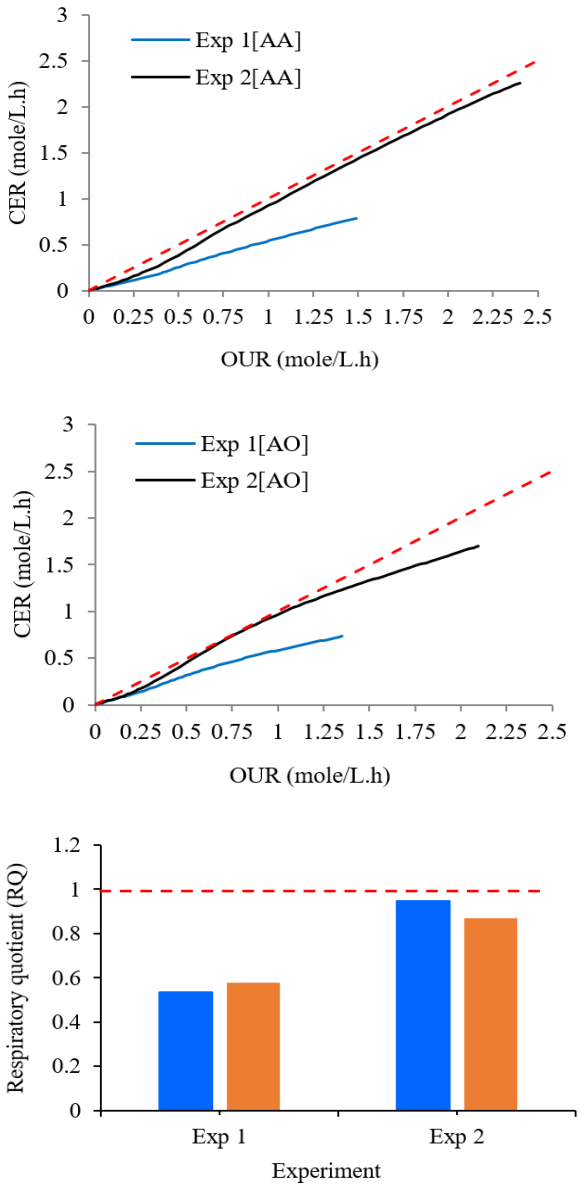


Fig. 10: Profiles of [a] RQ and [b] RQ in tray solid-state fermenter system with the fungi *A. awamori* [AA - ■] and *A. oryzae* [AO - ■] under different arrangements of moistened air. The red straight line (- - - - - - -) indicates RQ = 1. Exp 1: 8 L/min; Exp 2: 1 L/min

In solid-state fermentation, fungal mycelium growth is often hindered by limited O_2 diffusion within the solid substrate, particularly in its inner regions, due to the compact structure of solid particles (Finkler *et al*., 2021a). This limitation is comparable to restrict growth observed in rice koji with high moisture content in *A. niger* cultivation, where heat and O_2 transfer constraints prevent growth (Okazaki *et al*., 1980; Rajagopalan and Modak, 1995). RQ values near 1 signify peak O_2 uptake, while values surpassing 1 indicate metabolite utilization (Govind *et al*., 1997). Changes in RQ relate to metabolite generation during solid-state fermentation (Becerra and Gonzalez-Siso, 1996; Barrios-Gonzalez *et al*., 1993). Patterns of RQ offer insights into fermentation kinetics, categorized into associated, partially associated and non-associated growth (Rodriguez-Leon *et al*., 2018), crucial for process understanding (Torres-Mancera *et al*., 2018). Changes in the RQ value indicate the metabolic state of the fungus, suggesting adaptation to aeration strategies by effectively utilizing available nutrients like water and O_2 for complete oxidation. Continuous O_2 supply ensures uniform distribution within the substrate, highlighting RQ's value in assessing metabolic conditions in solid-state fermentation. The correlation between RQ and CO_2 evolution underscores its importance in fermentation optimization.

The metabolic activity, indicated by RQ values, results from microbial respiration involving O_2 consumption and CO_2 evolution. Incorporating a gas analysis system at the outlet would benefit basic laboratory solid-state fermentation setups. Mann *et al*. (2021) introduced a method for calculating the CO_2 ratio, akin to RQ, aiding in forecasting acetate or ethanol production in quasi-continuously ventilated shake flasks using *Clostridium ljungdahlii*. To derive accurate RQ values, solid substrate type, microorganism, fermenter system and metabolic pathway must be considered. Mendez-Gonzalez *et al*. (2020) demonstrated improved conidia production and productivity with forced aeration in packed bed columns compared to the tray and plastic bag fermenters, enabling online CO_2 measurement for optimal harvesting timing in *Metarhizium Roberts* solid-state fermentation.

The Relationship Between Heat Evolution and CER Throughout Fungal Growth

During the 72 h fermentation period, a strong correlation was observed between temperature increase and the concentration of evolved CO_2, indicating a direct link between carbon utilization and metabolic heat generation. This suggests that microbial respiration, involving O_2 consumption and CO_2 production, is highly exothermic and influenced by the level of metabolic activity. As a result, an assessment of CER data and temperature changes during solid-state fermentation for both fungi was undertaken. Across all experiments, CO_2 evolution commenced within the initial 6-24 h of fermentation, as presented in Fig. 11, showing temporal patterns of temperature fluctuations and CER for *A. awamori* and *A. oryzae*. Notably, the data revealed a synchronized behavior between temperature elevation and the CER profile: As CO_2 concentration increased, temperature rose accordingly and vice versa. This consistent correlation underscores the interplay between CER (and potentially OUR) and temperature, suggesting a feedback loop mechanism.

