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# 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

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## Abstract

**Background:** The growth of *Aspergillus awamori* and *Aspergillus oryzae* in a self-designed, multi-stacked circular tray solid-state bioreactor (SSB), operating in solid-state fermentation (SSF) conditions at a laboratory scale, was studied. The bioreactor was divided into six layers by six circular perforated trays. Wheat bran was used as both a carrier of bound mycelia and nutrient medium for the growth of *A. awamori* and *A. oryzae*. The new tray SSB is equipped with instrumentation (an oxygen (O<sub>2</sub>)/carbon dioxide (CO<sub>2</sub>) gas analyser and a thermocouple) to continuously monitor O<sub>2</sub> consumption and CO<sub>2</sub> and heat evolved, which can directly be used to monitor the fungal biomass. The integrated Gompertz model was used to describe the accumulated evolution of CO<sub>2</sub>.

**Results:** The results from the models strongly suggest that the evolved and accumulated CO<sub>2</sub> can be used to excellently describe fungal growth. Another important parameter that can be determined by the gas balance method is the respiratory quotient (RQ). This is the ratio of the CO<sub>2</sub> evolution rate (CER) to the O<sub>2</sub> uptake rate (OUR). The use of CER and OUR confirmed that correlated measurements of microbial activity are available, and the determination of RQ may propose an explanation for differences from expected levels. The kinetic behaviour of the fungal culture, using raw CO<sub>2</sub>, which represents an accumulation term, was integrated with respect to time and fitted to a Gompertz model, a log-like equation. The model can be used to generate parameter values that may be used to verify the experimental data, and also to simulate and optimise the process.

**Conclusion:** Overall, *A. awamori* and *A. oryzae* have their own ability to degrade and utilise the complex compositions contained in the solid substrate, and fermentation conditions may lead to possible comparisons. In addition, multi-stacked circular tray SSB systems demonstrated an excellent system for further investigations of mass transfer and possibly for large-scale operation, though considerable optimisation work remains to be done; for example, the height/diameter ratio and total number of trays should be optimised.

**Keywords:** Solid-state fermentation (SSF), Multi-stacked circular tray solid-state bioreactor (SSB), Gompertz model, Metabolic activity, Respiratory quotient (RQ)

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## Highlights

- Newly designed tray solid-state bioreactor with multiple trays.
- A strategy with different initial moisture content arrangements improved productivity of fungi.
- The multi-stacked circular tray solid-state bioreactor showed potential to provide the suitable physical stimulations to both fungi for monitoring growth, controlling water content, continuous supply of O<sub>2</sub>, continuous removal of CO<sub>2</sub>, and heat and better temperature control.
- Continuous monitoring of O<sub>2</sub> and CO<sub>2</sub> concentrations in the exhaust gas of SSF bioreactors is very useful to assess the physiological state and respiration rate of culture.
- CO<sub>2</sub> that evolved as a result of metabolic activity during *A. awamori* and *A. oryzae* SSF on wheat bran is easy to handle with a simple Gompertz model.
- The initial moisture content arrangement of fungus cultures has a profound effect on the fungal growth observed, including spores and enzymes production.

## Introduction

Solid-state fermentation (SSF) can be briefly described as microbial fermentation which takes place in the absence or near absence of free water; thus, it is close to the natural environment to which the selected microorganisms, especially fungi, are naturally acclimated (Abdul Manan and Webb 2017a). Nowadays, modern SSFs for future biorefineries aim to exploit the vast complexity of the technology to transform biomass produced by the agriculture and food industry for valuable by-products through microbial biotransformation (Abdul Manan and Webb 2017b). SSF has been chosen since it appeared to be exceptional and more favourable over submerged fermentation (SmF) in several aspects where it gave advantages in terms of biological, processing, environmental and economic aspects (Bushan et al. 2019; Abdul Manan and Webb 2018a; Koyani and Rajput 2015) to produce food, enzymes, food-grade pigments, livestock and aquaculture bio-feed, fine chemicals, high-value biogenic substances or a wide variety of secondary metabolites and bio-oils/biofuels. With the increasing interest in SSF nowadays, researchers are keen to discover as many new ways to explore the usage of this technology as possible to develop new added-value materials from by-products (Abdul Manan and Webb 2017b).

In SSF, the bioreactor provides the suitable conditions for growth and activity for the microorganisms involved, which allow the microbiological activity. SSF can be considered to be a “closed system”. At time  $t=0$ , the sterilised solid substrate in the bioreactor is inoculated with the selected microorganism, and fermentation is allowed to proceed under optimal physiological conditions. In the course of the entire fermentation, nothing is added into the bioreactor except oxygen (O<sub>2</sub>) (dry air or saturated air can be used). In general, designing a solid-state bioreactor should be focused on five main areas: (1) moisture content control; (2) O<sub>2</sub> transfer; (3) CO<sub>2</sub> evolved; (4) maximising heat removal and (5) monitoring temperature. Mitchell et al. (2006a, 2010, 2011) suggested that one of the main concerns of bioreactor design and operation is needed to eliminate enough metabolic heat waste. This is important to prevent temperature within the fermented bed from getting too high as this affects microbial growth and product formation (Ravindran and Jaiswal 2016; Mitchell et al. 2006b). However, according to Torres-Mancera et al. (2018), the monitoring and control of respirometry parameters of microorganisms are very important to achieve higher productivity and carry out optimisation and scaling of the process, as well as to control the process evolution.

The tray bioreactor is the most widely used bioreactor for SSF. Using trays is the oldest system, and they are very simple in design and used in static conditions—unmixed beds with no forced aeration on the solid substrate. The fermentation is done in stationary trays with no mechanical agitation. The bottom of the tray is perforated with mesh to hold the solid substrate to allow a normal aeration. The substrate thickness on the tray is the major limitation parameter (Xie et al. 2013). This system type only holds a limited amount of solid substrate to be fermented. This is because only thin layers must be used to avoid overheating and to maintain aerobic conditions (Robinson and Nigam 2003). Alcantara and da Silva (2012) and Vaseghi et al. (2013) found that substrate thickness; surface area and chamber temperature had a positive effect on enzyme activity and could improve metabolic heat and gas transfer.

Thickness of the solid substrates bed can be varied. Usually trays are placed in the incubation room, where temperature and humidity are controlled for optimal growth. Trays are arranged one above the other with suitable gaps between them. As there is no forced aeration in the medium, mass and heat transfer occurs by natural diffusion and convection. Chen et al. (2005) studied the effect of two dynamic changes of air (including air pressure pulsation and internal air circulating) in a tray bioreactor and observed changes in the temperature gradient.

In their results, internal air circulation was beneficial, accelerating heat transfer between the substrate surface and the outside air. Furthermore, Ruiz et al. (2012) and Assamoi et al. (2008) designed column-tray bioreactors with forced aeration. This allowed better control of environmental conditions in the bed due to manipulation of temperature and flow rate of the air.

In this work, the growth performance and ability of *A. awamori* and *A. oryzae* to grow in newly designed multi-stacked circular tray solid-state bioreactor (SSB) systems were explored and described in detail. Experiments were carried out under sterile conditions, equipped with an online temperature recorder and gas analyser, for continuous O<sub>2</sub> and CO<sub>2</sub> measurement. An initial moisture content strategy with fixed air flow rates was applied to control the temperature and moisture content of the solid substrate. Wheat bran with *A. awamori* or with *A. oryzae* was chosen as model system for the study and is discussed as a suitable alternative for the effective production of spores and four enzymes (glucoamylase, protease, xylanase and cellulase). Monitoring the microbial activity, including monitoring O<sub>2</sub> consumption and CO<sub>2</sub> and heat evolution during SSF, can directly reveal the activity of the fungal culture in SSF. A Gompertz model will be used to fit the integrated CO<sub>2</sub> data and predict the quantity of CO<sub>2</sub> evolution in all experiments. At the same time, bed temperature, O<sub>2</sub> uptake rate (OUR) and CO<sub>2</sub> evolution rate (CER) will be observed as indirect techniques for biomass estimation.

## Materials and methods

### Microorganisms and culture conditions

The strains of *Aspergillus awamori* and *Aspergillus oryzae* were proportioned by the School of Chemical Engineering and Analytical Science, Faculty of Engineering and Physical Science, University of Manchester. The fungal strains were cultured and preserved in a solid sporulation medium containing 5% (w/v) whole wheat flour and 2% (w/v) agar (Sigma-Aldrich). The strains were activated in sterilised media and incubated over 7 days at 32 °C, then preserved at 4 °C. They were sub-cultured on a time interval of every 2 months.

### Fungal spores for inoculum preparation

The spores were washed by lightly scrapping with wire loop in 10.0 mL of sterile 0.1% (v/v) Tween 80. Of the spore suspension, 0.5 mL was further transferred onto the surface of 100.0 mL of the same sporulation medium in a 500.0-mL Erlenmeyer flask and incubated for another 7 days at 30 °C. After the incubation period, 50.0 mL of sterile 0.1% (v/v) Tween 80 solution and several sterile glass beads (4 mm diameter) were added to the flask. The spores were suspended by shaking the flask

gently and collected in one bottle as a spore suspension. The concentration of the spore suspension was measured by haemocytometer.

### Solid substrate

Wheat bran was obtained from Cargill Wheat Processing Plant, Manchester, UK. The substrates were kept in an airtight container and stored in a cold room (4 °C) for future use. Wheat bran is a low-cost residue of the milling industry, an interesting solid substrate for SSF and was used, without any treatment, as a solid medium for growing *A. awamori* and *A. oryzae*. Wheat bran may be seen as a model of cheap and abundant agricultural waste and have potential in making the entire SSF process feasible.

### Substrate preparation and inoculum transfer procedures

A standard procedure was developed in these studies to prepare inoculum transfers for the inoculation process into solid substrates. This was done to make sure the spore inoculum was distributed homogeneously into all solid particles before the fermentation was started. First, 12.0 g wheat bran was weighed and placed into separate 250-mL flasks before being sterilised at 121 °C for 15 min. The substrates were allowed to cool at room temperature before inoculating with *A. awamori* and *A. oryzae* spores and being moistened with an amount of sterile distilled water to obtain the initial moisture content needed for each experiment. About  $1.2 \times 10^6$  spores/g substrate was inoculated into the flask and mixed well with a sterile spatula under aseptic conditions to uniformly distribute the spores within the substrate. After mixing well, the content was distributed into each tray before being incubated at 30 °C for 72 h. At the end of the fermentation period, a sample was taken out for sample analysis.

### Multi-stacked circular tray solid-state bioreactor

An image of a multi-stacked circular tray SSB and a schematic diagram of a bioreactor system are shown in Fig. 1. The bioreactor was constructed of multiple individual circular trays that can be fitted on top of one another. As shown in Fig. 1, the bioreactor contains six perforated base trays, 10.0 cm in diameter and 5.0 cm in height, which were tightly stacked one over another, bringing the total height to 33.5 cm. The closed tray at the bottom is as an air distributor with the same diameter and 3.5 cm height and will be referred to as the base tray. The air passes into this base tray before forcing through to another tray by continuous aeration (from bottom to the top). The mounted trays are sealed in such a manner that prevents leakage from the outside to the inside environment and vice versa.

