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Review

A Critical Evaluation of Recent Studies on Packed-Bed Bioreactors for Solid-State Fermentation

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Abstract: Packed-bed bioreactors are often used for aerobic solid-state fermentation, since the forced aeration supplies O_2 and removes metabolic heat from the bed. Motivated by the potential for applications in biorefineries, we review studies conducted on packed-bed bioreactors over the last decade, evaluating the insights these studies provide into how large-scale packed beds should be designed and operated. Many studies have used low superficial air velocities and suffer from preferential airflow, such that parts of the bed are not properly aerated. Moreover, some studies have proposed ineffective strategies, such as reversing the direction of the airflow or introducing air through perforated pipes within the bed. Additionally, many studies have used narrow water-jacketed packed-bed bioreactors, but these bioreactors do not reflect heat removal in wide large-scale packed beds, in which heat removal through the side walls makes a minor contribution. Finally, we conclude that, although some attention has been given to characterizing the porosities, water sorption isotherms and volumetric heat and mass transfer coefficients of substrate beds, this work needs to be extended to cover a wider range of solid substrates, and work needs to be done to characterize how these bed properties change due to microbial growth.

Keywords: biorefinery; lignocellulosic biomass conversion; forced aeration; superficial air velocity; multi-layered bed; Zymotis bioreactor; heat transfer coefficient; mass transfer coefficient; computational fluid dynamics; scale-up

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1. Introduction

This review deals with aerobic solid-state fermentation undertaken in packed-bed bioreactors. In aerobic solid-state fermentation (SSF) systems, microorganisms are cultivated within a bed of solid particles that has a continuous gas phase in the spaces between the particles [1]. Most of the water in the system is sorbed within the solid particles or held within the microbial biomass, although some free water may be present as a thin film at the particle surface and there may be a few water droplets held within the interparticle spaces.

Aerobic SSF has been used for centuries to produce various traditional fermented foods, especially in Asia [1]. It has also attracted increasing attention over the last few decades for the production of a wide range of biotechnological products and is especially attractive for use in biorefineries for biomass conversion, including second generation [2G] biorefineries converting lignocellulosic biomass and third generation [3G] biorefineries converting algal biomass. In such biorefineries, SSF allows high solids loadings and reduced water demand compared to submerged liquid fermentation and is the most appropriate technology for producing metabolites from residual organic solids generated within the biorefinery [2]. As such, SSF has the potential to contribute to the economic and operational feasibility of large-scale biorefineries.

Various bioreactor types can be used for SSF processes, including tray bioreactors, packed-bed bioreactors, rotating-drum bioreactors and continuously agitated bioreactors. The general principles of design and operation of these different bioreactor types have been described in detail [1]. Of these bioreactor types, the packed-bed bioreactor has received much attention due to its ease of operation in the use and conversion of lignocellulosic biomass. Packed-bed bioreactors can be defined as bioreactors in which the bed of substrate particles remains static during the fermentation, and air is blown into the bioreactor, being forced to flow through the interparticle spaces (or "void spaces") of the bed in order to reach the air outlet. It is also possible to have "intermittently mixed packed-bed bioreactors" in which, as the name suggests, there are infrequent mixing events. Mixing events may be used to promote uniformity within the bed, to allow the addition of water to the bed, or to reseat the bed if preferential flow paths have formed within it. With the absence of mixing, or infrequent mixing, packed-bed bioreactors are particularly suitable for the cultivation of filamentous fungi, which often do not tolerate continuous or frequent mixing, due to the excessive damage caused to the developing hyphae. However, packed-bed bioreactors have also been used with unicellular microorganisms.

Figure 1 shows how a packed-bed bioreactor could be used to produce enzymes in a 2G biorefinery for lignocellulose conversion, while Figure 2 shows details of the design of the packed-bed bioreactor and auxiliary equipment. For applications in 2G or 3G biorefineries, packed-bed bioreactors need to hold hundreds or thousands of kilograms of solid substrate. At this scale, the key challenge is to remove the metabolic heat generated during growth of the microorganism, to prevent the bed temperature from becoming high enough to affect growth and product formation negatively. High temperatures are desirable in composting-type SSF processes, but composting-type processes are not addressed in this review. This review limits itself to aerobic SSF processes involving a single process organism, in which it is desirable to maintain the bed temperature as close as possible to the optimum temperature for growth and product formation.