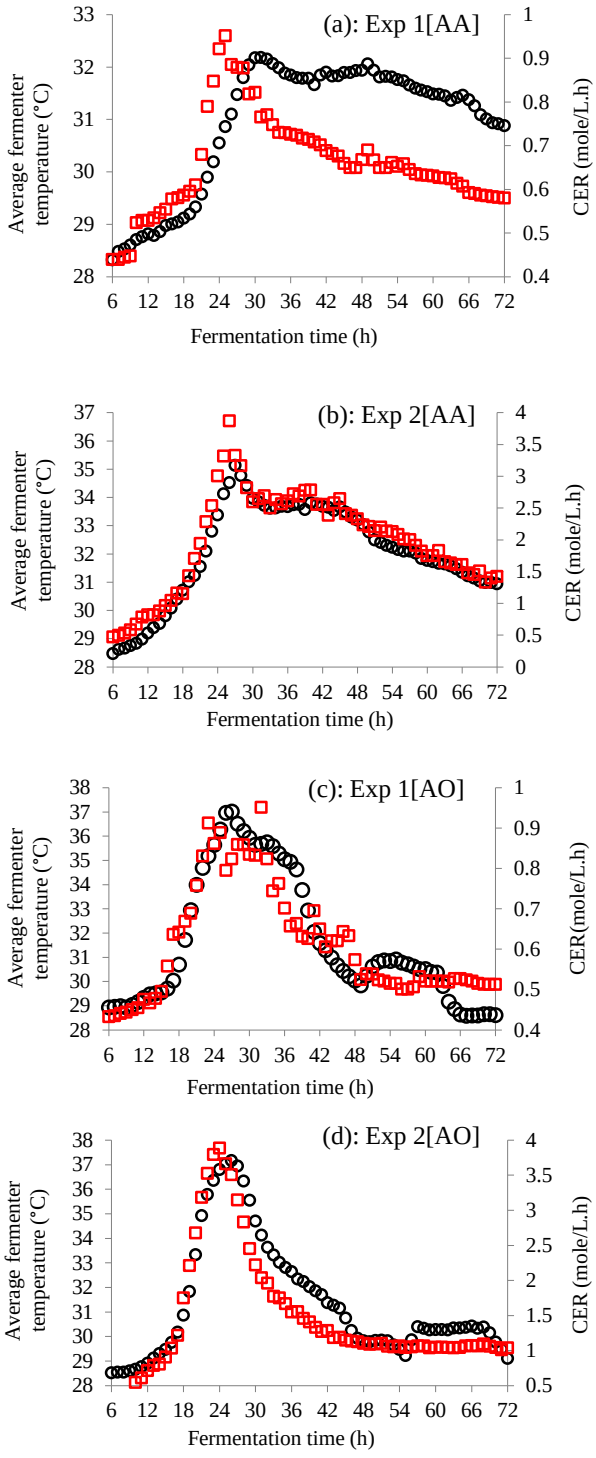


Fig. 11: Temperature and CER profiles of *A. awamori* (AA) and *A. oryzae* (AO) cultures from two distinct experiments conducted in a tray solid-state fermenter. [a] and [b]: Exp 1 with moistened air at a flow rate of 8 L/min and 1 L/min, respectively; [c] and [d]: Exp 2 with moistened air at a flow rate of 8 L/min and 1 L/min, respectively. (□): temperature profile and (◯): CER

Heat and CO_2 production indicate microbial metabolic activity, reflecting O_2 consumption, CO_2 evolution and carbon utilization. This study found a direct correlation between temperature increase during solid-state fermentation and CO_2 evolution, demonstrating a linear relationship across fungal growth rates, as presented in Fig. 12. The observed slope suggests a robust connection between temperature variation and CO_2 evolution, although comparisons between fermentation processes may not be direct. The data presented in Fig. 12 indicate a potential linear relationship between temperature elevation and CER, contingent upon the fungal species. This correspondence underscores the metabolic dynamics of both fungi throughout the solid-state fermentation. The concurrent increase in CO_2 levels and temperature suggests a symbiotic relationship, where CO_2 serves as a thermal insulator, trapping heat generated from the surface of the fermenting substrate (Hendry *et al*., 1993). This phenomenon effectively insulates the surface, leading to a subsequent rise in temperature. Moreover, the increment in temperature during microbial activity offers valuable insights into various growth parameters, including the growth curve and peak growth capacity (Abdul Manan and Webb, 2020; 2019; Braissant *et al*., 2013).

Influence of Air Arrangement on Fungal Spores Production

The fungal growth in the newly designed solid-state fermenter system was significantly influenced by the air arrangement strategy using moistened air at two different airflow rates, as presented in Fig. 13. After 72 h of fermentation, *A. awamori* in Exp 1 produced an average of 1.74×10^{12} spores/g of initial dry matter [IDM], while Exp 2 showed a notably higher average spore production of 5.5×10^{12} spores/g [IDM] (Fig. 13a). Similarly, for *A. oryzae*, the fermenter system in Exp 1 resulted in an average spore production of 1.05×10^{10} spores/g [IDM], whereas Exp 2 demonstrated a significantly increased average spore production of 3.07×10^{10} spores/g [IDM] after the same 72 h fermentation period (Fig. 13b).

The trends in spore concentration were similar across the solid-state fermenter system for both fungi under investigation, albeit with some notable differences. In the case of *A. awamori*, the solid-state fermenter system supplied with moistened air at a flow rate of 1 L/min exhibited superior spore production compared to the system with a flow rate of 8 L/min. Conversely, *A. oryzae* displayed relatively poor spore production in the fermenter system compared to *A. awamori*. However, when considering *A. oryzae* individually, it appeared to perform better with humidified air at a flow rate of 1

L/min, followed by 8 L/min. Overall, the results suggest that Exp 2 (1 L/min) was optimal for spore production in both fungal cultures. Mendez-Gonzalez *et al*. (2020) demonstrated that forced aeration during solid-state fermentation in solid-state fermenters led to increased spore generation efficiency. Sella *et al*. (2009) investigated the influence of aeration and moisture levels on *Bacillus atrophaeus* spore production in solid-state fermentation. Their findings revealed that while aeration rate had no significant effect on spore yield, moisture levels showed a significant impact, depending on the fermenter used. However, De-la-Cruz-Quiroz *et al*. (2019) discovered that applying water tension during solid-state fermentation reduced spore production but did enhanced spore viability in *Trichoderma harzianum*. Environmental factors, including nutrient compositions, moisture levels, water activity and O_2 levels, play a significant role in microbial growth and product formation (Sella *et al*., 2009).

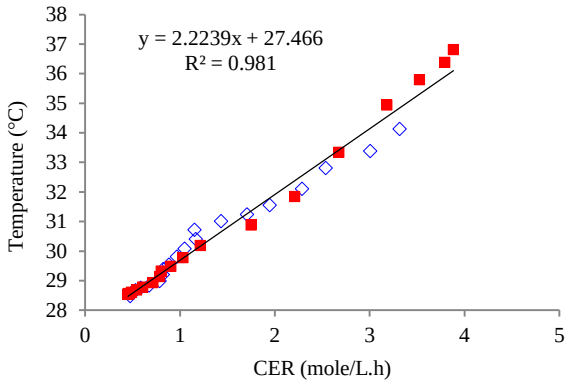
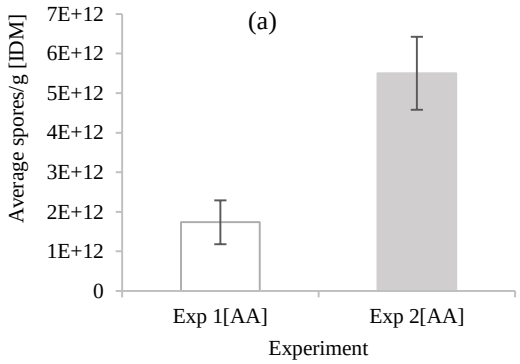


Fig. 12: The analysis examined temperature and CER profiles of fungal cultures in four experiments utilizing tray solid-state fermenter systems. Exp 1 employed moistened air at a flow rate of 8 L/min, while Exp 2 utilized moistened air at a flow rate of 1 L/min. (◇): *A. awamori* and (■): *A. oryzae*