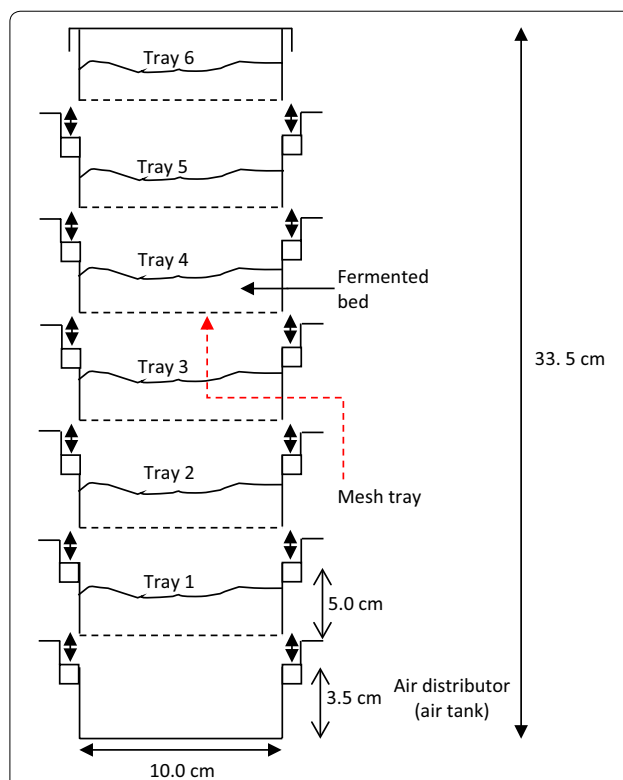
The bioreactor was constructed from stainless steel. A perforated base tray with different aperture sizes (500  $\mu\text{m}$ —tray number 1; 600  $\mu\text{m}$ —tray number 2; 710  $\mu\text{m}$ —tray number 3; 850  $\mu\text{m}$ —tray number 4; 1000  $\mu\text{m}$ —tray number 5 and 1400  $\mu\text{m}$ —tray number 6) was used and placed accordingly during bioreactor set-up for uniform distribution of air, as well as to support and prevent the solid particles from flowing through. After sterilising, a standard procedure for substrate preparation and inoculum transfer was performed, as described above, before loading onto every tray with 1.5 cm bed substrate height.

#### Bioreactor set-up and initial moisture content arrangement

The bioreactor and solid substrate were autoclaved at 121 °C for 15 min prior to operation. In this work, wheat bran was used as a model substrate with *A. awamori* and *A. oryzae*. The sterilised substrate was inoculated with  $1.2 \times 10^6$  spores/g substrate in another Erlenmeyer flask before loading in the sterile tray and transferred into the bioreactor as described above. All work was carried out in a laminar flow cabinet under sterile conditions. The bioreactor was then placed in an incubator growth room for 72 h at 30 °C. During fermentation, the moistened air sparged into the bioreactor system (from bottom to top of the bioreactor). A schematic diagram of the experimental set-up consisting of the multi-stacked circular tray SSB with aeration, control and an online monitoring system is shown in Fig. 2.

For moistened air, dry air from the compressor (Air compressor JUN-AIR, USA) was passed through a humidifier chamber before entering the system. The flow rate of the air into the bioreactor system needs to be accurately measured, normally using a rotameter. The air was sterilised by passing it through a 0.45- $\mu\text{m}$  cellulose acetate membrane filter and then into a humidifier chamber with sterilised distilled water, then monitored in a water bath at 30 °C. The bioreactor is equipped with thermocouples and  $\text{O}_2/\text{CO}_2$  dioxide analyser.

In this study, experiments were carried out on an arrangement of different initial moisture contents in every tray instead of air arrangement. The airflow rate was 2 L/min. There are four different initial moisture content arrangements in every tray, which are described in Table 1. As fermentation systems were further developed, internal moist air circulation under forced aeration was developed from the bottom to the top of a series of perforated mesh trays.



**Fig. 1** A multi-stacked circular tray SSB: a perspective view of a bioreactor according to a first embodiment of the multi-stacked trays, with emphasis on the location of the seals, which are represented by the symbol "□", removed and a split tray installed. The tray at the bottom represents an air distributor at the bottom of the bioreactor

#### Measuring metabolic activity

##### Temperature

Every tray was equipped with a thermocouple type K (Pico Lab Technology, UK) placed at the surface of the fermented substrate to continuously record the bed temperature over time. All of the online data are monitored on screen and recorded using a computer.

##### Respiratory gases

The system used for quantifying  $\text{CO}_2$  and  $\text{O}_2$  is able to continuously measure exhaust gas compositions online. An air outlet is situated in the headspace of the highest tray. The exit gas may need to be conditioned (moisture removed) before going into the gas analyser. Therefore, the humid exhaust air from the bioreactor was first dried by passing through silica gel tubes before entering an  $\text{O}_2/\text{CO}_2$  analyser (FerMac 368 Electrolab, UK). This analyser was connected to the Electrolab eLogger data logging, and it translates the concentration of  $\text{O}_2$  and  $\text{CO}_2$  to be recorded using a computer system. Table 2 shows

the equations applied for the gas balance that can be calculated directly when the composition of the exhaust gas is known. Details of operating conditions are described elsewhere (Abdul Manan and Webb 2018b).

**Final moisture content measurement**

The moisture content of the samples was determined using the oven method, by measuring weight loss after heating to a constant weight at 95 °C as described previously (Abdul Manan and Webb 2018b).

**Preparation of enzymes supernatant**

After the 72-h fermentation period, samples were taken for enzymes (glucoamylase, protease, xylanase and cellulase) analysis. A standard operational procedure for enzyme extraction was developed in this study, as reported elsewhere (Abdul Manan and Webb 2016a).

**Spores count**

About 2.0 g (wet weight) of fermented substrate of *A. awamori* and *A. oryzae* was used to harvest the spores in a 250-mL flask containing 40 mL 0.1% (v/v) Tween 80. The flasks were continuously shaken in an orbital shaker at 100 rpm for 30 min at 30 °C. Then, the spore suspensions were filtered using a stainless steel sieve with aperture size 45 µm to separate the solid particles.

The procedure of spores count was carried out using a haemocytometer.

**Statistical analysis**

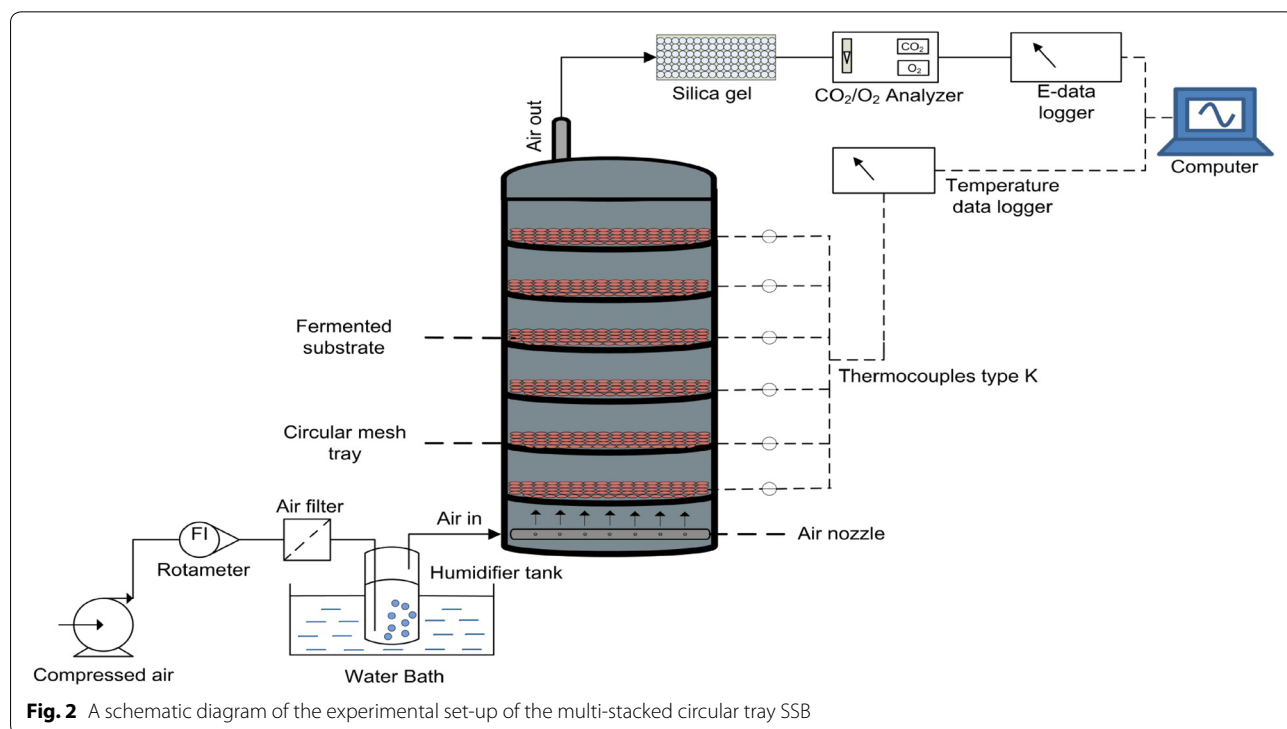
All experiments were repeated three times independently. The data were reported as mean ± standard deviation (SD). The MINITAB version 14 (Minitab Inc., PA, United States) was performed to evaluate statistical significance level of 0.05.

**Results and discussion**

**Profile final moisture content**

The profiles of final moisture content in every tray of the multi-stacked circular tray SSB are shown in Fig. 3. In Exp. 1 [initial moisture content arrangement from low (50%) to high (75%)] with *A. awamori*, trays at positions 1 and 2 lost about 17.72 and 2.13% moisture content, respectively. However, trays at positions 3, 4, 5 and 6 showed an increment in moisture content of about 4.37, 8.48, 7.41 and 3.83%, respectively. In the case of *A. oryzae*, trays at positions 1, 2, 3, 4, 5 and 6 showed an increment in moisture content of about 11.44%, 15.66%, 17.68%, 12.36% and 5.73%, respectively.