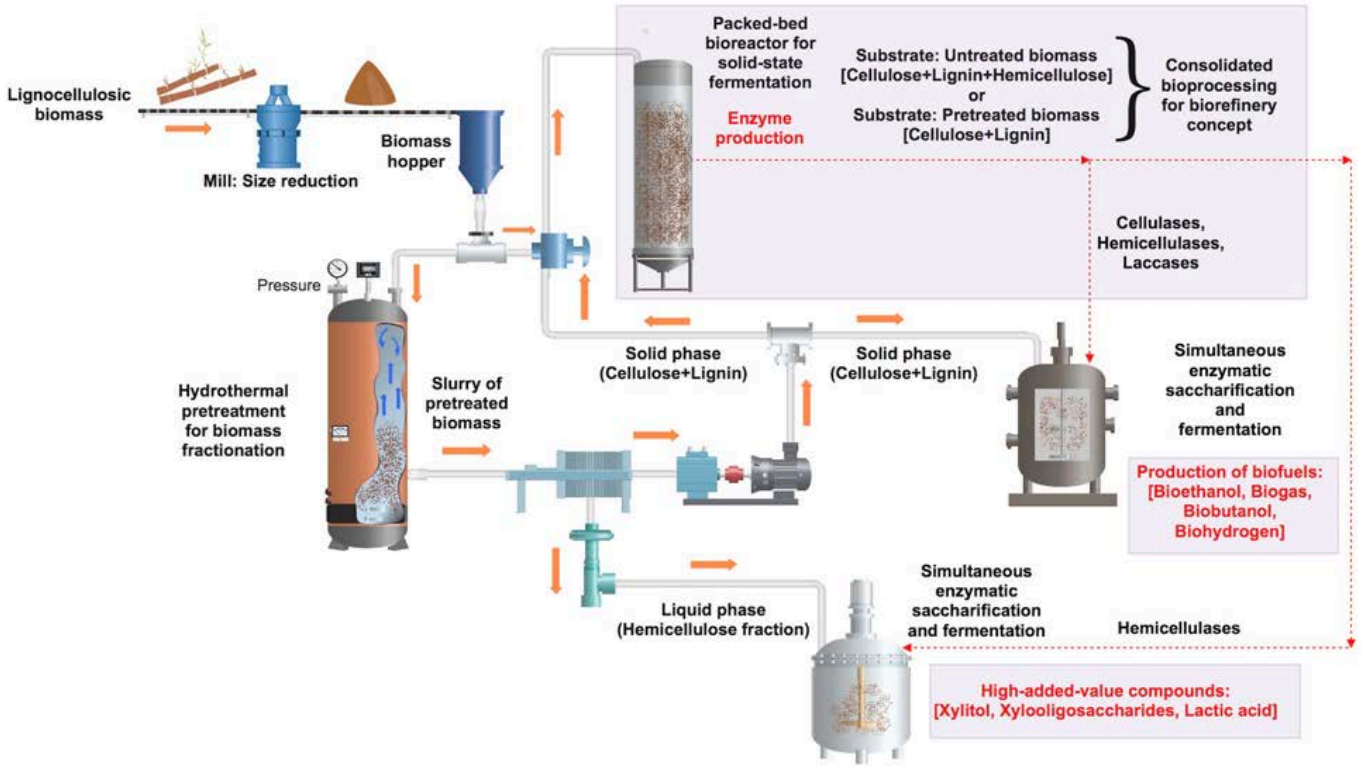

Figure 1. Schematic representation of a general biorefinery process for lignocellulosic biomass fractionation showing the production of enzymes by SSF, using a packed-bed bioreactor.