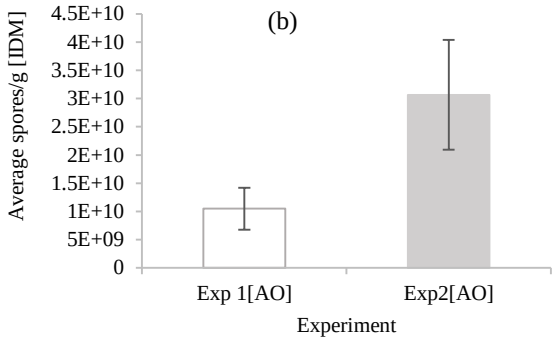


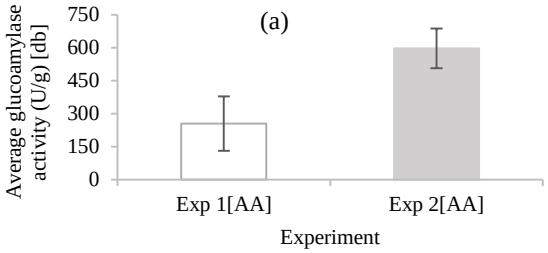
Fig. 13: Summary of fermentation outcomes presenting the average spore production in a tray solid-state fermenter employing two distinct air arrangements. [AA]: Refers to *A. awamori* and [AO]: To *A. oryzae*. The data depicted represent the mean ± standard deviation across eight trays. Exp 1: 8 L/min; Exp 2: 1 L/min. [IDM]: initial dry matter

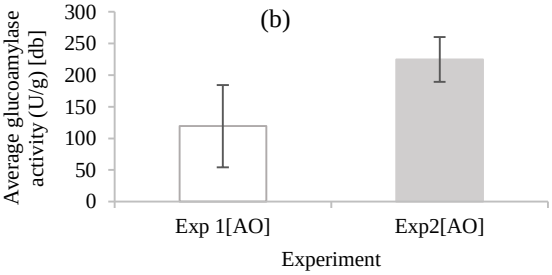
Influence of Air Arrangement on Enzyme Production

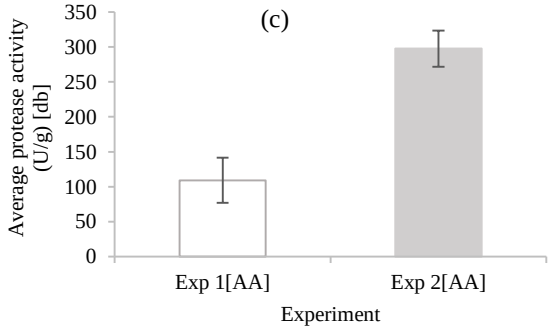
The figures presented in Fig. 14 illustrate the average levels of enzyme production resulting from the two different airflow strategies. Specifically, with *A. awamori*, glucoamylase activity levels reached 255.55 U/g dry basis [db] after 72 h of fermentation at an airflow rate of 8 L/min (Exp 1), whereas a notably higher activity level of 597.40 U/g [db] was achieved at an airflow rate of 1 L/min (Exp 2) (Fig. 14a). This disparity suggests that glucoamylase activity was more pronounced when solid-state fermentation was conducted with a lower airflow rate (Exp 2). The enhanced air circulation and nutrient diffusion facilitated by the perforated base lead to increased O_2 availability and improved mass transfer within the substrate. At a low airflow rate, the concentration of O_2 remained high due to the absence of forced aeration, ensuring a continuous supply of O_2. However, a nearly two-fold decrease in glucoamylase activity was observed at higher airflow rates (Exp 1). Additionally, glucoamylase activity levels of 119.30 and 224.74 U/g [db] were recorded after 72 h of fermentation with *A. oryzae* under airflow rates of 8 L/min (Exp 1) and 1 L/min (Exp 2), respectively (Fig. 14b). This stands in contrast to the findings with *A. awamori* under both airflow rates. Notably, glucoamylase activity decreased nearly two-fold in the case of *A. oryzae* within the same fermenter system. *A. awamori* exhibited higher glucoamylase activity on wheat bran due to its high starch content (23.3%) (Abdul Manan and Webb, 2016b), resulting in double the glucoamylase production compared to *A. oryzae*.

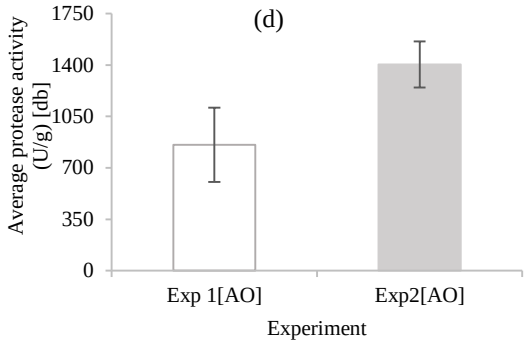
A. awamori and *A. oryzae* showed different protease activity levels, with *A. awamori* recording 109.21 U/g [db] and 297.40 U/g [db] at airflow rates of 8 L/min and 1 L/min, respectively, while *A. oryzae* exhibited 857.77

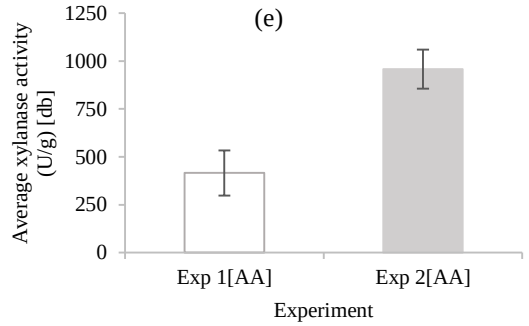
U/g [db] and 1,403.30 U/g [db] for Exp 1 and Exp 2, respectively. These findings suggest *A. oryzae's* potential for protease production, particularly in wheat bran substrates with 15.1% protein content (Abdul Manan and Webb, 2016b), which was further enhanced by improved mass transfer, increased O_2 concentration and optimal moisture content in the newly designed fermenter system. In both Exp 1 and Exp 2, *A. awamori* demonstrated significantly higher xylanase activity levels compared to *A. oryzae*, with averages of 415.89 and 958.48 U/g [db], respectively (Fig. 14e). On the other hand, *A. oryzae* exhibited lower xylanase activity levels, averaging of 84.46 and 184.07 U/g [db] for Exp 1 and Exp 2, respectively (Fig. 14f). These results underscored notable difference in enzymatic activities between *A. awamori* and *A. oryzae* in the same fermenter system. Additionally, *A. awamori* showcased superior cellulase production of 84.46 and 184.07 FPU/g [db] Exp 1 and Exp 2, respectively (Fig. 14g). In contrast, *A. oryzae* exhibited lower cellulase activity levels, recording 52.52 and 74.83 FPU/g [db] for Exp 1 and Exp 2, respectively (Fig. 14h)