In Exp. 2 [initial moisture content arrangement from high (75%) to low (50%)], *A. awamori* recorded loss in moisture content of about 16.88 and 8.6% in trays at positions 1 and 2, respectively. However, there were



**Fig. 2** A schematic diagram of the experimental set-up of the multi-stacked circular tray SSB

**Table 1 Arrangement of initial moisture content in multi-stacked tray SSB**

Experiment	Tray position with initial moisture content arrangements					
	Tray 1 (%)	Tray 2 (%)	Tray 3 (%)	Tray 4 (%)	Tray 5 (%)	Tray 6 (%)
Exp 1	50	55	60	65	70	75
Exp 2	75	70	65	60	55	50
Exp 3	65	65	65	65	65	65
Exp 4	15	15	15	15	15	15

**Table 2 Equations used for simulation data from respiratory gases**

Mathematical modelling	Equations	Equation number
Oxygen uptake rate (OUR) (Sukatsch and Dziengel 1987)	$OUR = \frac{F_1}{V_m \times V_0} \left( X_{O_2(in)} - \frac{1 - (X_{O_2(in)} + X_{CO_2(in)})}{1 - (X_{O_2(out)} + X_{CO_2(out)})} \times X_{O_2(out)} \right)$	1
Carbon dioxide evolution rate (CER) (Sukatsch and Dziengel 1987)	$CER = \frac{F_1}{V_m \times V_0} \left( X_{CO_2(out)} \times \frac{1 - (X_{O_2(in)} + X_{CO_2(in)})}{1 - (X_{O_2(out)} + X_{CO_2(out)})} - X_{CO_2(in)} \right)$	2
The Gompertz model is a sigmoid function, as the logistic curve (Skiadas and Skiadas 2008)	$(\ln x)' = -b_0 \ln x$	3
Direct integration of Eq. 3	$x = \exp(\ln(x_0) \exp(-bt))$	4
The integrated Gompertz model-logistics-like model the product CO <sub>2</sub> is a function of time (t)	$[CO_2] = [CO_{2,max}] \exp(-b \exp[-kt])$	5
Respiratory quotient (RQ)	$RQ = \frac{CER}{OUR}$	6

OUR, oxygen uptake rate (mole/L h); CER, carbon dioxide evolution rate (mole/L h);  $F_1$ , air flow rate of inlet gas (L/h) at 1 atm and 30 °C;  $V_m$ , molar volume of gases = 24.88 L/mole at 1 atm and 30 °C;  $V_0$  working volume solid phase (L);  $X_{O_2(in)}$ , molar fraction of oxygen at gas inlet;  $X_{O_2(out)}$ , molar fraction of oxygen at gas outlet;  $X_{CO_2(in)}$ , molar fraction of CO<sub>2</sub> at gas inlet;  $X_{CO_2(out)}$ , molar fraction of CO<sub>2</sub> at gas outlet;  $x$ , the function of time;  $b_0$ , a positive constant expressing the rate of growth of the system;  $[CO_{2,max}]$ , the maximum CO<sub>2</sub> concentration (at  $t > \infty$ ) (mole);  $b$ , a constant related to the initial conditions (when  $t = 0$ , then  $[CO_2] = [CO_{2_0}] = [CO_{2,max}] \exp(-b)$  (dimensionless);  $k$ , the specific CO<sub>2</sub> evolution rate ( $h^{-1}$ );  $t$ , fermentation time (h), RQ, respiratory quotient (dimensionless)

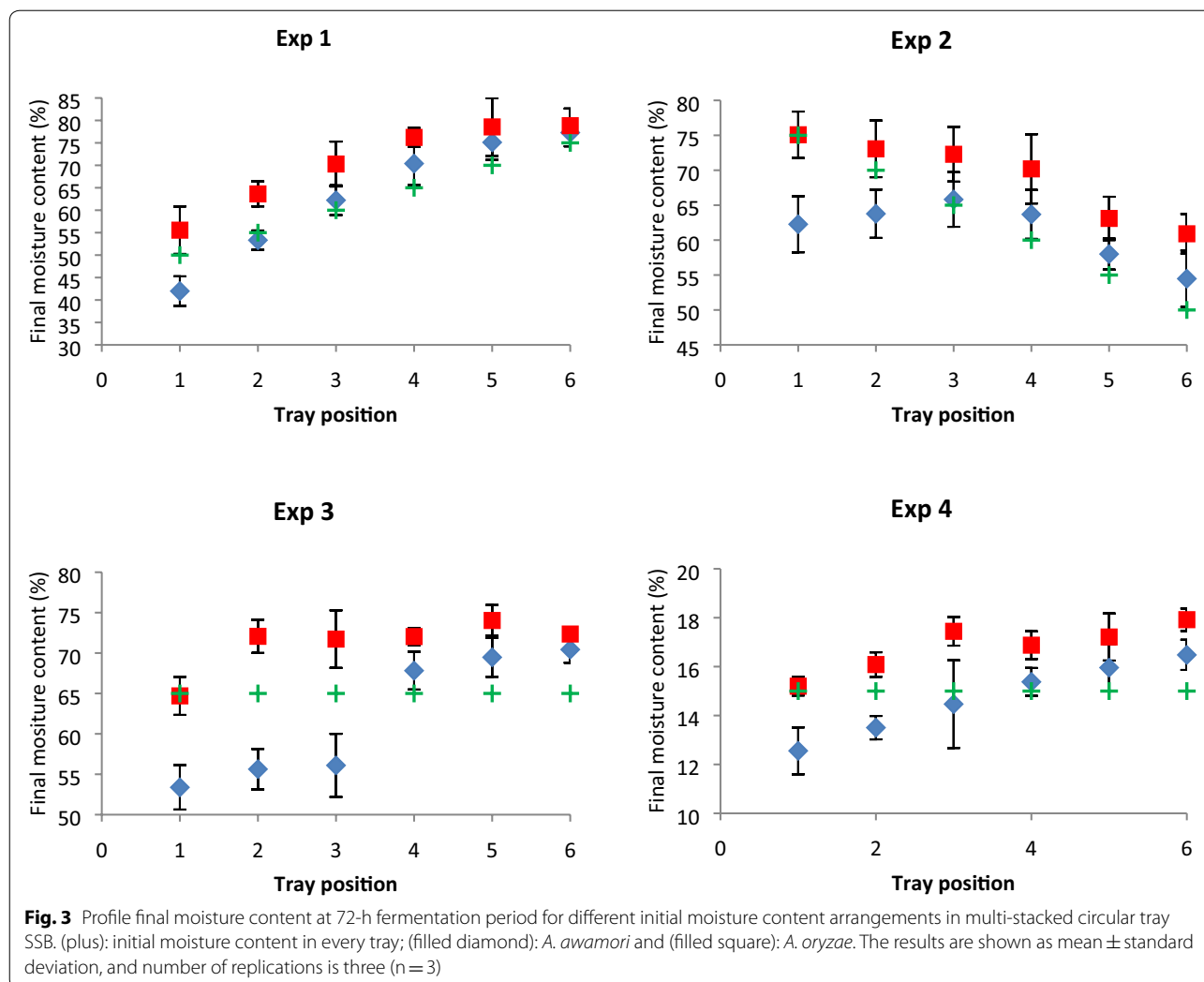
increments of about 9.67, 6.10, 5.76 and 9.88% in final moisture content for trays at positions 3, 4, 5 and 6, respectively. In the case of *A. oryzae*, the final moisture content in trays 1, 2, 3, 4, 5 and 6 increased by about 0.11, 4.41, 11.77, 17.15, 14.75 and 21.50%, respectively.

In Exp. 3 (initial moisture content was set up at 65% in every tray), the final moisture content in fermented solid with *A. awamori* decreased by about 18.08, 13.99 and 13.68% in trays at positions 1, 2 and 3, respectively. However, it increased in trays at positions 4, 5 and 6 by about 4.35, 6.49 and 8.06, respectively. In the case of *A. oryzae*, only the tray at position 1 showed a decrease in final moisture content of about 0.96%. Final moisture content in trays at positions 2, 3, 4, 5 and 6 showed increments of about 11.26, 10.56, 10.76, 13.97 and 11.10%, respectively.

Finally, in Exp. 4 (initial moisture content was set up at 15% in every tray), the final moisture content in the first

three trays from the bottom decreased by about 15.61, 9.53 and 0.53% (trays 1, 2 and 3, respectively) for fermented *A. awamori*. However, increments were observed in trays 4, 5 and 6 of about 1.40, 6.57 and 9.85%, respectively. As observed in the first three experiments above, the final moisture content in every tray increased for *A. oryzae*. The increments were recorded at about 1.19, 7.06, 17.30, 11.5, 14.96 and 19.51 for tray in position 1, 2, 3, 4, 5 and 6, respectively.

It can be noticed that the tray at position 1 suffered higher loss of moisture, especially with the *A. awamori* culture, due to access to moistened air being very high. The strategy to create humidified air through a humidifier chamber was not observed to create 100% saturation. If the air is 100% saturated, it cannot carry away any moisture from the substrate. The air forced into the system is only moistened and is still able to carry some



amount of water from the fermented substrate. This is the reason why the tray at position 1 (and sometimes at positions 2 and 3) was observed to lose moisture content at a high level. Something to notice in this study is that *A. oryzae* proved to have high ability to retain water in its cells compared to *A. awamori*, as previously reported elsewhere (Abdul Manan and Webb 2016b). Thus, a combination of the ability of wheat bran and *A. oryzae* to retain water resulted in high final moisture content in fermented *A. oryzae*.

In this system, it was assumed that the trays were tightly stacked above each other. There is no access for air from inside or outside. Aeration with moistened air was used to avoid dryness in the cultural medium and regulate the heat generated during the growth. The moistened air that was forced inside the bioreactor passed through tray-by-tray, and the heat (from microbial activity) was moved along the tray from bottom to the top. It was found that in the present cross-flow arrangement in

the system, the fermented substrate is effectively used in heat and moisture content exchange. The metabolic heat evolved from every tray will give advantage to other trays at the levels above it. The heat will go up from the surface of the fermented bed in the tray below and become trapped at the fermented substrate in the above trays. The perforated mesh tray will allow mass transfer between the trays. Here, the condensation process will occur and will generate water and directly provide moisture into the fermented substrate.

Both heat and moisture contents are transferred between the trays when they flow through the fermented substrate. Thus, heat and moisture content could be recovered from the provided forced moistened air; the excess heat and moisture could be transferred to the other trays in order to cool and dehumidify the next fermented substrate in the tray at the top. This process continuously occurs parallel with continuous air supply into the system. Here, a synergistic relationship is

applied where each tray will provide an advantage to the other trays. The metabolic heat generated from microbial activity was observed to be advantageous for maintaining moisture content, especially in the trays above the tray at position 1.

#### Distributed parameter gas balance—the Gompertz curve as a growth curve

In this system, moistened air supply into the system was constant at a flow rate of 2 L/min, and air was sparged from the bottom to the top of the multi-stacked tray bioreactor. However, different initial moisture contents were set up at every tray according to the experimental design explained in Table 1. Figure 4a shows the profile of CO<sub>2</sub> evolved during the fermentation process for both fungi. Figure 4b shows the fitting of Gompertz model to these data for both fungi during SSF with wheat bran. It is quite clearly visible that with the different initial moisture contents in every tray, CO<sub>2</sub> that evolved during SSF was variable for *A. awamori* in different experiments. With *A. oryzae*, CO<sub>2</sub> evolution was comparable with Exp. 1[AO], 2[AO] and 3[AO] but very low in Exp. 4[AO] (15% moisture content in every tray). It seems that in response to different initial moisture content, clear differences occur in parameters of growth characteristics of *A. awamori* and *A. oryzae*. The application of the Gompertz model to the raw experimental data confirmed that maximum CO<sub>2</sub> evolution could be obtained. As the initial moisture content was reduced to 15%, [CO<sub>2,max</sub>] decreased.