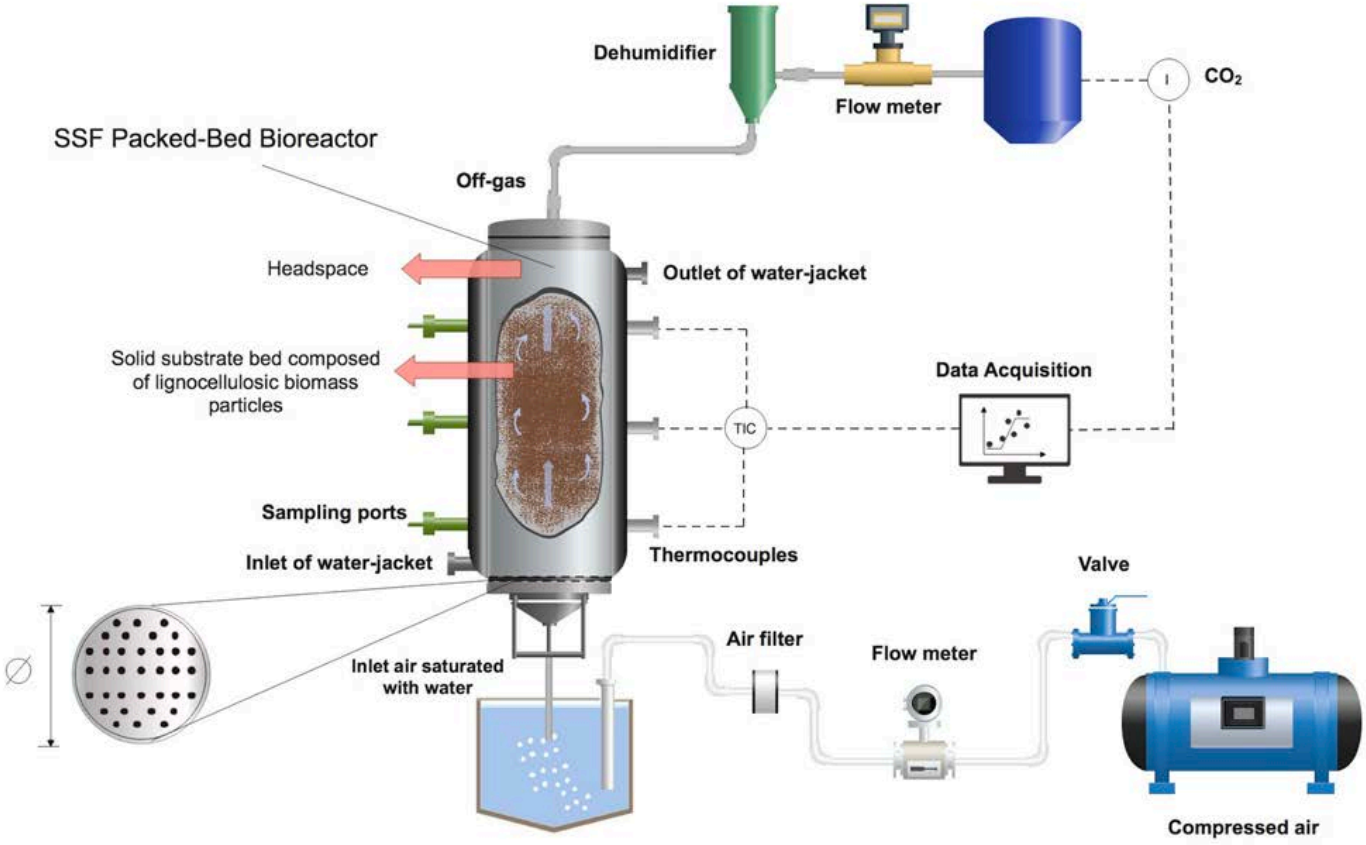

Figure 2. Schematic representation of a typical SSF packed-bed bioreactor for lignocellulosic biomass conversion.

This review focuses on studies of packed-bed bioreactors and intermittently mixed packed-bed bioreactors conducted over the last decade or so. It evaluates whether these studies have given insights into how to optimize the design and operation of packed-bed bioreactors for aerobic SSF processes.

In this review, aeration of the bed is characterized in terms of the "nominal superficial air velocity", which is calculated as the reported volumetric airflow rate divided by the horizontal cross section of the empty bioreactor and represented by V_Z.

2. Traditional Designs for Packed-Bed Bioreactors

Several authors have investigated designs for packed-bed bioreactors that have been studied for several decades. This section evaluates whether these studies provide new insights into the design and operation of large-scale packed-bed bioreactors.

2.1. Will Jacketed Packed-Bed Bioreactors Be Useful at a Large Scale?

Over recent years, several studies have been conducted with jacketed packed-bed bioreactors (Table 1) [3–9]. Several of these studies had the intention of guiding scale-up but were conducted with relatively narrow bioreactors. For example, Zanelato et al. [3] and Casciatori-Frassatto et al. [9] used 7.62 cm diameter columns, while Casciatori and Thoméo [4] used a 4.7 cm diameter column to study bed–wall heat transfer. Similarly, Ranjbar and Hejazi [5] used a 6 cm diameter column. Slightly wider columns have been used more recently. Although Perez et al. [6] and Cunha et al. [7] did use narrow columns of 10 cm diameter or less, both also undertook fermentations in wider water-jacketed columns, 20 cm in diameter. Using an air jacket instead of a water jacket, Chysirichote [8] used a 30 cm diameter bioreactor to cultivate *Aspergillus niger*.

Table 1. Studies conducted in jacketed packed-bed bioreactors with the aim of guiding scale-up.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Myceliophtora sp. Zanelato et al. [3]	Sugarcane bagasse (70%) and wheat bran (30%); 45 g (presumably wet) per module IMC = 75% (presumably wet basis)	Column: 5 water-jacketed modules, each h = 10 cm and ∅ = 7.62 cm. Total h_{bed} = 80 cm Water jacket at (i) 45 °C or (ii) 50 °C (same temperature as the inlet air) Air at (i) 45 °C or (ii) 50 °C. V_Z = 0.49 cm s^{-1}	Temperature control not good, considering the small column diameter. For the fermentation performed with the water jacket and inlet air at 45 °C, T_{bed} peaked at 51 °C
None Casciatori and Thoméo [4]	(1) Sugarcane bagasse (2) Sugarcane bagasse (20%), orange pulp and peel (40%) and wheat bran (40%) IMC = 70%	Column h = 21 cm, ∅ = 4.67 cm h_{bed} up to 18 cm Water jacket at 65 °C Air at 40 °C V_Z from 6.5 to 19.5 cm s^{-1}	Focus was on wall–bed heat transfer, which is not important in wide packed beds. Wall effects occurred due to the small bed diameter. V_Z values are of the order necessary for good control of T_{bed}
Pseudomonas aeruginosa Ranjbar and Hejazi [5]	Mixture (0.34 kg) of dried extracted oil-corn germ meal (70%) and corn bran (30%) IMC = 70%	Column h = 60 cm, ∅ = 6 cm Water jacket at 30 °C Humidified air at 30 °C V_Z = 1.67 cm s^{-1}	Spatiotemporal temperature profiles suggest uniform air flow, with $T_{5cm} < T_{20cm} < T_{45cm} < T_{60cm}$ throughout the fermentation. However, temperature control poor, with T_{bed} reaching 43 °C
Myceliophtora thermophila Perez et al. [6]	Sugarcane bagasse (70%) and wheat bran (30%) (1) Bench scale = 45 g per module (2) Pilot scale = 620 g per module IMC = 75% Bed porosity = 0.75	(1) Bench-scale column: 8 water-jacketed modules of h = 10 cm and ∅ = 7.62 cm. Total h_{bed} = 80 cm. V_Z up to 0.426 cm s^{-1} (2) Pilot-scale column: 4 water-jacketed modules of h = 20 cm and ∅ = 20 cm. Total h_{bed} = 80 cm. V_Z = 0.213 cm s^{-1} Water jacket at 45 °C Saturated air at 45 °C	Spatiotemporal temperature profiles suggest non-uniform airflow occurred in pilot-scale column. V_Z < 1 cm s^{-1}
Metarhizium anisopliae Cunha et al. [7]	Rice (90%) and bagasse (10%) (1) Narrow bioreactor: 400 g of solids (360 g rice and 40 g bagasse) (2) Wide bioreactor: 4500 g of solids (4050 g rice and 450 g bagasse) IMC = 48% Bed porosity = 0.63	(1) Narrow column: 3 water-jacketed modules, each h = 10 cm and ∅ = 10 cm. Total h_{bed} = 33 cm. V_Z = 0.21 cm s^{-1} (2) Wide column: 3 water-jacketed modules, each h = 20 cm and ∅ = 20 cm. Perforated metal plates between modules. Total h_{bed} = 69 cm. V_Z = 0.64 cm s^{-1} Water jackets at 28 °C	Spatiotemporal temperature profiles suggest non-uniform airflow occurred in both bioreactors. V_Z < 1 cm s^{-1}