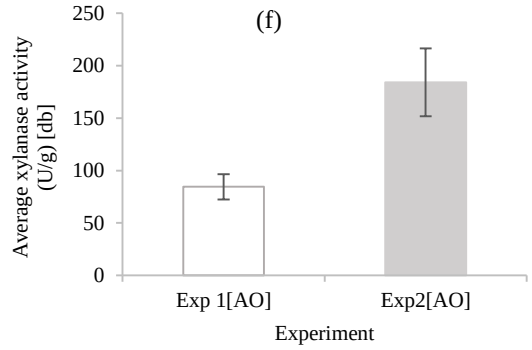


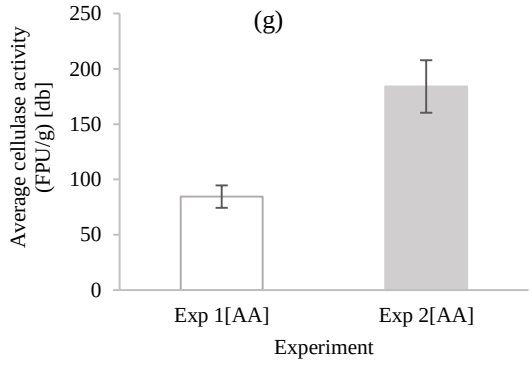












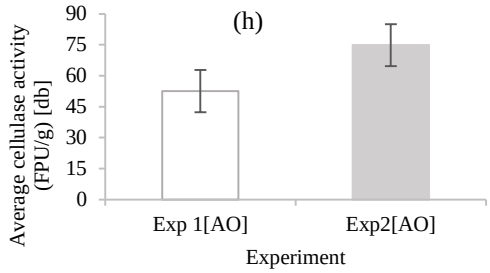


Fig. 14: Summary of fermentation results illustrating the average production of enzymes in a tray solid-state fermenter with two distinct air arrangements. [AA] represents *A. awamori* and [AO] denotes *A. oryzae*. The presented data represent the mean ± standard deviation across eight trays. Exp 1: 8 L/min; Exp 2: 1 L/min. [db]: dry basis

A. *awamori* exhibits strong enzyme production, particularly glucoamylase, xylanase and cellulase, but lacks in protease production, unlike *A. oryzae*, which excels in protease production due to its preference for protein-rich substrates. The use of moistened air at 1 L/min (Exp 2) enhanced enzyme production for both strains by ensuring uniform conditions, adequate O_2 supply, optimal moisture and preventing desiccation, whereas a higher airflow rate of 8 L/min (Exp 1) led to O_2 depletion and moisture loss, especially impacting *A. awamori*. The findings suggest that employing an effective strategy for air supply in solid-state fermentation fermenter systems offers significant advantages in enhancing fermentation performance and fungal activity. By carefully controlling factors such as O_2 concentration, bed temperature and moisture content through the manipulation of moistened air flow rates, optimal conditions can be achieved (Abdul Manan and Webb, 2020; 2019). Dallastra *et al.* (2023) emphasized in their work that careful consideration should be given to factors like heat distribution and airflow within the trays in tray fermenters to maintain consistent environmental conditions and effectively address issues related to overheating and gaseous exchanges.

The findings of this study present a contrast to previous research conducted by Sosa-Martinez *et al.* (2024). Their work on solid-state fermentation in packed-bed column fermenters with enhanced air circulation indicated elevated activities of a diverse array of hydrolases, including xylanases, cellulases and pectinases, produced by *Aspergillus* sp. It should be noted that the productivity of both fungi in enzyme production for the conducted study is influenced by the water content in the fermented wheat bran during the fermentation process. Numerous studies highlighted the correlation between enzyme production and water content. Insufficient water activity reduces gas and solute diffusions, impacting cell metabolism by nutrient availability or accumulating toxic compounds while also dissolving enzymes critical for cellular metabolic processes (Casciatori *et al*., 2016). Yet, to conduct a thorough assessment and meaningful comparisons, it is essential to explore other solid substrates, diverse microorganisms or fungi and various types of fermenters.

Discussion

This study tested two moistened aeration methods in a tray solid-state fermenter using wheat bran to grow *A. awamori* and *A. oryzae*. It included online gas analysis and temperature recording to measure O_2, CO_2 and heat continuously under sterile conditions. Monitoring fungal growth through O_2 consumption, CO_2 evolution and heat generation provided valuable insights into their metabolic activity and indirectly estimated biomass in solid-state fermentation. Implementing moistened air at the inlet with airflow rates of 8 and 1 L/min effectively controlled the final moisture content and maintained optimal temperatures in the fermenter. These strategies, along with the multi-layer tray design, ensured efficient air distribution, although they had minimal impact on the final moisture content and temperature profiles of both fungi, suggesting improved gas distribution within the fermenter. The Gompertz model efficiently predicts evolved CO_2 from solid-state fermentation of *A. awamori* and *A. oryzae* on wheat bran, aiding in management. Measurement of OUR and CER provides valuable insights into fungal growth dynamics by determining the RQ, acknowledged as the most accurate method for evaluating fungal growth in solid-state fermenters. Consistently obtained RQ values below 1 across all experiments suggest the predominance of oxidative metabolism.

The RQ serves as a key indicator of the metabolic activity within the fermenter, offering valuable insights into the fungus's metabolic activity. Precise measurements of CO_2, O_2 and airflow rate facilitate the immediate and accurate determination of key parameters associated with culture respiration, including the specific CO_2 production rate and the RQ (Torres-Mancera *et al*., 2018). During glucose oxidation with simple sugars, RQ will be equal to 1 (Rodriguez-Leon *et al*., 2018; Pandey *et al*., 2001). Generally, the RQ for carbohydrate metabolism can be demonstrated by the chemical equation for the oxidation of glucose, as in Eq. 9.

$$Sugars\ (C_6H_{12}O_6) + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (9)$$

As per Eq. 9, the theoretical RQ for the oxidation of glucose in aerobic microorganisms equals 1. This value signifies an equilibrium in gas exchange during this

metabolic reaction (Rodriguez-Leon *et al*., 2018; Pandey *et al*., 2001). The equation indicates that during glucose fermentation via aerobic respiration, ideally, six molecules of O_2 are consumed per molecule of glucose, while six molecules of CO_2 are produced. These considerations are relevant to simple sugars, as well as related carbohydrates and polysaccharides (Rodriguez-Leon *et al*., 2018).

Acknowledging the diverse compositions of carbon and nitrogen in solid substrates used for solid-state fermentation is crucial. Certain substrates comprise intricate compounds like hemicellulose, cellulose and lignin. In these cases, microorganisms must initially break down these complex compounds into simpler fermentable sugars for utilization. Effectively, achieving an RQ of 1 in the fermentation process is often unattainable due to the complexities of substrate composition and microbial metabolism. An RQ value less than 1 indicates that the fungus may have produced additional metabolites beyond CO_2, including biomass, sugars, water, energy and secondary metabolites (Eq. 10), while consuming O_2. Additionally, O_2 serves a dual role in both respiration and maintaining cell structure. An RQ value exceeding 1 signifies reduced O_2 uptake and the onset of glucose fermentation. In application, RQ values exceeding one indicate anaerobic fermentation. In highly aerated fermentation scenarios, a significant increase in CO_2 production compared to O_2 typically signals inefficiencies in aeration systems and the existence of anaerobic regions within the fermenter, necessitating immediate resolution (Rodriguez-Leon *et al*., 2018; Torres-Mancera *et al*., 2018; Pandey *et al*., 2001):

$$Organic\ material + nO_2 \rightarrow Biomass + sugars + nCO_2 + nH_2O + Energy \quad (10)$$

A crucial finding from this research emphasizes the importance of adapting air demand or quality during the solid-state fermentation process to sustain optimal fungal growth rates, rather than relying on fixed airflow rates or air quality standards, as traditionally practiced. Acknowledging the variability in airflow or air quality is crucial, as it impacts substrate moisture and consequently affects fungal growth. Failure to consider this aspect could lead to unintended drying of the system beyond acceptable levels. Additionally, the study highlights the significance of designing multi-layer squared tray solid-state fermenters to enhance heat transfer, thereby reducing airflow requirements factor that should not be overlooked.