In Table 3, modelling parameters of the integrated CO<sub>2</sub> evolution data are presented. The [CO<sub>2,max</sub>] values confirmed the influence of the initial moisture content arrangement. For example, it can be seen that CO<sub>2</sub> evolution was lower on wheat bran when the initial moisture content was set up at 15% for both fungi (Exp. 4[AA] and Exp. 4[AO]). In Exp 1[AA] with *A. awamori* (initial moisture content from low to high), greatly enhanced evolution of CO<sub>2</sub> was observed. However, in Exp. 3[AO] with *A. oryzae* (initial moisture content at 65% in every tray), there was observed among the highest evolution of CO<sub>2</sub>. The evolution rate (*k*) values were variable, with all being in the range 0.038–0.063 h<sup>-1</sup> and 0.043–0.073 h<sup>-1</sup> for *A. awamori* and *A. oryzae*, respectively, though a clear tendency can be seen in relation to the initial moisture content arrangement. There is still no apparent relationship between this parameter and the amount of CO<sub>2</sub> evolved characterised by [CO<sub>2,max</sub>]. The calculated *t*<sub>max</sub> values were close to those obtained experimentally. These values were in the range of 30.5–43.2 h and 24.9–30.7 h for *A. awamori* and *A. oryzae*, respectively, and seem to be very strongly influenced by the studies carried out. The same observation was recorded with *b* values.

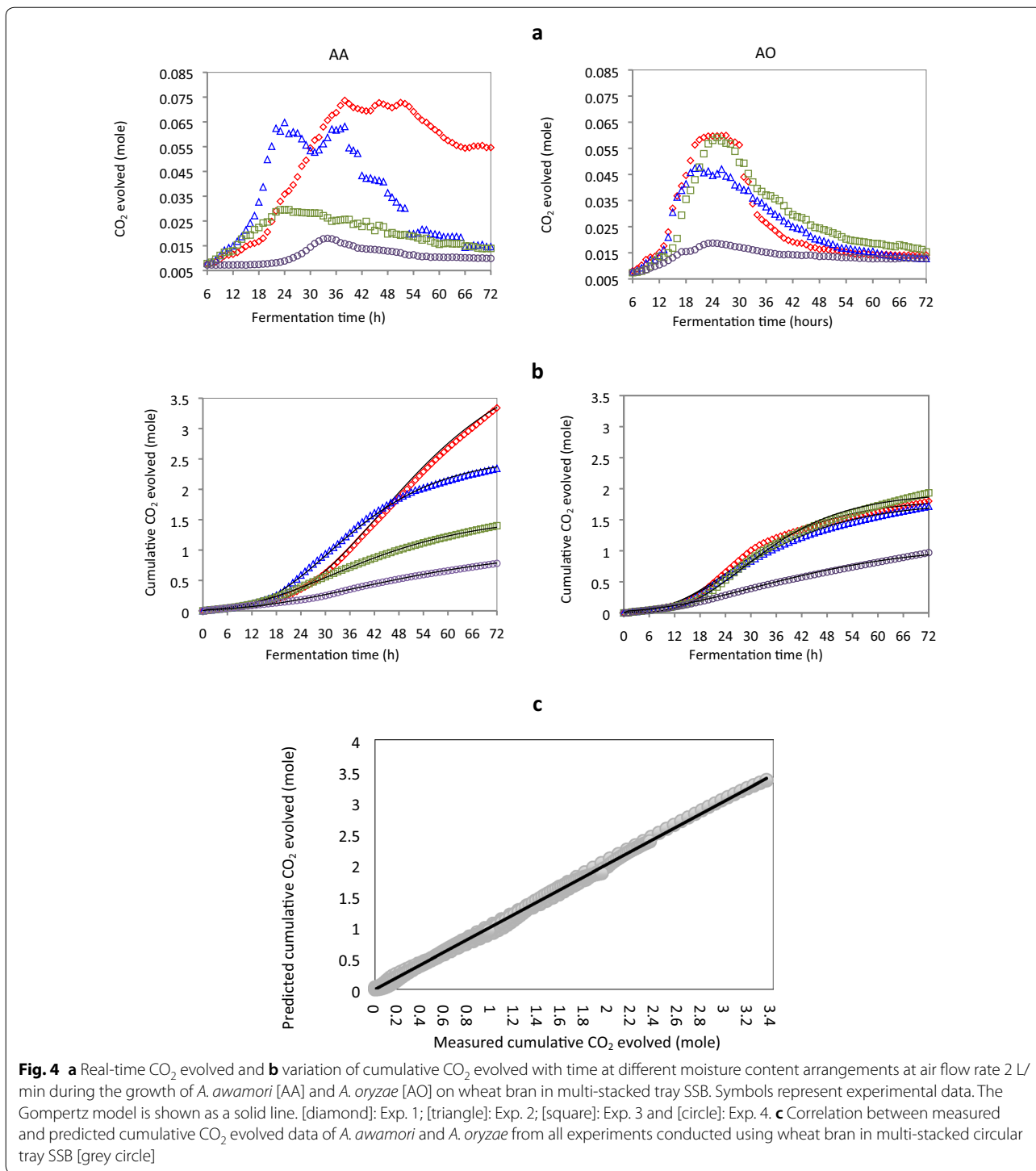
Interestingly, it is noticeable that the two fungi react differently to the same arrangement of growth characteristics. As reflected by the intensity of CO<sub>2</sub> evolved, it seems that initial moisture content has a direct influence on metabolic activities and thus on the rate and amount of CO<sub>2</sub> evolved. Moisture content was significant as a source of variation for biomass production (measured in this case by CO<sub>2</sub> evolution). This was confirmed by the distribution of each kinetic constant, presented for the four most productive experiments. In these cases, *A. awamori* with Exp. 1[AA] showed a high overall growth performance on wheat bran based on CO<sub>2</sub> evolution.

As previously reported by Abdul Manan and Webb (2018b), fitting growth models to accumulated CO<sub>2</sub> evolution raw data is meaningful and easier to handle for further analysis. It is possible to use Gompertz model to describe fungal growth in SSF based on CO<sub>2</sub> evolution. Data of CO<sub>2</sub> evolution as a result of metabolic activity during SSF with *A. awamori* and *A. oryzae* on wheat bran were easy to handle with this model. It was observed in this study that the concentration of CO<sub>2</sub> increases during SSF over time following a sigmoidal curve that describes fungal growth. Also, the variation patterns of sigmoidal curve were produced in response to different strategies (for example, different initial moisture content arrangements).

Figure 4c shows the correlation between experimental and predicted data for the cumulative CO<sub>2</sub> evolved by the behaviour of *A. awamori* and *A. oryzae*, during SSF on wheat bran at initial moisture content arrangements, in multi-stacked tray SSB systems, calculated with the Gompertz model. An excellent agreement between experimental and predicted values of *A. awamori* and *A. oryzae* was obtained with *R*<sup>2</sup> > 0.99. Thus, this model allowed an excellent prediction of the effects of various initial moisture content arrangements on CO<sub>2</sub> evolution during SSF. This was supported by Hussein et al. (2017), where the modified Gompertz model showed a close agreement between the experimental and predicted data in growth rate for two *Centella asiatica* phenotypes. Ultimately, given the assumptions that need to be considered, the Gompertz model shows potential and may be capable of describing what happens under different culture conditions.

Feng et al. (2013) demonstrated that the modified Gompertz equation was developed to calculate methane yield from different feed to inoculum ratios. The value predicted from the modified Gompertz equation seemed to be slightly lower than those of the experimental cumulative methane yield. Soltan et al. (2017) demonstrated that a Gompertz model could be efficiently used to stimulate the behaviour of cumulative hydrogen production





in the batch reactors at different volumetric concentrations of mixed fruit peels and palm mill sludge.

For this study, a Gompertz model was selected because it can reflect the sigmoidal nature of growth. In this study, for multi-stacked tray SSBs and for both fungi, growth followed a typical pattern with four distinct phases: a

lag phase, an acceleration phase, a log (exponential) phase and a deceleration phase. However, there was no clear stationary phase and no accelerated death phase observed. In conclusion, it can be summarised that the Gompertz model is the model of choice for the description of the growth curves of *A. awamori* and *A. oryzae*.

**Table 3** Kinetics constants for carbon dioxide evolved by *A. awamori* and *A. oryzae* with different initial moisture content arrangements in multi-stacked tray

Experiment (flow rate L/ min)	<i>A. awamori</i>					<i>A. oryzae</i>				
	[CO <sub>2max</sub> ] (mole)	<i>k</i> (h <sup>-1</sup> )	<i>b</i>	<i>t</i> <sub>max</sub> (h)	<i>R</i> <sup>2</sup> coefficient	[CO <sub>2max</sub> ] (mole)	<i>k</i> (h <sup>-1</sup> )	<i>b</i>	<i>t</i> <sub>max</sub> (h)	<i>R</i> <sup>2</sup> coefficient
Exp 1	4.31	0.048	7.92	43.2	0.999	1.83	0.069	5.57	24.9	0.994
Exp 2	2.55	0.063	6.83	30.5	0.999	1.78	0.063	5.08	25.8	0.998
Exp 3	1.59	0.048	4.48	31.3	0.999	1.98	0.073	7.67	27.9	0.997
Exp 4	1.03	0.038	4.24	37.6	0.999	1.11	0.043	3.74	30.7	0.997

It is noteworthy that the potential of models to describe growth curves is not the only criterion for their assessment. Some other criteria, such as prediction of growth curve and estimation of some biological parameter, may be used for the selection of an appropriate model.

### Respiratory quotient

During aerobic respiration, O<sub>2</sub> is consumed and CO<sub>2</sub> is released. The ratio of released CO<sub>2</sub> to the O<sub>2</sub> consumed during respiration is called RQ. The RQ can be calculated directly when the composition of the exhaust gas is known. All these values are interesting because they characterise physiology, specific performance of an SSF bioreactor or total performance of fermentation as a function of microbial growth (Kabanova et al. 2012). The rates of O<sub>2</sub> consumption and CO<sub>2</sub> evolution are presented along with the calculated RQ values, giving further insights into the behaviour of the fermentation process (Torres-Mancera et al. 2018). Figure 5a shows the profiles of RQ obtained using Eq. 6 for both fungi in multi-stacked tray SSB with various initial moisture content arrangements. The RQ values are presented in Fig. 5b by obtaining the slope from every experiment illustrated in Fig. 5a. It was observed in these studies that all RQ values obtained with different initial moisture content arrangements are below 1 (Fig. 5b).