Table 1. *Cont.*

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Aspergillus niger Chysirichote [8]	Banana peel, 3.5 kg (unclear if wet or dry mass)	Column h = 30 cm and ∅ = 30 cm. h_{bed} ~15 cm. Air jacket fed with air at 30 °C Inlet air at 30 °C V_Z = 0.5 cm s^{-1}	Without air jacket, T_{bed} reached 42 °C. With air jacket, T_{bed} peaked at ~38 °C. In both cases, peak $T_{bed} > T_{air\ outlet}$, suggesting non-uniform airflow occurred.
Myceliophthora thermophila Casciatori-Frassatto et al. [9]	Sugarcane bagasse (70%) and wheat bran (30); 45 g per module IMC = 75%	Column: 8 water-jacketed modules, each h = 10 cm and ∅ = 7.62 cm. Total h_{bed} = 80 cm Water jacket at 45 °C Moist air at 45 °C V_Z = 1.46 cm s^{-1}	Large variations in production of cellulases with bed height Temperature controlled well, remaining below 47 °C Spatiotemporal temperature profiles suggest non-uniform airflow: $T_{20cm} > T_{50cm} > T_{80cm}$, (opposite of expected gradient for bottom-to-top airflow)

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). Bed porosity is cm^3-voids cm^{-3}-bed. [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor.

Although heat removal to the jacket can contribute significantly to bed cooling in relatively narrow bioreactors, these authors did not explore how water jackets or air jackets might be used at the much larger scales that would be used in biorefineries, with hundreds or thousands of kilograms of substrate. They ignored the fact that the most likely scale-up strategy for packed-bed bioreactors will be to maintain bed heights at less than one meter and increase the bed diameter [10,11]. With this scale-up strategy, large-scale bioreactors are likely to be several meters in diameter. At this scale, the removal of heat to a jacket at the bioreactor wall will make a negligible contribution to overall bed cooling, and temperatures will be reduced only in the region within a few centimeters of the wall [12]. Given this situation, if one wishes to use a narrow packed-bed bioreactor to generate data for guiding scale-up, the best strategy is to insulate the side walls, mimicking the negligible contribution that bed–wall heat transfer will make to metabolic heat removal at a large scale [13].

Bed–wall heat transfer can be promoted in pilot- and large-scale packed-bed bioreactors: in the Zymotis bioreactor, designed 30 years ago, the bed is divided by vertical heat transfer plates spaced about 5 cm apart. This design was originally demonstrated with 40 kg of moist solids of lignocellulosic biomass [14], but it has not received much attention recently, although a patent application was made for a modified version [15]. This may be due to difficulties in ensuring uniform airflow through the bed: if preferential airflow paths develop due to bed shrinkage, then the closely spaced heat transfer plates make it impractical to agitate the bed to reseat it [16]. However, if this problem can be overcome, it remains a promising bioreactor design for large-scale biomass conversion.

2.2. The Uniformity of Airflow through Packed-Bed Bioreactors Is Important

If one is doing studies in a small-scale packed-bed bioreactor with the intention of generating data that can be used to guide scale-up, then it is necessary for the small-scale packed-bed bioreactor to function properly. In other words, the air must flow uniformly from the air inlet to the air outlet, through the porous matrix of the bed itself. It must not

flow through preferential flow paths, such as cracks in the bed or gaps between the bed and the wall, a phenomenon known as channeling.