Solid-state fermentation presents the vast potential to produce spores, microbial enzymes, secondary metabolites and various other products. However, optimizing productivity in fungi, including spore and enzyme consortium production, often requires strategic variations in aeration arrangements. The efficacy of fermentation processes hinges on the biological activity, which is driven by interactive enzyme actions. Microorganisms like bacteria, fungi and yeast are commonly utilized for industrial purposes due to their ability to produce diverse enzymes. Nonetheless, selecting the most suitable strain for achieving commercially competitive enzyme yields can be a challenging endeavor (Singh *et al*., 2008). The selection process is significantly impacted by variables like substrate characteristics and environmental factors, underscoring the crucial role of careful strain selection in attaining desired results (Singh *et al*., 2008).

Numerous techniques from chemical engineering for modeling and simulation are applicable to the realm of solid-state fermentation. Nevertheless, further endeavors are essential to achieve a comprehensive quantitative comprehension of microbial responses to environmental stimuli. Recent advancements in instrumentation for detecting and regulating environmental parameters have catalyzed progress in modeling efforts. It appears that nearly all known enzymes can potentially be generated through the solid-phase fermentation process, given the current state of knowledge and available production methods. With the expanding scale of microbial processes, advanced technologies in fermenter operation and design are increasingly vital. Integrating computers with fermenters enables real-time data collection, analysis and optimization, while multi-layer squared tray solid-state fermenter systems effectively maintain ideal conditions for fungal growth. Further research is needed to optimize fermenter parameters and understand mass transfer phenomena for improved efficiency.

Conclusion

The study utilized a distributed parameter model to simulate a newly designed tray solid-state fermenter, focusing on airflow rate effects. Control procedures significantly improved fermenter productivity by regulating water content, O_2 transfer, CO_2 levels and substrate temperature through air saturation and temperature adjustment. The addition of water when moisture drops below a threshold was proposed. CO_2/O_2 ratio, heat and moisture content were identified as critical factors for optimal bioconversion conditions, akin to a living cell's dynamics. Mathematical modeling proved valuable for exploring various operating conditions economically, given the fermenter's scale. While solid-state fermenters are suitable for diverse bioconversion conditions, ongoing advancements are expected for operational flexibility. Although conducted at a laboratory scale, the study's insights have potential applications in complementing existing literature and enhancing fermenter productivity through control strategies. Ongoing experimental validation of predictions aims to guide future designs and applications,

strengthening solid-state fermentation as a viable alternative to submerged techniques.

Acknowledgment

I extend my gratitude to the Malaysian Agricultural Research and Development Institute (MARDI) and the Government of Malaysia for affording me the opportunity to pursue further studies at the University of Manchester, United Kingdom.

Funding Information

This study was supported by the Malaysian Agricultural Research and Development Institute (MARDI) and the University of Manchester, United Kingdom, through a special Scholarship for Study Leave Training.

Author's Contributions

Musaalbakri Abdul Manan: Involved in designing and developing the method, conducting experiments in the laboratory, collecting and analyzing the data and preparing the paper.

Colin Webb: Conceptualized and designed the experiments, provided supervision throughout the process and conducted revisions on the paper.

Ethics

The authors accept responsibility for addressing any ethical concerns that may arise subsequent to the publication of this study.

References

Abdul Manan, M., & Webb, C. (2020) Newly designed multi-stacked circular tray solid-state bioreactor: analysis of a distributed parameter gas balance during solid-state fermentation with influence of variable initial moisture content arrangements. *Bioresources and Bioprocessing 7(16)*, 1-18. https://doi.org/10.1186/s40643-020-00307-9

Abdul Manan, M., & Webb, C. (2019). Control strategies with variables air arrangements, forcefully aerated in single circular tray solid state bioreactors with modified Gompertz model and analysis of a distributed parameter gas balance. *Biotechnology and Biotechnological Equipment, 32(6)*, 1456-1467. https://doi.org/10.1080/13102818.2018.1530950

Abdul Manan, M., & Webb, C. (2016a). Multi-enzymes production studies in single tray solid state fermentation with opened and closed system. *Journal of Life Science, 10*, 342-356. https://doi.org/10.17265/1934-7391/2016.07.005

Abdul Manan, M., & Webb, C. (2016b). Water retention value: A study model based by *Aspergillus awamori* and *Aspergillus oryzae* embrace three models of solid substrates. *Journal of Life Science*, *8*, 420-429. https://doi.org/10.17265/1934-7391/2016.08.008

Alam, M. Z., Mamun, A. A., Qudsieh, I. Y., Muyibi, S. A., Salleh, H. M., & Omar, N. M. (2009). Solid state bioconversion of oil palm empty fruit bunches for cellulase enzyme production using a rotary drum bioreactor. *Biochemical Engineering Journal*, *46*(1), 61-64. https://doi.org/10.1016/j.bej.2009.03.010

Arora, S., Rani, R., & Ghosh, S. (2018). Bioreactors in solid state fermentation technology: Design, applications and engineering aspects. *Journal of Biotechnology*, *269*, 16-34. https://doi.org/10.1016/j.jbiotec.2018.01.010

Ashok, A., Doriya, K., Rao, D. R. M., & Kumar, D. S. (2017). Design solid state bioreactor for industrial applications: An overview to conventional bioreactors. *Biocatalysis and Agricultural Biotechnology, 9*, 11-18. https://doi.org/10.1016/j.bcab.2016.10.014

Augustine, A., Joseph, I., Raj, P. R., & David, N. S. (2015). Growth kinetic profiles of *Aspergillus niger* S14 a mangrove isolate and *Aspergillus oryzae* NCIM 1212 in solid state fermentation. *Indian Journal of Fisheries*, *62*(3), 100-106. ISSN: 0970-6011.

Ano, T., Jin, G. Y., Mizumoto, S., Rahman, M. S., Okuno, K., & Shoda, M. (2009). Solid state fermentation of lipopeptide antibiotic iturin A by using a novel solid state fermentation reactor system. *Journal of Environmental Sciences*, *21*(Suppl 1), S162-S165. https://doi.org/10.1016/S1001-0742(09)60064-4

Bandelier, S., Renaud, R., & Durand, A. (1997). Production of gibberellic acid by fed-batch solid state fermentation in an aseptic pilot-scale. *Process Biochemistry*, *32*(2), 141-145. https://doi.org/10.1016/S0032-9592(96)00063-5

Barrios-Gonzalez, J., Gonzalez, H., & Mejia, A. (1993). Effect of particle size, packing density and agitation on Penicillin production in solid state fermentation. *Biotechnology Advances*, *7*, 539-547. https://doi.org/10.1016/0734-9750(93)90022-F

Becerra, M., & Gonzalez-Siso, M. I. (1996). Yeast β-galactosidase in solid state fermentations. *Enzyme and Microbial Technology*, *19*(1), 39-44. https://doi.org/10.1016/0141-0229(95)00180-8