The RQ values were greater in the multi-stacked circular tray SSB with different initial moisture content arrangements. For *A. awamori* with Exp. 1[AA], 2[AA] and 3[AA], the RQ values were 0.856, 0.954 and 0.694, respectively. RQ values for *A. oryzae* were 0.807, 0.807, 0.844 and 0.712 for Exp. 1[AO], 2[AO], 3[AO] and 4[AO], respectively. Exp. 4[AA] (initial moisture content at 15% in each tray) recorded the lowest RQ at 0.520 for *A. awamori*. This low value of RQ can also be linked to the low water content and created unfavourable conditions for *A. awamori*. According to Torres-Mancera et al. (2018), when the value for the RQ is higher than 1, it must be considered an aerated fermentation. Lower production of CO<sub>2</sub> related to corresponds to a bad functioning of aeration and the existence of anaerobic zones in the fermentation bed. In any case, a low RQ indicates that less CO<sub>2</sub>

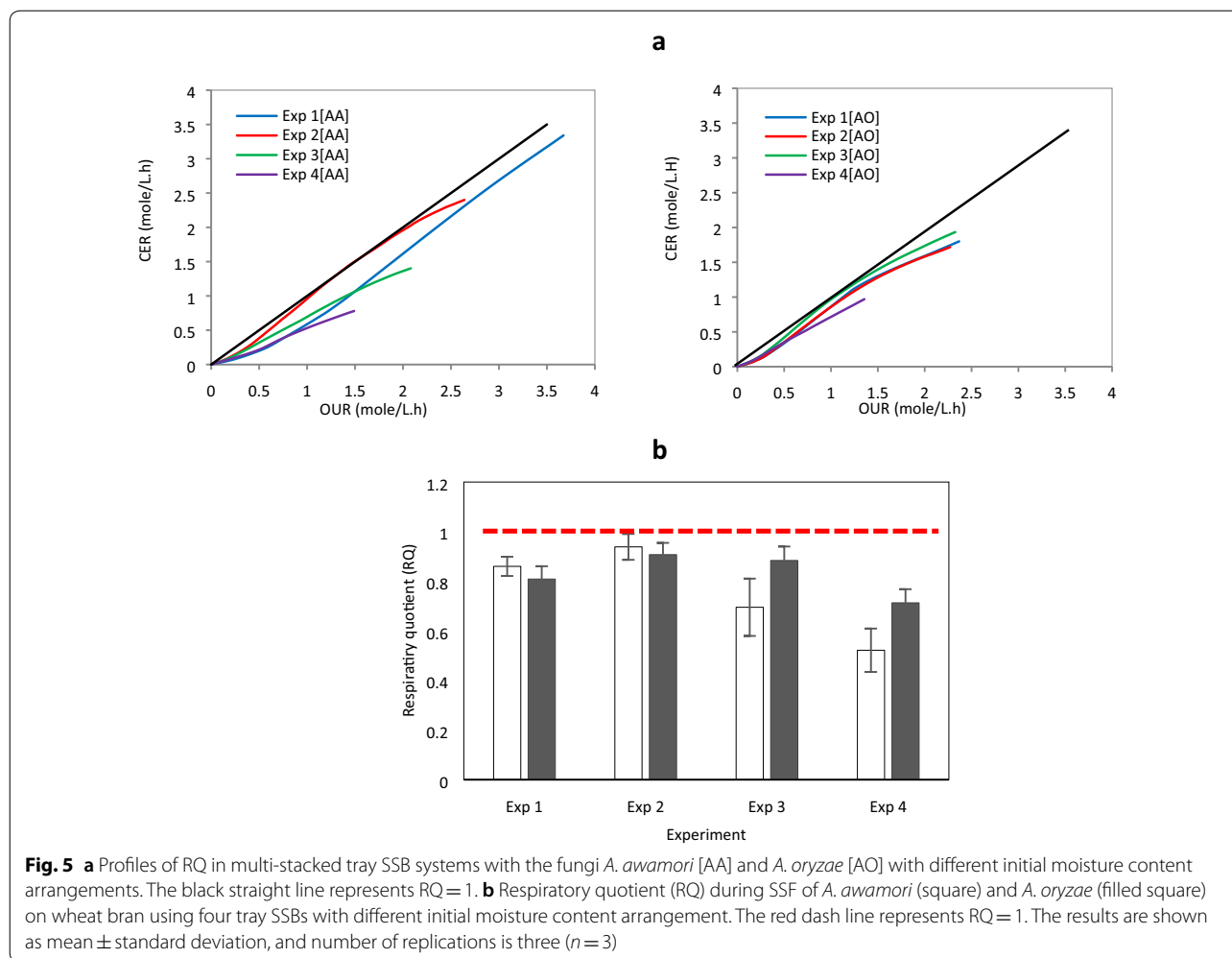
is evolved per unit of O<sub>2</sub> consumed. However, low RQ values cannot directly be linked to anaerobic conditions. According to Govind et al. (1997), RQ values are close to 1 until O<sub>2</sub> uptake reaches a maximum level and, thereafter, RQ becomes higher than 1 due to utilisation of metabolite products. Becerra and Gonzalez-Siso (1996) argued that changes in RQ values could be associated with the production of multiple products by *Streptomyces catleya* during SSF. Barrios-Gonzalez et al. (1993) reported that an RQ of 0.7 indicates oil-supported metabolism for *Nocardia lactamdurans* during the production of efrotomycin in SSF. With a large area of fermented surface and a flow of air, it seems O<sub>2</sub> taken by fungus is less and that more O<sub>2</sub> is carried into the gas outlet. This might be because O<sub>2</sub> has to diffuse from the gas phase to inside the fermented bed, whereas the CO<sub>2</sub> is generated throughout and has to diffuse to the outside compared to the other air delivery arrangement where moistened air was forced through the fermented substrate from bottom to the top. Other factors, such as the effect of readily degradable nutrient compounds contained in the fermented substrate, seem to be a key for determining the RQ value (Abdul Manan and Webb 2018b).

### Metabolic measurement

It is possible to estimate biomass development in SSF by considering OUR, CER and heat evolved, which are easily measurable parameters (Abdul Manan and Webb 2018b; Cooney et al. 2000). For example, OUR and CER offer the advantage of a fast response time and are directly linked to the metabolism of the microorganism (Mitchell et al. 2006a; Krishna 2005). This is also true for the heat evolved during fermentation as a result of microbial growth and can be related to oxygen consumption (Cooney et al. 2000).

### OUR and CER as a tool for online monitoring of fungal growth

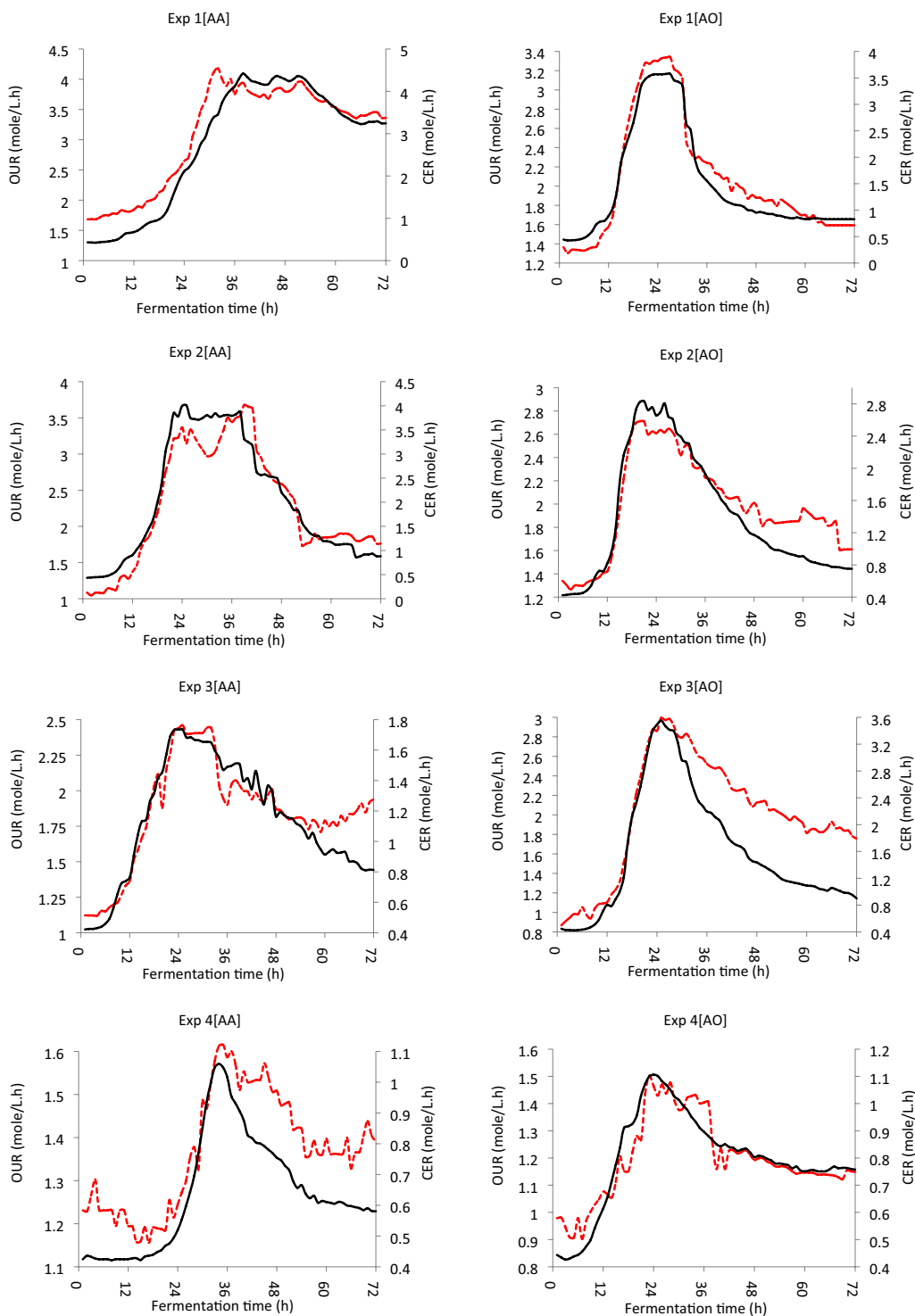
Referring to Fig. 6, during the first 28 h of SSF, the OUR and CER profile increased rapidly, which indicates that both fungi are extensively growing. Clearly reflected in OUR and CER curves, the stationary phase, for both



**Fig. 5** **a** Profiles of RQ in multi-stacked tray SSB systems with the fungi *A. awamori* [AA] and *A. oryzae* [AO] with different initial moisture content arrangements. The black straight line represents RQ = 1. **b** Respiratory quotient (RQ) during SSF of *A. awamori* (square) and *A. oryzae* (filled square) on wheat bran using four tray SSBs with different initial moisture content arrangement. The red dash line represents RQ = 1. The results are shown as mean  $\pm$  standard deviation, and number of replications is three ( $n=3$ )

fungi in all experiment tested, was very short and lasted for 1–2 h of fermentation time. The curves showed a sigmoidal shape and reached the maximum value of  $O_2$  consumption and  $CO_2$  generation between approximately 24 and 30 h of fermentation. After this peak, the gradual decrease of  $O_2$  and  $CO_2$  concentrations indicated the beginning of the last phase, which corresponds to slower fungal growth. This is a very slow progress phase because the active spores still consume the  $O_2$  (plus other nutrients) and produce  $CO_2$ . Ikasari and Mitchell (1998), using the *Rhizopus oligosporus* SSF process with rice bran as the substrate, observed similar results and reported an increase in OUR and heat evolved around 24 h, as well as a significance decrease was reported at 72 h fermentation. This finding was supported by Arora et al. (2018), where a correlation was obtained between OUR and biomass concentration of *R. oligosporus* MTCC 1987 during phytase production in an intermittently mixed novel SSF bioreactor.