The temperature profile within the bed between the air inlet and the air outlet can be used to identify if channeling is occurring: If a packed-bed bioreactor is supplied with saturated air (i.e., air with 100% relative humidity), and the air flows uniformly through the bed, then the combination of the production of waste metabolic heat and heat removal by convective flow leads to a monotonic rise in the bed temperature between the air inlet and the air outlet [1]. The air is usually blown into the bottom of the reactor and leaves at the top; in this case, at any particular time during growth, the bed temperature should rise with increasing bed height.

Several studies report axial temperature gradients that give insights into the uniformity of airflow through the bed (Table 2) [10,17–20]. The expected monotonic rise in bed temperature occurred in the fermentation by Karimi et al. [17], where, during the greater part of the fermentation, $T_{3cm} < T_{6cm} < T_{9cm} < T_{12cm} < T_{15cm}$ (the subscript indicates the bed height at which the temperature was measured). However, in some studies, a monotonic increase in bed height was not maintained throughout the cultivation, indicating that channeling occurred [10,18–20]. For example, in the study of Castro et al. [19], at the time of peak heat production at 48 h, the bed temperature increased monotonically with bed height ($T_{5.6cm} < T_{11.6cm} < T_{17.6cm} < T_{23.6cm}$). However, at 72 h, the order had inverted ($T_{23.6cm} < T_{17.6cm} < T_{11.6cm} < T_{5.6cm}$). In the study of Melikoglu et al. [20] with a 15 cm high bed, the temperature at 11.9 cm bed height stayed around 28–30 °C throughout the fermentations, while the temperature at 6.4 cm bed height increased during the fermentations, reaching peak values between 36 and 37 °C for all three superficial air velocities tested: 0.07, 0.13 and 0.40 cm s^{-1}. In the study of Melikoglu et al. [20] with a 30 cm high bed, the temperature profiles were also strange. For all three superficial velocities tested, 0.33, 0.50 and 0.99 cm s^{-1}, the bed temperatures at four heights (5.5, 9.4, 13.8 and 18.2 cm) increased from their initial value of 30 °C. At the superficial velocity of 0.33 cm s^{-1}, the temperature gradient with bed height was not monotonic: at the time of peak heat generation, the temperatures at all bed heights were around 37 °C, but the temperature at the top of the bed was lower than the other temperatures. At the superficial velocity of 0.50 cm s^{-1}, the three-lowest bed heights reached maximum temperatures around 37 °C, while the highest bed height reached a maximum temperature around 35 °C. At the superficial velocity of 0.99 cm s^{-1}, all bed heights reached maximum temperatures around 35–36 °C, but with the temperature at the top of the bed being lower than the temperature at the bottom of the bed.

Table 2. Studies in which axial temperature gradients give insights into the uniformity of airflow.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Aspergillus niger Karimi et al. [17]	Milled wheat bran (250 g) IMC = 55%	Column h = 20 cm, ∅ = 2.5 cm. Placed in a 30 °C water bath Air saturated at 30 °C. V_Z = 1.70 cm s^{-1}	Focus on porosity changes during the fermentation. Spatiotemporal temperature profiles indicate uniform airflow through bed. Peak T_{bed} = 33 °C
Bacillus subtilis Piedrahíta-Aguirre et al. [18]	Mixture (100 g, presumably dry mass) of defatted soybean meal (64%), wheat bran (13%) and rice (23%) IMC = 70%	Column h = 30 cm, ∅ = 5 cm, h_{bed} = 15 cm. Moist air saturated at 30 °C. V_Z = 0.34 and 0.68 cm s^{-1}	Maximum T_{bed} reached 33–33.5 °C (difficult to interpret this result because T_{air} fluctuated by 3–4 °C). Spatiotemporal temperature profiles suggest non-uniform airflow

Table 2. *Cont.*

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Aspergillus awamori Castro et al. [19]	Babassu cake (400 g) IMC = 62%	Column h = 30 cm, ∅ = 10 cm. h_{bed} = 23.9 cm No water jacket. Room temperature = 22 °C Humidified air at 22.9 °C. V_Z up to 2.2 cm s^{-1}	Temperature control very poor, with peak T_{bed} ~45 °C At the time of peak heat production at 48 h, T_{bed} increased monotonically with bed height, but at 72 h, T_{bed} decreased monotonically with bed height.
Aspergillus awamori Melikoglu et al. [20]	Pieces (20 mm) of waste bread: (1) 80 g; (2) 170 g IMC = 64%	Columns water-jacketed with silicon tubing (1) h = 15 cm, ∅ = 8 cm, h_{bed} = 10 cm. V_Z from 0.07 to 0.40 cm s^{-1} (2) h = 30 cm, ∅ = 8 cm, h_{bed} = 20 cm. V_Z from 0.33 to 0.99 cm s^{-1} Air 40% RH. Temperature presumably ~30 °C	Temperature control not good, considering the small bioreactor size (1) T_{bed} peaked at 36–37 °C (2) T_{bed} peaked at 36–38 °C V_Z < 1 cm s^{-1}
Aspergillus niger Pitol et al. [10]	(1) 20 kg wheat bran (2) 18 kg wheat bran + 2 kg sugarcane bagasse (3) 27 kg wheat bran + 3 kg sugarcane bagasse IMC = 62%	Rectangular fermentation box, base 70 cm × 60 cm, h = 50 cm (1) h_{bed} = 23 cm (2) h_{bed} = 27 cm (3) h_{bed} = 40 cm Saturated air at 30 °C V_Z = 9.9 cm s^{-1}	Bed shrank away from walls, allowing preferential airflow around the bed. T_{bed} reached values as high as 47 °C Poor uniformity of pectinase production

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor. RH is the relative humidity of the inlet air.