Berovic, M., & Ostroversnik, H. (1997). Production of *Aspergillus niger* pectolytic enzymes by solid state bioprocessing of apple pomace. *Journal of Biotechnology*, *53*(1), 47-53. https://doi.org/10.1016/S0168-1656(96)01661-6

Braissant, O., Bonkat, G., Wirz, D., & Bachmann, A. (2013). Microbial growth and isothermal microcalorimetry: Growth and their application to microcalorimetric data. *Thermochimica Acta*, *555*, 64-71. https://doi.org/10.1016/j.tca.2012.12.005

Brijwani, K., Vadlani, P. V., Hohn, K. L., & Maier, D. E. (2011). Experimental and theoretical analysis of a novel deep-bed solid-sate bioreactor for cellulolytic enzymes production. *Biochemical Engineering Journal*, *58*-59, 110-123. https://doi.org/10.1016/j.bej.2011.09.004

Casciatori, F. P., Buck, A., Thomeo, J. C., & Tsotsas, E. (2016). Two-phase and two-dimensional model describing heat and water transfer during solid-state fermentation within a packed-bed bioreactor. *Chemical Engineering Journal*, *287*, 103-116. https://doi.org/10.1016/j.cej.2015.10.108

Christen, P., Meza, J. C., & Revah, S. (1997). Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata*: Influence of the substrate type and the presence of precursors. *Mycological Research*, *101*(8), 911-919. https://doi.org/10.1017/S0953756297003535

Costa, P. C., dos Reis, E. E., de Carvalho, L. S., Fontana, R. C., Schneider, W. D. H., & Camassola, M. (2021). Making the process of enzyme production in solid-state cultivation and more sustainable-reuse of raw materials and a syringe-type bioreactor enter in the scene. *ACS Sustainable Chemistry and Engineering*, *9*(42), 14134-14142. https://doi.org/10.1021/acssuschemeng.1c04257

Costa, J. A., Treichel, H., Kumar, V., & Pandey, A. (2018). Advances in solid-state fermentation. *In Current Developments in Biotechnology and Bioengineering* (pp. 1-17). *Elsevier*. https://doi.org/10.1016/B978-0-444-63990-5.00001-3

Dabaghi, S., Ataei, S., A., & Taheri, A. (2021). Performance of a laboratory scale rotating drum bioreactor for production of rhamnolipid in solid-state fermentation using an agro-industrial residue. *Biomass Conversion and Biorefinery*, *13,* 1153-11520. https://doi.org/10.1007/s13399-021-02113-5

Da Cunha, D. C., Souza, J. A., Rocha, L. A. O., & Costa, J. A. V. (2009). Hexahedral modular bioreactor for solid state bioprocesses. *World Journal of Microbiology and Biotechnology*, *25*, 2173-2178. https://doi.org/10.1007/s11274-009-0122-3

Dallastra, E. D. G., Dias, A. C. OP., de Morais, P. B., da Silva, J. F. M., Casciatori, F. P. & Grajales, L. M. (2023). Development of a novel pilot-scale tray bioreactor for solid-state fermentation aiming at process intensification. *Chemical Engineering and Processing-Process Intensification*, *193*, 109526. https://doi.org/10.1016/j.cep.2023.109526

Deive, F. J., & Sanromán, M. Á. (2017). Bioreactor development for the cultivation of extremophilic microorganisms. In Current Developments in *Biotechnology and Bioengineering* (403-432). Elsevier. https://doi.org/10.1016/B978-0-444-63663-8.00014-8

De-la-Cruz-Quiroz, R., Roussos, S., Tranier, M. T., Rodríguez-Herrera, R., Ramírez-Guzmán, N., & Aguilar, C. N. (2019). Fungal Spores Production by solid-state fermentation under hydric stress condition. *Rev. Cient. Univ. Auton. Coahuila, 11*(21), 2-6. https://www.researchgate.net/publication/334524139_Fungal_Spores_Production_by_solid-state_fermentation_under_hydric_stress_condition

El Zein, A., Seif, H., & Gooda, E. (2015). Moisture content and thermal balance during composting of fish, banana mulch and municipal solid wastes. *European Scientific Journal*, *11*(5), 169-187. https://eujournal.org/index.php/esj/article/view/5183

Erkmen, O. (2008). Modelling the effects of sucuk production technique on *Listeria monocytogenes*, aerobic bacteria and lactic acid bacteria during ripening and storage. *Food and Bioproducts Processing*, *86*(3), 220-226. https://doi.org/10.1016/j.fbp.2007.10.002

Finkler, A. T. J., de Lima Luz Jr, L. F., Krieger, N., Mitchell, D. A., & Jorge, L. M. (2021a). A model-based strategy for scaling-up traditional packed-bed bioreactors for solid-state fermentation based on measurement of O_2 uptake rates. *Biochemical Engineering Journal*, *166*, 107854. https://doi.org/10.1016/j.bej.2020.107854

Finkler, A. T. J., Weber, M. Z., Fuchs, G. A., Scholz, L. A., de Lima Luz Jr, L. F., Krieger, N., Mitchell, D. A., & Jorge, L. M. (2021b). Estimation of heat and mass transfer coefficients in a pilot packed-bed solid-state fermentation bioreactor. *Chemical Engineering Journal*, *408*, 127246. https://doi.org/10.1016/j.cej.2020.127246

Foong, C. W., Krishnaiah, K., Janaun, J., Subbarao, D., & Prabhakar, A. (2009). Heat and mass transfer studies of palm kernel cake (PKC) in fluidized bed fermenter. *Industrial Crops Products*, *30*, 227-234. https://doi.org/10.1016/j.indcrop.2009.03.012

Gervais, P. & Molin, P. (2003). The role of water in solid-state fermentation. *Biochemical Engineering Journal, 13*(2-3), 85-101. https://doi.org/10.1016/S1369-703X(02)00122-5

Govind, R., Gao, C., Lai, L., & Tabak, H. H. (1997). Continuous, automated and simultaneous measurement of oxygen uptake and carbon dioxide evolution in biological systems. *Water Environment Research*, *69*(1), 73-80. https://www.jstor.org/stable/25044844

Gutierrez-Rojas, M., Aboul Hosn, S. A., Auria, R., Revah, S., & Favela-Torres, E. (1996). Heat transfer in citric acid production by solid state fermentation. *Process Biochemistry*, *31*(4), 363-369. https://doi.org/10.1016/0032-9592(95)00071-2

He, Q., Peng, H., Sheng, M., Hu, S., Qiu, J., & Gu, J. (2019). Humidity control strategies for solid-state fermentation: Capillary water supply by water-retention materials and negative-pressure auto controlled irrigation. *Frontiers in Bioengineering and Biotechnology*, *7*(263). https://doi.org/10.3389/fbioe.2019.00263

Hendry, M. J., Lawrence, J. R., Zanyk, B. N., & Kirkland, R. (1993) Microbial production of CO_2 in unsaturated geologic media in a Mesoscale model. *Water Resources Research*, *29*(4), 973-984. https://doi.org/10.1029/92WR02847