In this case, it was considered that the high content of readily available carbon source in wheat bran (starch in this case) was the reason for the comparatively high OUR and CER indicating high fungal growth during the first 24–30 h of SSF. Also, in SSE, fungi cannot grow continuously as the amount of nutrients available is finite and waste products will accumulate. These conditions might be the reason for the deceleration of fungal growth after achieving maximum activity. Growth still continued at a slow rate and did not eventually stop because of the presence of nutrients. At the same time, inhibitory metabolites built up due to waste accumulation in the system. The temperature often reaches values which severely limit growth or even kill the microorganism (Mitchell et al. 2002). Measurement of metabolic activities (OUR and CER) is the easiest to operate online. If both values are used, it is possible to determine the RQ of the microorganism, which can provide information about the metabolic state (discussed above).



**Fig. 6** Indirect fungal growth of multi-stacked tray solid-state bioreactor using four strategies of initial moisture content with respect to oxygen uptake rate [OUR: red lines] and carbon dioxide evolution rate [CER: black lines] with *A. awamori* [AA] and *A. oryzae* [AO]

### Temperature distribution during SSF

As the positions of multi-trays vary, so does their temperature. As a result, each tray is exposed to several temperature cycles during fermentation. The temperature of the fermented bed in the tray at position 1 is predicted to be the lowest and the temperature increases gradually in the trays above. The fermented bed in the tray at position 6 is predicted to reach the highest temperature. The moistened air blown into the bioreactor is maintained at 30 °C (the optimum temperature for *A. awamori* and *A. oryzae*), in an attempt to control bed temperatures. The temperature of the moistened air increases with height and this increases the air water holding capacity, causing some evaporation to occur in the trays. This phenomenon can be proved by the final profile of moisture content (especially in the *A. oryzae* culture). However, the average of fermented bed temperature was presented in this system from six temperature profiles. Growth in all trays is almost similar, with various growth profiles being quite close to the profile for the average of all six trays. This result is clearly observed in the culture of *A. oryzae* (Exp 1[AO], 2[AO] and 3[AO]).

Figure 7 shows the average temperature profiles for both fungi during SSF on wheat bran with different initial moisture content arrangements. During the period 6–12 h, various profiles of temperature were observed. After that period, the temperature rose gradually as an indication of increase in metabolic activity. Exp. 1[AA] recorded temperature peaks of between 32 and 35 °C, while Exp. 2[AA] and 3[AA] recorded a maximum temperature of almost 39 °C. Exp. 1[AA], 2[AA] and 3[AA] recorded temperature profiles above 30 °C until the end of the experiments. Exp. 4[AA] recorded temperatures below 30 °C and only reached maximum (32 °C) at 32 h. This indicates that growth in Exp. 4[AA] was very slow compared to other experiments because of lack of water in the fermented bed.

In Exp. 1[AO], 2[AO] and 3[AO], the maximum temperatures reached were 41.57, 40.71 and 41.20 °C after 26 h. As the initial moisture content was low in Exp. 4[AO] (15% moisture content) and because of slow growth, as observed with the *A. awamori* culture, the maximum temperature reached was only 33.42 °C.

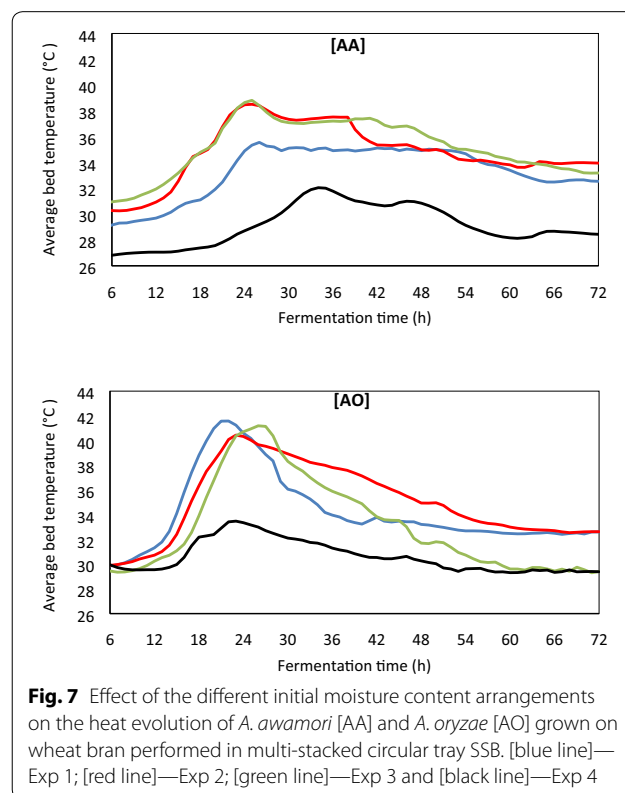
The growths of both fungi more or less corresponded with each other. The cultures in every tray all started growing slowly and entered a rapid growth phase together. As a result, fermented cultures in every single circular tray reached their peak generation at about the same time. It has to be kept in mind that the chosen initial moisture content in Exp. 1 and Exp. 2 was assumed to be optimal to support growth of both fungi. Only in Exp. 4, the limited water content (15%) in the culture system was the reason the growth of both fungi was slow.

In this multi-stacked circular tray SSB, proper stacking and sealing of the attached trays to a lip at the bottom of the tray would ensure a good sealing against the supporting tray above. If proper sealing is not achieved, then the air will pass around outside the trays rather than be forced to flow upwards through them. The temperature and moisture gradients can lead the fermented substrate at different tray heights to have very different temperature profiles.

Stulova et al. 2015 and Kabanova et al. (2012) developed a method for the investigation of bacterial growth in solid-state matrices using isothermal microcalorimetry. They showed that microcalorimetry measurement was a very powerful instrument in studying quantitative detailed peculiarities of SSF and that it is possible to calculate heats evolved during growth with number of bacteria in the sample.

### Effect of arrangement initial moisture content on spore production

The profile effect of the arrangement of initial moisture content of the multi-stacked circular tray SSB on spore production is shown in Fig. 8. In Exp. 1 [initial moisture content arrangement from low (50%) to high (75%)] with *A. awamori*, trays at positions 1, 2, 3 and 4 showed high production of spores [range between 2.3 and 2.7 × 10<sup>10</sup>



spores/g initial dry weight (IDW)]. However, trays at position 5 and 6 showed decreased spore production (range between  $1.3$  and  $1.7 \times 10^{10}$  spore/g IDW) due to high moisture content (70 and 75%, respectively). In the case of *A. oryzae*, trays at positions 1, 2, 3, 4, 5 and 6 showed spore production within the range of  $1.3$ – $2.1 \times 10^9$  spores/g IDW.

In Exp. 2 [initial moisture content arrangement from high (75%) to low (50%)], *A. awamori* recorded low spore production in trays at positions 1 and 2 (range between  $1.1$  and  $1.4 \times 10^{10}$  spores/g IDW) (initial moisture content 70 and 75%, respectively). It was assumed that conditions of the solid substrate are too wet due to high moisture content and not suitable to support growth of *A. awamori*. This condition also explains why the spore production was lower in trays at position 5 and 6 in Exp. 1 with fermented *A. awamori*. However, there were increments in spore production within the range  $2.5$ – $3.7 \times 10^{10}$  spores/g IDW for trays at positions 3, 4, 5 and 6 (initial moisture content 65, 60, 55 and 50%, respectively). In the case of *A. oryzae*, the final spore production was within the range  $2.3$ – $2.9 \times 10^9$  spores/g IDW for trays at positions 1 until 6.

In Exp. 3 (initial moisture content was set up at 65% in every tray), the spore production with *A. awamori* was among the highest being within the range of  $2.2$ – $2.3 \times 10^{10}$  spores/g IDW in trays at positions 1, 2, 3, 4, 5 and 6. The same observation was observed in trays at positions 1–6 for *A. oryzae* where the range of spore production was within the range  $3.0$ – $4.6 \times 10^9$  spores/g IDW. It was found in the previous study that at 65% initial moisture content was the best for *A. awamori* and *A. oryzae* for spores and enzyme production (Abdul Manan and Webb 2016a).

Finally, in Exp. 4 (when initial moisture content was set up at 15% in every tray), the final spore production for fermented *A. awamori* was within the range  $1.0 \times 10^9$ – $2.0 \times 10^{10}$  spores/g IDW in trays at positions 1 until 6. The same observation was observed in trays at positions 1, 2, 3, 4, 5 and 6 for fermented *A. oryzae* with a range of spore production between  $2.0$  and  $4.0 \times 10^8$  spores/g IDW. This might be due the very low initial moisture content. In this case, *A. awamori* and *A. oryzae* are not favoured at very low 15% initial moisture content, as fungal growth on solid particles without the presence of free water and cause slower growth.

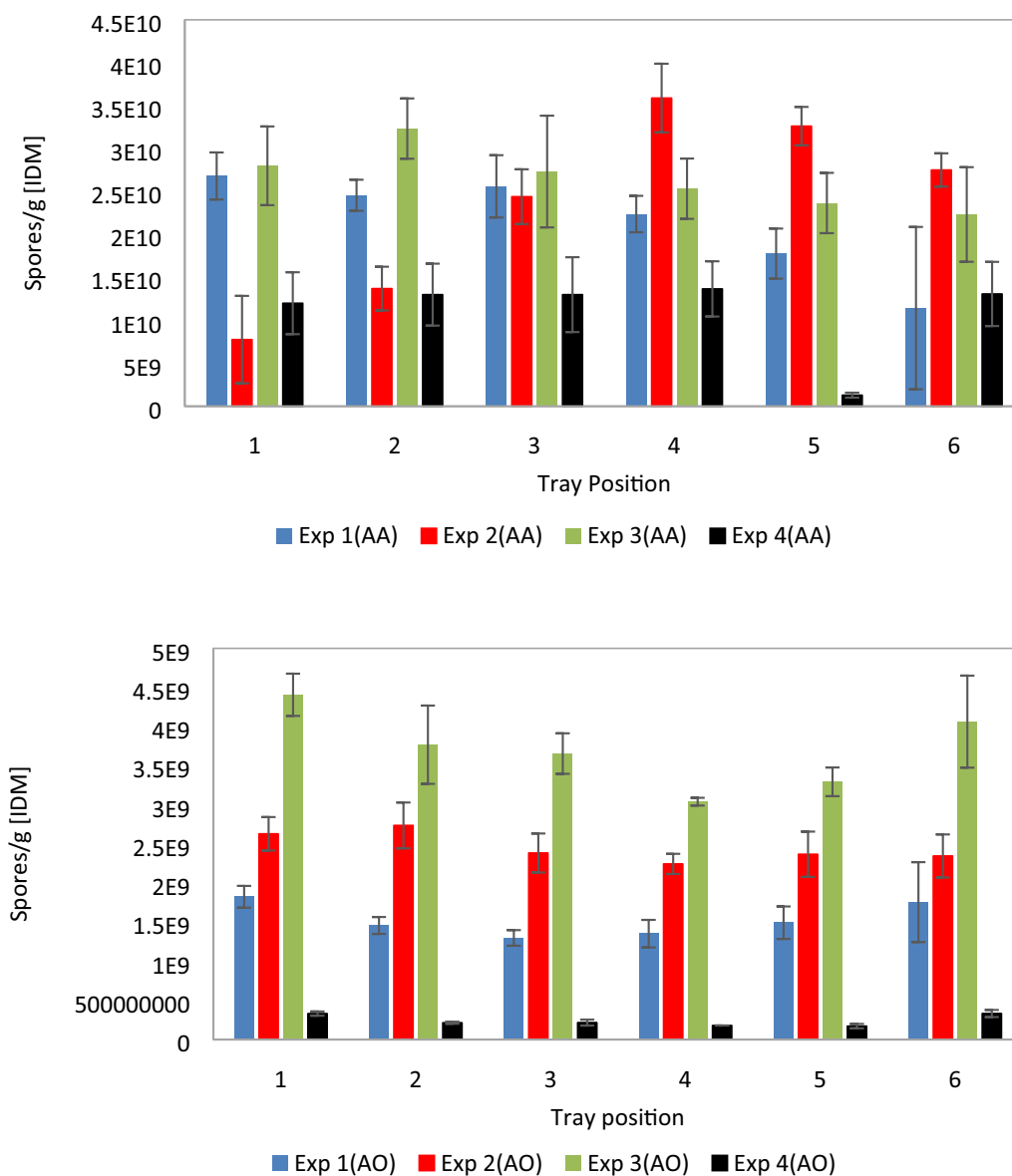
During this study, it was observed that the use of *A. awamori* was favourable to growth and producing spores but less favourable to mycelial formation, whereas *A. oryzae* produced less spores and was favourable to formation of mycelial material during SSF. The higher mycelial formation led to a high concentration of glucosamine in all samples isolated from *A. oryzae* cultures. This is might be