Although it has been recognized for many years that channeling in packed-bed bioreactors can lead to poor aeration of the bed [1], the possibility of uneven airflow in the absence of visible cracks in the bed or gaps between the bed and the side walls was raised only recently in the context of SSF. One important phenomenon is the "wall effect", in which the presence of the wall leads to a higher porosity (i.e., lower packing density) in the regions near the wall [4]. As the ratio of the particle diameter to the column diameter increases, there is an increase in the fraction of the cross-sectional area of the bed that has a higher porosity due to the wall effect. The wall effect is therefore exacerbated when the substrate is composed of long fibers, such as sugarcane bagasse, which are often added as inert materials to ensure that the bed is sufficiently porous [4]. Due to the wall effect, if one wants to generate data for guiding scale-up, it is best to avoid using narrow packed-bed bioreactors, with diameters of 10 cm or less.

In the modeling of relatively narrow packed-bed bioreactors that suffer from wall effects, one possibility is to model two zones, a higher-porosity zone with an annular cross-section located closer to the wall, and a lower-porosity zone with a circular cross section located in the center of the bed [21,22]. Recently, Gómez-Ramos et al. [23] took wall effects into account, developing a model that described the variation of void fractions near the bioreactor wall and the resulting effect on the permeability of the bed and the superficial velocity of the air. However, the model was not used to describe a fermentation.

Non-uniform airflow can even occur in larger bioreactors, in which one would expect wall effects to be minor, such as the pilot bioreactor of Finkler et al. [11], which has a

rectangular cross section 60 cm by 70 cm, with wheat bran representing 90% (by mass) of the substrate. In cooling, heating and drying experiments conducted in the absence of growth, it was only possible to fit a heat and mass transfer model to temporal temperature profiles obtained at different positions of the bed by assuming that the airflow rate was not uniform across the whole cross section of the bed [11]. Although the nominal airflow rate was 0.1 kg-dry-air m^{-2} s^{-1}, the best fits for two different bed positions in the drying experiment were obtained with simulated airflow rates of 0.12 and 0.18 kg-dry-air m^{-2} s^{-1}, while the best fits for two different bed positions in the heating experiment were obtained with simulated airflow rates of 0.045 and 0.11 kg-dry-air m^{-2} s^{-1}. Drying patterns at the top of the bed indicated that the non-uniformity was not due to wall effects, since the regions near the wall did not dry first [11]. One possibility is that random variations of packing density occurred during the preparation of the bed; another possibility is that the air box below the bed was not well-designed, favoring non-uniform flow of air into the bed. These two possibilities are difficult to describe using classical heat and mass transfer models but can be incorporated into models developed using computational fluid dynamics (CFD) software packages. Pessoa et al. [24] used CFD to show that the design of the air box did indeed lead to non-uniform airflow into the bed. Later, Pessoa et al. [25] simulated the early stages of a fermentation in a packed-bed bioreactor, using a non-uniform initial bed porosity: the initial porosity of each cell of the CFD mesh was randomly assigned, using a continuous uniform distribution with a mean of 0.564 and a standard deviation of 0.163.

2.3. High Porosity and High Superficial Air Velocities Can Lead to Good Temperature Control

One strategy for reducing the problem of non-uniform airflow is to use a highly porous bed (Table 3) [26–30]. van Breukelen et al. [26] used hemp (2.5 kg dry mass) impregnated with a nutrient solution to produce conidia of *Metarhizium anisopliae* in a 20 L bioreactor. Although they did not measure the porosity, it is known that hemp fibers have a high water-holding capacity and give porous beds that do not shrink: in an earlier study, the initial bed porosity was 0.48, and 1 kg of dry hemp could hold 7.2 kg of water [31]. van Breukelen et al. [26] varied the airflow in response to the measured O_2 uptake rate, trying to control the outlet air temperature; V_Z varied from 2.7 to 8.0 cm s^{-1}. With the combination of the porous medium and these high nominal superficial velocities, it is not surprising that the authors reported "perfect" control of the bed temperature. Sala et al. [27,28] also used a porous medium in a 22 L bioreactor containing 3 kg of rice husk, with an initial porosity around 0.85. With this high porosity, they obtained reasonable temperature control during the production of conidia of the biocontrol fungi *Trichoderma harzianum* and *Beauveria bassiana*, even though they used relatively low nominal superficial air velocities (<0.05 cm s^{-1}): the maximum temperature differences measured in the bed were 3 °C [28].