Hector, A. R., Rodriguez-Jassoa, R. M., Rodriguez, R., Contreras-Esquivel, J. C., & Aguilar, C. N. (2012). Pectinase production from lemon peel pomace as support and carbon source in solid-state fermentation column-tray bioreactor. *Biochemical Engineering Journal*, *65*, 90-95. https://doi.org/10.1016/j.bej.2012.03.007

Ikasari, L., & Mitchell, D.A. (1998). Oxygen uptake rate kinetics during solid state fermentation with *Rhizopus oligosporus*. *Biotechnology Techniques*, *12*(2), 171-175. https://doi.org/10.1023/a:1008805004361

Jang, H. D., & Yang, S. S. (2008). Polyunsaturated fatty acids production with a solid-state column reactor. *Bioresources Technology*, *99*(14), 6181-6189. https://doi.org/10.1016/j.biortech.2007.12.024

Kafle, G. K., & Kim, S. H. (2013). Anaerobic treatment of apple waste with swine manure for biogass production: Batch and continuous operation. *Applied Energy*, *103*, 61-72. https://doi.org/10.1016/j.apenergy.2012.10.018

Li, M., Niu, H., Zhao, G., Tian, L., Huang, X., Zhang, J., Tian, W., & Zhang, Q. (2013). Analysis of mathematical models of *Pseudomonas* spp. growth in pallet-package pork stored at different temperatures. *Meat Science*, *93*(4), 855-864. https://doi.org/10.1016/j.meatsci.2012.11.048

Ma, H., Li, Z., Yin, F., Kao, W., Yin, Y., & Bai, X. (2014). Study on aerobic treatment of hazardous Steel-mill Waste Rolling Oil (SmWRO) for multi-benefit disposal route. *Bioresources Technology, 151,* 106-112. https://doi.org/10.1016/j.biortech.2013.10.051

Mahmoodi, M., Najafpour, G. D., & Mohammadi, M. (2019). Bioconversion of agroindustrial wastes to pectinases enzyme via solid state fermentation in trays and rotating drum bioreactors. *Biocatalysis and Agricultural Biotechnology*, *21*, 101280. https://doi.org/10.1016/j.bcab.2019.101280

Maiga, Y., Carbour, Q., Hamrouni, R., Tranier, M. S., Menadi, Y. B., & Roussos, S. (2021). Development and evaluation of a disposable solid-state culture packed-bed bioreactor for the production of conidia from Tricoderma asperellum grown under water stress. *Waste and Biomass Valorization, 12,* 3223-3231. https://doi.org/10.1007/s12649-020-01210-2

Mann, M., Huser, A., Schick, B., Dinger, R., Miebach, K., & Buchs, J. (2021). Online monitoring of gas transfer rates during CO and CO/H_2 gas fermentation in quasi-continuously ventilated shake flasks. *Biotechnology and Bioengineering*, *118*, 2092-2104. https://doi.org/10.1002/bit.27722

Mendez-Gonzalez, F., Loera, O., Saucedo-Castaned, G., & Favela-Torres, E. (2020). Forced aeration promotes high production and productivity of infective conidia from *Metarhizium robertsii* in solid-state fermentation. *Biochemical Engineering Journal*, *156*, 107492. https://doi.org/10.1016/j.bej.2020.107492

Mitchell, D. A., Ruiz, H. A., & Krieger, N. (2023). A critical evaluation of recent studies on packed-bed bioreactors for solid-state fermentation. *Processes*, *11*(3), 872. https://doi.org/10.3390/pr11030872

Mitchell, D. A., Berovic, M., & Krieger, N. (2006). Introduction to solid-state bioreactors, in Solid State Fermentation Bioreactors-Fundamentals of Design and operation (Mitchell, D.A., Krieger, N., & Berovic, M., Ed). Springer-Verlag Berlin Heidelberg, *Germany*, pp. 33-44. https://doi.org/10.1007/3-540-31286-2

Mitchell, D. A., von Meien, O. F., Krieger, N., & Dalsenter, F. D. H. (2004). A review of recent developments in modelling of microbial growth kinetics and intraparticle phenomena in solid-state fermentation. *Biochemical Engineering Journal*, *17*(1), 15-26. https://doi.org/10.1016/S1369-703X(03)00120-7

Mitchell, D. A., & von Meien, O. F. (2000). Mathematical modelling as a tool to investigate the design and operation of the zymotis packed bed-bioreactor for solid state fermentation. *Biotechnology and Bioengineering*, *68*(2), 127-135. https://doi.org/10.1002/(SICI)1097-0290(20000420)68:2<127::AID-BIT1>3.0.CO;2-K

Nascimento, F. V., De Castro, A. L., Secchi, A. R., & Coelho, M. A. Z. (2021). Insights into media supplementation in solid-state fermentation of soybean hulls by *Yarrowia lipolytica*: Impact on lipase production in tray and insulated packed-bed bioreactors. *Biochemical Engineering Journal*, *166*, 107866. https://doi.org/10.1016/j.bej.2020.107866

Nadal-Rey, G., McClure, D. D., Kavanagh, J. M., Cornelissen, S., Fletcher, D. F., & Gernaey, K. V. (2020). Understanding gradients in industrial bioreactors. *Biotechnology Advances*, *46*, 107660. https://doi.org/10.1016/j.biotechadv.2020.107660

Nagel, F. J. J. I., Tramper, J., Bakker, M. S. N., & Rinzema, A. (2001). Temperature control in a continuously mixed bioreactor for solid-state fermentation. *Biotechnology and Bioengineering*, *72*(2), 219-230. https://doi.org/10.1002/1097-0290(20000120)72:2<219::AID-BIT10>3.0.CO;2-T

Naveena, B., Sakthiselvan, P., Elaiyaraju, P., & Partha, N. (2012). Ultrasound induced production of thrombinase by marine actinomycetes: Kinetic and optimization studies. *Biochemical Engineering Journal*, *61*, 34-42. https://doi.org/10.1016/j.bej.2011.12.007

Okazaki, N., Sugama, S., & Tanaka, T. (1980). Mathematical model for surface culture of koji mold: Growth of koji mold on the surface of steamed rice grains (IX). *Journal of Fermentation Technology*, *58*(5), 471-476. http://dl.ndl.go.jp/info:ndljp/pid/11076987

Pakaweerachat, P., Klinthong, W., Ohtaguchi, K., & Chysirichote, T. (2023). Simultaneous isoquercitin and gallic acid production of *Aspergillus niger* on Triphala byproduct under solid state fermentation in packed-bed bioreactor. *AIMS Agriculture and Food*, *8*(2), 359-373. https://doi.org/10.3934/agrfood.2023020

Pandey, A., Soccol, C. R., Rodriguez-Leon, J. A., & Nigam, P. S. N. (2001). "Solid State Fermentation in Biotechnology: Fundamentals and Applications" Reference Book. https://pure.ulster.ac.uk/en/publications/solid-state-fermentation-in-biotechnology-fundamentals-and-applic-2

Rajagopalan, S., & Modak, J. (1995). Modelling of heat and mass transfer for solid state fermentation process in tray bioreactor. *Bioprocess Engineering*, *13*(3), 161-169. https://doi.org/10.1007/BF00369700