the reason for *A. oryzae* being able to retain water better than *A. awamori* through mycelial material. The *A. oryzae* culture was observed to be very excellent in protease production in all experiments using the tray systems but very poor in glucoamylase, xylanase and cellulase production. According to Cruz-Quiroz et al. (2017), the forced air supply into SSF did not improve the production of spores from *Trichoderma* since similar results were obtained in the process without forced aeration. Prior to starting the work, this study showed the importance of considering the initial moisture content in assessing the productivity of any culture. The arrangement of initial moisture involved in this type of tray position showed a good insight of overall performance of *A. awamori* and *A. oryzae*. This might determine the success of the SSF process.

#### Effect of arrangement initial moisture content on enzymes' production

This work also presents the SSF process of wheat bran using *A. awamori* and *A. oryzae* for the production of an enzymatic consortium containing glucoamylase, protease, cellulase and xylanase for each initial moisture content arrangement performed. Activity levels for enzymes that were produced for each arrangement are shown in Fig. 9. These results proved that *A. awamori* is an excellent fungus for glucoamylase, xylanase and cellulase but for protease production. *A. oryzae* is excellent in protease production but poor for glucoamylase, xylanase and cellulase production. This might be because the *A. oryzae* does not favour wheat bran as a main substrate and does favour on rapeseed meal (Abdul Manan and Webb 2016a). *A. oryzae* seemed to very much favour a solid substrate with high protein content (as a nitrogen source), while *A. awamori* seemed to very much favour a solid substrate with high carbohydrate content (as a carbon source) (Abdul Manan and Webb 2016a). Rodriguez Couto et al. (2003) showed that on barley bran as medium, the tray configuration led to the highest laccase activity when compared to a packed-bed bioreactor. However, in whatever studies, for a better monitoring and good comparison, other solid substrates, different types of microorganisms or fungi and different types of bioreactors, may be used. The tray bioreactor is often considered the most suitable option, as it is low-cost equipment with low maintenance costs. A number of enzymes have been successfully produced using tray bioreactors, especially xylanases, cellulases, laccases and pectinases (Khanahmadi et al. 2018).

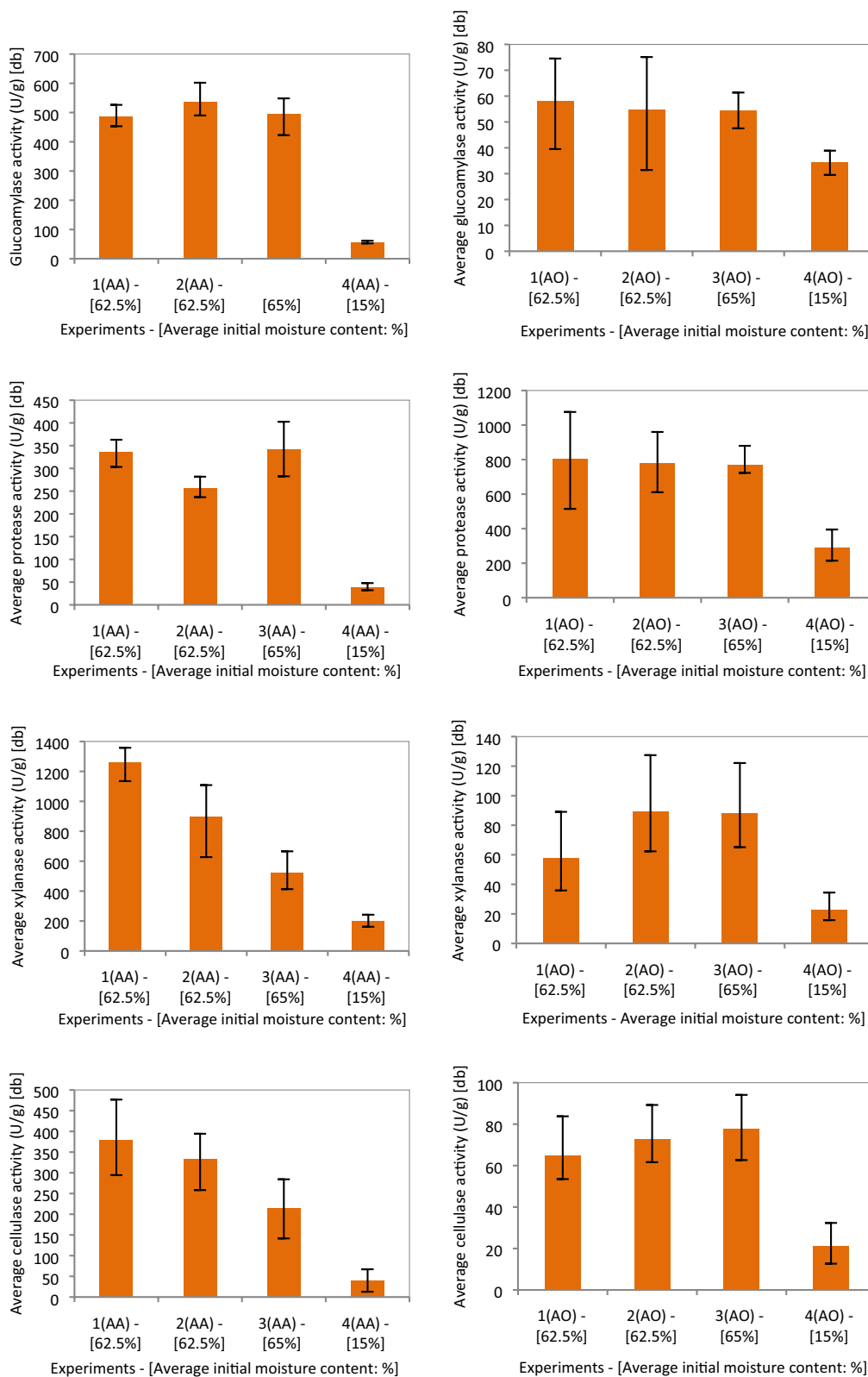
In this study, prior to starting the SSF process, moisture content was homogeneously distributed among the solid particle's substrate. Arrangement of initial moisture content in the system might explain this behaviour.



**Fig. 8** Fermentation summary of average spores production from multi-stacked circular tray solid-state bioreactor with different moisture content arrangements. [AA]—*A. awamori* and [AO]—*A. oryzae*. The results are shown as mean  $\pm$  standard deviation, and number of replications is three ( $n = 3$ )

Production of all enzymes seemed very poor with Exp. 4 for both *A. awamori* and *A. oryzae*, where the initial moisture content was very low at 15% in every tray. We conclude that proper distribution of initial moisture content to start the SSF gives an advantage to performance of the fermentation process. Preparation of the solid substrate and subsequent initial moisture content are very important because the initial conditions strongly affect the entire SSF process (Maurya et al. 2012). In SSF, providing the right initial moisture

content will definitely involve intensive labour due to the necessity of making homogenous conditions, especially on a large scale. Cunha et al. (2012) proposed an unconventional pre-culture with an initial fungal growth phase under SSF, followed by transition to submerged fermentation by adding the liquid culture medium to the mycelium grown on solid substrate. The proposed technique improved an approximately three-fold improvement in endoglucanase productivity compared to conventional submerged fermentation.



**Fig. 9** Fermentation summary of average enzymes production from multi-stacked circular tray solid-state bioreactor with different initial moisture content arrangements. [AA]—*A. awamori* and [AO]—*A. oryzae*. The results are shown as mean  $\pm$  standard deviation, and number of replications is three ( $n = 3$ )



## Conclusion

The aim of this paper was to highlight the effect of arrangement initial moisture content of the newly designed multi-stacked circular tray SSB. The strategy with different initial moisture content arrangements with supply moistened air was able to supply an amount of water and promote effective O<sub>2</sub> and mass transfer to fermenting solid in the bioreactor. However, the effect on the final moisture content was very different for the two fungi. The kinetic behaviour of the fungal culture, using raw CO<sub>2</sub>, which represents an accumulation term, was integrated with respect to time and fitted to a Gompertz model, a log-like equation. The model can be used to generate parameter values that may be used to verify the experimental data and also to simulate and optimise the process. The data for maximum CO<sub>2</sub> concentration ([CO<sub>2max</sub>]), accumulated from all experiments, were generally highly correlated with spores and enzymes (glucosylase, protease, xylanase and cellulase) production. This study showed that the rate of O<sub>2</sub> consumption and CO<sub>2</sub> evolution was generally highly correlated. The multi-stacked circular tray SSB with different initial moisture content arrangements exhibited greater RQ values. RQ values < 1 were recorded during SSF for both fungi. This indicates relatively high O<sub>2</sub> consumption during the current fungal SSF on wheat bran. Different types of fungi and fermentation conditions showed varying RQ values during metabolic activity and indicated variation in fungal growth. A linear relationship between [CO<sub>2max</sub>] and RQ was observed, which can be explained by varying fungal metabolism in fermentation conditions. The strategy presented in this study allowed quantitative evaluation of the effect of the forced internal moist air circulation on the removal of metabolic heat. With the proposed strategy, it was possible to maintain the bed temperatures at the optimum value for growth. Initial moisture content arrangements with moistened air had a positive effect on temperature control during SSF. As mentioned earlier, in bioreactor models, the five most important environmental variables are moisture content, O<sub>2</sub> transfer, CO<sub>2</sub> evolved, monitoring temperature and heat removal of the bed, and five of them are intimately tied to the metabolic activity of the microorganism.

## Abbreviations

SSF: Solid-state fermentation; SSB: Solid-state bioreactor; SmF: Submerged fermentation; O<sub>2</sub>: Oxygen; CO<sub>2</sub>: Carbon dioxide; OUR: Oxygen uptake rate; CER: Carbon dioxide evolution rate; RQ: Respiratory quotient; Exp: Experiment; AA: *Aspergillus awamori*; AO: *Aspergillus oryzae*; Fig: Figure; IDW: Initial dry weight.