Table 3. Use of packed-bed bioreactors with high porosity substrates or with bed porosity modifiers.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Metarhizium anisopliae van Breukelen et al. [26]	Hemp fiber (2.5 kg) impregnated with nutrient solution containing glucose (5 kg), yeast extract (0.625 kg) and bacteriological peptone (0.625 kg) Initial moisture content not reported, but 7 L water added in the impregnation step, and hemp has high water-holding capacity	Air-jacketed column, h = 60 cm, ∅ = 20 cm. h_{bed} = 60 cm Top to bottom airflow with saturated air. Inlet air temperature unclear. V_Z varied manually based on off-gas temperatures. V_Z ranged from 2.7 to 8.0 cm s^{-1}	Authors say that "Perfect temperature control was achieved"

Table 3. *Cont.*

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Trichoderma harzianum Sala et al. [27,28]	Rice husk (presumably wet masses): (i) 300 g; (ii) 3 kg IMC: (i) 64%; (ii) 59% Initial porosity ~0.85	(1) 1.5 L column: h = 21 cm, ∅ = 10.5 cm. h_{bed} = 15.5 cm. $V_Z \leq 0.02$ cm s^{-1} [c] (2) 22 L column: h = 48 cm, ∅ = 24.5 cm. h_{bed} = 43 cm. $V_Z \leq 0.045$ cm s^{-1} [c] Inlet air temperature and RH unclear	T_{bed} varied between 20 and 30 °C at both scales, but difficult to interpret temporal T_{bed} profiles, since T_{air} is unclear and may have varied. Spatial temperature gradients in the 22 L bioreactor ≤ 3 °C.
Trichoderma harzianum Sala et al. [27,28]	(i) Mixture (300 g, presumably wet mass) of beer draff (70%) and wood chips (30%) (ii) Mixture (4 kg, presumably wet mass) of beer draff (40%) and wood chips (60%) IMC: (i) 64%; (ii) 55% Initial porosity (i) ~0.70; (ii) ~0.80	(1) 1.5 L column: h = 21 cm, ∅ = 10.5 cm. h_{bed} = 15.5 cm. $V_Z \leq 0.055$ cm s^{-1} [c] (2) 22 L column: h = 48 cm, ∅ = 24.5 cm. h_{bed} = 43 cm. $V_Z \leq 0.125$ cm s^{-1} [c] Inlet air temperature and RH unclear	Difficult to interpret temporal T_{bed} profiles, since T_{air} is unclear and may have varied. Spatial (radial) temperature gradients in the 22 L bioreactor >10 °C
Aspergillus oryzae Biz et al. [29]	7.26 kg of sugarcane bagasse and 7.74 kg of citrus pulp IMC = 78%	Rectangular box, base 70 cm × 60 cm, h = 50 cm. h_{bed} = 40 cm Saturated air at 30–32 °C V_Z = 9.9 cm s^{-1}	Good temperature control due to high porosity and high V_Z
Rhizopus microsporus Pitol et al. [30]	7.5 kg of wheat bran and 7.5 kg of sugarcane bagasse	Rectangular box, base 70 cm × 60 cm, h = 50 cm. h_{bed} = 40 cm Saturated air at (1) 34 °C and (2) 40 °C. V_Z = 6 cm s^{-1}	Good temperature control. Axial temperature gradient negligible

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). Bed porosity is cm^3-voids cm^{-3}-bed. [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor. RH is the relative humidity of the inlet air. [c] Aeration rate given in mL per min per gram of dry matter. Since it is not explicitly stated whether masses loaded are dry or wet masses, it is difficult to calculate V_Z with certainty.

If the bed is not sufficiently porous, a "bed porosity modifier" can be added (Table 3). Biz et al. [29] did this to produce pectinases with *Aspergillus oryzae* by using a substrate that was approximately 50% citrus waste, which leads to compact beds if used alone, and 50% sugarcane bagasse. Later, Pitol et al. [30] used 50% wheat bran and 50% sugarcane bagasse to produce lipases with *Rhizopus microsporus*. In both cases, 15 kg of dry substrate was used in a pilot packed-bed bioreactor with a 60 cm by 70 cm cross section, with a bed-height of 40 cm. In other words, the apparent bulk density on a dry basis was only 90 kg m^{-3}. In both fermentations, the bed remained porous, and very good temperature control was obtained, with the bed temperature remaining within 1 °C of the inlet air temperature. The moisture content of the bed also remained close to the initial value throughout the fermentation. This is not surprising, since in fermentations in which saturated air is used to aerate the bed, the bed dries due to the increase in the temperature of the air as it flows through the bed: the heating of the air increases its water-carrying capacity, promoting

evaporation [1]. Consequently, if the temperature of the air increases only slightly, then evaporation is minimized. Of course, although addition of an inert bed porosity modifier can improve temperature control, it also "dilutes" the substrate in the bed, such that the volumetric productivity of the bed (product produced per unit of time per unit of bed volume) may decrease. In other words, a larger bed might be required to produce the same amount of product. Further, the addition of a fibrous solid to improve porosity is only a good strategy for wider bioreactors; the use of such fibers in narrow bioreactors exacerbates wall effects, promoting non-uniform airflow.