Rodriguez-Leon, J. A., de Carvalho, J. C., Pandey, A., Soccol, C. R., & Rodriguez-Fernandez, D. E. (2018). Kinetics of the solid-state fermentation process in Current Developments in Biotechnology and Bioengineering-Current Advances in Solid-state Fermentation, (Pandey, A., Larroche, C., & Soccol, C.R., Ed.), *Elsevier*, pp. 57-82. https://doi.org/10.1016/B978-0-444-63990-5.00004-9

Rodriguez-Fernandez, D. E., Rodriguez-Leon, J. A., de Carvalho, J. C., Sturm, W., & Soccol, C. R. (2011). The behaviour of kinetic parameters in production of pectinase and xylanase by solid-state fermentation. *Bioresources Technology*, *102*, 10657-10662. https://doi.org/10.1016/j.biortech.2011.08.106

Singh, S. K., Sczakas, G., Soccol, C. R., & Pandey, A. (2008). Production of enzymes by solid-state fermentation. *Current Developments in Solid-State Fermentation*, 183-204. https://doi.org/10.1007/978-0-387-75213-6_9

Sabrini, N. S. A., Gutarra, M. L. E., Fernandez-Lafuente, R., Cavalcanti, E. D. C., & Freire, D. M. G. (2019). Multipurposes fixed-bed bioreactor to simplify lipase production by solid-sate fermentation and application in biocatalysis. *Biochemical Engineering Journal*, *144*, 1-7. https://doi.org/10.1016/j.bej.2018.12.024

Sella, S. R. B. R., Guizelini, B. P., de Souza Vandenberghe, L. P., Medeiros, A. B. P., & Soccol, C. R. (2009). Lab-scale production of *Bacillus atrophaeus* spores by solid state fermentation in different types of bioreactors. *Brazillian Archives of Biology and Technology*, *52*, 159-170. https://www.scielo.br/j/babt/a/CjQ773d4nyGDdvNMjXqsTKB/?lang=en

Sentis-More, P., Ramero-Fabregat, M., Rodriguez-Marca, C., Guerra-Sanchez, A., & Ortega-Olive, N. (2023). Design optimization of a tray bioreactor for solid-state fermentation: Study of process parameters through protein mondification by-products. *Fermentation*, *9*(10), 921. https://doi.org/10.3390/fermentation9100921

Shahryani, Z., Fazaelipoor, M.H., Shaabani, M.S. & Ghasemi, Y. (2019). Production of fungal phytase in an innovative trickle bed bioreactor. *Waste and Biomass Valorization*. *11*, 3273-3280. https://doi.org/10.1007/s12649-019-00642-9

Silva, E. M., & Yang, S. T. (1998). Production of amylases from rice by solid-state fermentation in a gas-solid spouted-bed bioreactor. *Biotechnology Progress*, *14*, 580-587. https://doi.org/10.1021/bp9800440

Sindhu, R., Pandey, A., & Binod, P. (2017). Design and types of bioprocesses, in Current Developments in Biotechnology and Bioengineering-Bioprocess, Bioreactors and Controls, (Larroche, C., Sanroman, M. A., Du, G., & Pandey, A., Ed.), *Elsevier*, 29-43. https://doi.org/10.1016/B978-0-444-63663-8.00002-1

Skiadas, C. H., & Skiadas, C. (2008). Comparing the Gompertz models with a first passage time density model, in Advances in data analysis, (Skiadas, C.H., Ed.), *Springer*, *Birkhauser Boston*. pp. 203-209. https://doi.org/10.1007/978-0-8176-4799-5_18

Sukatsch, D. A., & Dziengel, A. (1987). Biotechnology: A handbook of practical formulae. *Longman Scientific and Technical, Essex*, *John Wiley and Sons*, *Inc*. *New Yor*k. Pp. 104-105. ISBN-10: 0-582-98899-3.

Soares, M., Christen, P., Pandey, A., & Soccol, C. R. (2000). Fruity flavor production by *Ceratocystis fimbriata* grown on coffee husk in solid-state fermentation. *Process Biochemistry*, *35*(8), 857-861. https://doi.org/10.1016/S0032-9592(99)00144-2

Sosa-Martinez, J. D., Morales-Oyervides, L., Montanez, J., Contreras-Esquivel, J. C., Balagurusamy, N., Gadi, S. K., & Salmeron, I. (2024). Sustainable co-production of xylanase, cellulase and pectinase through agroindustrial residue volarization using solid-state fermentation: A techno-economic assessment. *Sustainability*, *16*(4), 1564. https://doi.org/10.3390/su16041564

Torres-Leon, C., Chavez-Gonzalez, L. M., Hernadez-Almanza, A., Martinez-Medina, G. A., Ramirez-Guzman, N., Londono-Hernadez, L., & Aguilar, C.N. (2021). Recent advances on the microbiological and enzymatic processing for conversion of food wastes to valuable bioproducts. *Current Opinion Food Science*, *38*, 40-45. https://doi.org/10.1016/j.cofs.2020.11.002

Torres-Mancera, M., Figueroa-Mantero, A., Favela-Torres, E., Rosales-Zamora, E. G., Nampoothiri, K. M. & Saucedo-Castaneda, G. (2018). Online monitoring of solid-state fermentation using respirometry. *In Current Developments in Biotechnology and Bioengineering*, (Pandey, A., Larroche, C., & Soccol, C. R., Ed.), Elsevier, 97-108. https://doi.org/10.1016/B978-0-444-63990-5.00006-2

Varzakas, T. H., Roussos, S., & Arvanitoyannis, I.S. (2008). Glucoamylases production of *Aspergillus niger* in solid state fermentation using a continuous counter-current reactor. *International Journal of Food Science and Technology*. *43*(7), 1159-1168. https://doi.org/10.1111/j.1365-2621.2007.01582.x

Vauris, A., Valcauda, S., Husson, F., & De Coninck, J. (2022). A novel method to assess heat transfer and impact of relevant physiocochemical parameters for the scalling up of solid state fermentation systems. *Biotechnology Reports*, *36*, e00764. https://doi.org/10.1016/j.btre.2022.e00764

Wang, R., Gmoser, R., Taherzadeh, M. J., & Lennartsson, P.R. (2021). Solid-state fermentation of stale bread by an edible fungus in a semi-continuous plug-flow bioreactor. *Biochemical Engineering Journal*, *169*, 107959. https://doi.org/10.1016/j.bej.2021.107959

Winsor, C. P. (1932). The Gompertz curve as a growth curve. *Proceedings of the National Academy of Sciences of the United States of America, 18*(1), 1-8. https://doi.org/10.1073/pnas.18.1.1

Zhong, J. J. (2011). Bioreactor engineering, 3rd ed., in Comprehensive Biotechnology, (Moo-Young, M., Ed.), *Elsevier*, pp. 165-177. https://doi.org/10.1016/B978-0-08-088504-9.00097-0

Zhu, Z., Sun, L., Huang, X., Ran, W., & Shen, Q. (2014). Comparison of the kinetics of lipopeptide production by *Bacillus amyloliquefaciens* XZ-173 in solid-state fermentation under isothermal and non-isothermal conditions. *World Journal of Microbiology and Biotechnology*, *30*, 1615-1623. https://doi.org/10.1007/s11274-013-1587-7