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## Authors' contributions

MAM performed all the experiments. MAM and CW conceived the idea, designed the study, and wrote the manuscript. Both authors read and approved the final manuscript.

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## Availability of data and materials

Not applicable.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## References

- Abdul Manan M, Webb C (2016a) Multi-enzymes production studies in single tray solid state fermentation with opened and closed system. *J Life Sci* 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. *J Life Sci* 8:420–429. <https://doi.org/10.17265/1934-7391/2016.08.008>
- Abdul Manan M, Webb C (2017a) Design aspects of solid state fermentation as applied to microbial bioprocessing. *J App Biotech Bioeng* 4(1):511–532. <https://doi.org/10.15406/jabb.2017.04.00094>
- Abdul Manan M, Webb C (2017b) A review paper: modern microbial solid state fermentation technology for future biorefineries for the production of added-value products. *Biofuel Res J* 16:730–740. <https://doi.org/10.18331/BRJ2017.4.4.5>
- Abdul Manan M, Webb C (2018a) Estimating fungal growth in submerged fermentation in the presence of solid particles based on colour development. *Biotech Biotechnol Equip* 32(3):618–627. <https://doi.org/10.1080/13102818.2018.1440974>
- Abdul Manan M, Webb C (2018b) Control strategies with variable air arrangements, forcefully aerated in single circular tray solid state bioreactors with modified Gompertz model and analysis of a distributed parameter gas balance. *Biotech Biotechnol Equip* 32(6):1455–1467. <https://doi.org/10.1080/13102818.2018.1530950>
- Alcantara SR, da Silva FLH (2012) Solid state fermentation process for polygalacturonase production using tray bioreactor. *Chem Eng Trans* 27:355–359. <https://doi.org/10.3303/CET1227060>
- Arora S, Singh P, Rani R, Ghosh S (2018) Oxygen uptake rate as a tool for on-line estimation of cell biomass and bed temperature in a novel solid-state fermentation bioreactor. *Bioprocess Biosys Eng* 41(7):917–929. <https://doi.org/10.1007/s00449-018-1923-2>
- Assamoi AA, Destain J, Delvigne F, Lognay G, Thonart P (2008) Solid-state fermentation of xylanase from *Penicillium canescens* 10–10c in a multi-layer-packed bed reactor. *App Biochem Biotechnol* 145:87–98. <https://doi.org/10.1007/s12010-007-8077-z>
- Barrios-Gonzalez J, Gonzalez H, Mejia A (1993) Effect of particle size, packing density and agitation on Penicillin production in solid state fermentation. *Biotech Adv*. 11(3):539–547. [https://doi.org/10.1016/0734-9750\(93\)90022-f](https://doi.org/10.1016/0734-9750(93)90022-f)
- Becerra M, Gonzalez-Siso MI (1996) Yeast β-galactosidase in solid-state fermentation. *Enz Microb Tech* 9:39–44
- Bhushan I, Kour M, Kour G (2019) Strategies to improve enzymes via solid-state fermentation. In: Srivastava M, Mishra PK, Upadhyay SN, Ramteke PW, Gupta VK (eds) Srivastava N. From current status to practical

- implementation. Springer International Publishing AG, Sustainable approaches for biofuels production technologies, pp 111–119
- Chen HZ, Xu J, Li ZH (2005) Temperature control at different bed depths in a novel solid-state fermentation system with two dynamic changes of air. *Biochem Eng J* 23(2):117–122. <https://doi.org/10.1016/j.bej.2004.11.003>
- Cooney CL, Wang DI, Mateles RI (2000) Measurement of heat evolution and correlation with oxygen consumption during microbial growth. *Biotechnol Bioeng* (Reprinted) 67(6):691–703. <https://doi.org/10.1002/bit.260110302>
- Cruz-Quiroz RD, Robledo-Padilla F, Aguilar CN, Roussos S (2017) Forced aeration influence on the production of spores by trichoderma strains. *Waste Biomass Valor* 8(7):2263–3370. <https://doi.org/10.1007/s12649-017-0045-4>
- Cunha FM, Esperanca MN, Zangirolami TC, Badino AC, Farinas CS (2012) Sequential solid-state and submerged cultivation of *Aspergillus niger* on sugarcane bagasse for the production of cellulose. *Biores Tech* 112:270–274. <https://doi.org/10.1016/j.biortech.2012.02.082>
- Feng L, Li Y, Chen C, Liu X, Xiao X, Ma X, Zhang R, He Y, Liu G (2013) Biochemical methane potential (BMP) of vinegar residue and the influence of feed to inoculum ratios on biogas production. *Biores* 8(2):2487–2498
- Govind R, Gao C, Lai L, Tabak HH (1997) Continuous, automated and simultaneous measurement of oxygen uptake and carbon dioxide evolution in biological systems. *Water Env Res* 69:73–80
- Hussein S, Halmi MEI, Ling APK (2017) The modified Gompertz model demonstrates a variable growth rate between two *Centella asiatic* phenotypes. *J Biochem Microb Biotechnol* 5(1):18–20
- Ikasari L, Mitchell DA (1998) Oxygen uptake kinetics during solid state fermentation with *Rhizopus oligosporus*. *Biotech Tech* 12(2):171–175
- Kabanova N, Stulova I, Vilu R (2012) Microcalorimetric study of the growth of bacterial colonies of *Lactococcus lactis* IL1403 in agar gels. *Food Microb* 29(1):67–79. <https://doi.org/10.1016/j.fm.2011.08.018>
- Khanahmadi M, Arezi I, Amiri M, Miranzadeh M (2018) Bioprocessing of agro-industrial residues for optimization of xylanase production by solid-state fermentation in flask and tray bioreactor. *Biocatal Agric Biotechnol* 13:272–282. <https://doi.org/10.1016/j.bcab.2018.01.005>
- Koyani RD, Rajput KS (2015) Solid state fermentation: comprehensive tool for utilization of lignocellulosic through biotechnology. *J Bioproc Biotech* 5(10):1–15. <https://doi.org/10.4172/2155-9821.1000258>
- Krishna C (2005) Solid-state fermentation systems—an overview. *Crit Rev Biotechnol* 25(1–2):1–30. <https://doi.org/10.1080/07388550590925383>
- Maurya DP, Singh D, Pratap D, Maurya JP (2012) Optimization of solid state fermentation conditions for the production of cellulase by *Trichoderma reesei*. *J Environ Biol* 33(1):5–8
- Mitchell DA, Berovic M (2010) Solid state bioprocessing. In: Berovic M, Enfors SO (eds) *Comprehensive bioprocess Engineering*. European Federation on Biotechnology: working group on bioreactor performance in collaboration with european section on biochemical engineering sciences. University of Ljubljana, Slovenia, pp 209–238
- Mitchell DA, Tongta A, Stuart DM, Krieger N (2002) The potential for establishment of axial temperature profiles during solid-state fermentation in rotating drum bioreactors. *Biotechnol Bioeng* 80(1):114–122. <https://doi.org/10.1002/bit.10356>
- Mitchell DA, Berovic M, Nopharatana M, Krieger N (2006a) Chapter 3—introduction to solid-state fermentation bioreactors. In: Mitchell DA, Krieger N, Berovic M (eds) *Solid-state fermentation bioreactors: fundamentals of design and operation*. Springer-Verlag, Berlin Heidelberg, pp 33–43
- Mitchell DA, Krieger N, Berovic M (2006b) Chapter 6: bioreactors: unaerated and unmixed. In: Mitchell DA, Krieger N, Berovic M (eds) *Solid state fermentation bioreactors: fundamentals of design and operation*. Springer-Verlag, Berlin Heidelberg, pp 65–76
- Mitchell DA, Lima Luz LF, Krieger N (2011) Bioreactors for solid-state fermentation. In: Moo-Yong M (ed) *Comprehensive biotechnology*. Elsevier, Amsterdam, pp 347–360
- Ravindran R, Jaiswal AK (2016) Review: microbial enzyme production using lignocellulosic food industry wastes as feedstock: a review. *Bioengineering* (Basel) 3(4):1–22. <https://doi.org/10.3390/bioengineering3040030>
- Robinson T, Nigam P (2003) Bioreactor design for protein enrichment of agricultural residues by solid state fermentation. *Biochem Eng J* 13(2–3):197–203. [https://doi.org/10.1016/S1369-703X\(02\)00132-8](https://doi.org/10.1016/S1369-703X(02)00132-8)
- Rodriguez Couto SR, Moldes D, Liebanas A, Sanroman A (2003) Investigation of several bioreactor configurations for laccase production by *Trametes versicolor* operating in solid-state conditions. *Biochem Eng J* 15(1):21–26. [https://doi.org/10.1016/S1369-703X\(02\)00180-8](https://doi.org/10.1016/S1369-703X(02)00180-8)
- Ruiz HA, Rodriguez-Jasso RM, Rodriguez R, Contreras-Esquivel JC, Aguilar CN (2012) Pectinase production from lemon peel pomace as support and carbon source in solid-state fermentation column-tray bioreactor. *Biochem Eng J* 65:90–95. <https://doi.org/10.1016/j.bej.2012.03.007>
- Skiadas CH, Skiadas C (2008) Comparing the Gompertz models with a first passage time density model. In: Skiadas CH (ed) *Advances in data analysis*. Springer, Birkhauser Boston, pp 203–209
- Soltan M, Elsamadony MS, Tawfik A (2017) Biological hydrogen promotion via integrated fermentation of complex agro-industrial wastes. *App Energy* 185:929–938. <https://doi.org/10.1016/j.apenergy.2016.10.002>
- Stulova I, Kabanova N, Krisciunaite T, Adamberg K, Laht TM, Vilu R (2015) Microcalorimetric study of the growth of *Streptococcus thermophilus* in renneted milk. *Front Microbiol* 6(79):1–13. <https://doi.org/10.3389/fmicb.2015.00079>
- Sukatsch DA, Dziengel A (1987) *Biotechnology: a handbook of practical formulae*. Longman scientific and technical. Wiley, New York, pp 104–105 (ISBN 0-582-98899-3)
- Torres-Mancera MT, Figueroa-Montero A, Favela-Torres E, Rosales-Zamora G, Nampoothiri KM, Saucedo-Castaneda G (2018) Online monitoring of solid-state fermentation using respirometry. In: Pandey A, Larroche C, Soccol CR (eds) *Current developments in biotechnology and bioengineering: current advances in solid-state fermentation*. Elsevier, Amsterdam, pp 97–108
- Vaseghi Z, Najafpour GD, Mohseni S, Mahjoub S (2013) Production of active lipase by *Rhizopus oryzae* from sugarcane bagasse: solid state fermentation in a tray bioreactor. *International J Food Sc Tech* 48(2):283–289. <https://doi.org/10.1111/j.1365-2621.2012.03185.x>
- Xie L, Chen H, Yang J (2013) *Conidia* production by *Beauveria bassiana* on rice in solid-state fermentation using tray bioreactor. *Adv Mater Res* 610:3478–3482. <https://doi.org/10.4028/www.scientific.net/AMR.610-613.3478>

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