Biz et al. [29] and Pitol et al. [30] obtained good temperature control not only because of the high bed porosity, but also because they used relatively high nominal superficial air velocities of 10 and 6 cm s^{-1}, respectively. These superficial air velocities are much higher than the values below 1 cm s^{-1} that many recent authors have used (Table 4) [32–42]. For example, Kumar et al. [35] produced lovastatin using *Aspergillus terreus* in a pilot-scale packed-bed bioreactor, with 50 kg of a substrate mixture consisting of wheat bran with a particle size of 0.4 to 0.5 mm, and wheat straw with a particle size of 1.5 to 2.0 cm. However, the maximum nominal superficial air velocities were below 1 cm s^{-1}, and temperatures as high as 42 °C were reached in the bed, compared to the setpoint temperature of 30 °C. Similarly, Silveira et al. [36] used a superficial velocity of 0.61 cm s^{-1} in the cultivation of *Kluyveromyces marxianus* on a medium based on sugarcane bagasse, while Zhang and Jiang [39] used a superficial velocity of 0.90 cm s^{-1} in the cultivation of *Aspergillus niger* on particles from the woody part of corn cobs. In both cases, the bed temperature increased monotonically with bed height, indicating the absence of channeling, but temperature control at the time of peak heat production was poor. Silveira et al. [36] measured a temperature of 27.5 °C at the bottom of the bed, compared to 49.6 °C at the top, while Zhang and Jiang [39] measured a temperature of 26.3 °C at the bottom of their 22 cm high bed, compared to 43.4 °C at the top. It is surprising that these authors used superficial air velocities below 1 cm s^{-1}, since it has long been clear that superficial velocities of up to 10 cm s^{-1} will be necessary to obtain reasonable temperature control in pilot-scale and large-scale packed-bed bioreactors [13].

Table 4. Examples of the use of low nominal superficial air velocities with packed-bed bioreactors.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
(1) *Phlebiopsis gigantea* (basidiomycete) (2) *Streptomyces* sp. Virtanen et al. [32]	40 kg (unclear whether wet or dry basis) (1) Water (58.3%), condensed starch mash (16.2%), silica (24.3%), dolomite lime (1.2%) (2) Water (66%), silica (29.1%), corn steep solids (1.4%), lactose (1.4%), dolomite lime (1.4%)	Column h = 48 cm, ∅ = 50 cm. h_{bed} unclear Top-to-bottom airflow. T_{air} and humidity unclear (no attempt made to saturate the air). V_Z = 0.03 cm s^{-1}	Temperature control not good, considering the slow-growing organisms used. T_{bed} increased from 23 to 30 °C for *Phlebiopsis gigantea* and from 23 to 27.5 °C for *Streptomyces* sp. (in both cases, the ambient temperature was 22 °C when the peak was reached)
Kluyveromyces marxianus Mazutti et al. [33,34]	Sugarcane bagasse (2 kg) supplemented with cane molasses, corn steep liquor and soybean bran IMC = 65%	Column h = 50 cm, ∅ = 34 cm, h_{bed} = 40 cm Inlet air temperatures of 27, 30 and 33 °C and V_Z = 0.61, 0.73 and 0.92 cm s^{-1} Air 95–100% RH	Temperature control poor, with T_{bed} peaking at values from 47 to 50 °C in different experiments V_Z < 1 cm s^{-1}

Table 4. *Cont.*

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Aspergillus terreus Kumar et al. [35]	Wheat straw and wheat bran. Mixtures ranged from "36% wheat straw + 64% wheat bran" to "64% wheat straw + 36% wheat bran". Total of 50 kg IMC = 65%	Column 1200 L. ∅ = 94 cm. h_{bed} = 122.5 cm. Provision for intermittent agitation (but agitation not described) Vz up to 0.59 cm s^{-1} Inlet air at 65% RH, with T_{air} controlled with PI controllers	Poor temperature control, with peak T_{bed} of 41.7, compared to optimum temperature for growth of 30 °C
Kluyveromyces marxianus Silveira et al. [36]	Sugarcane bagasse (3 kg) supplemented with pretreated cane molasses (10%), corn steep liquor (30%) and soybean bran (20%) IMC = 65%	Column h = 50 cm, ∅ = 34 cm. h_{bed} = 50 cm Air with 95–100% RH. V_Z = 0.61 cm s^{-1}	Temperature control poor. T_{bed} = 27.5 °C at the bottom compared to 49.6 °C at the top
Aspergillus awamori and *Aspergillus oryzae* Manan and Webb [37]	Wheat bran. Average particle size ~0.5 mm IMC = 65%	Column reactor h = 6 cm and ∅ = 10 cm. h_{bed} = 2 cm Moist air at 30 °C V_Z = 0.21, 0.42, 0.85 and 1.27 cm s^{-1}	Considering the small h_{bed} of 2 cm, temperature control poor at V_Z < 1 cm s^{-1}. With *Aspergillus awamori*, T_{bed} reached ~35 °C. With *Aspergillus awamori*, T_{bed} reached ~34 °C
Trichoderma asperellum Barrera et al. [38]	Polyurethane foam cubes with 2 cm sides or 80-mesh rice husk, also a mixture of the two. Additionally, mixture of white rice (81.2%) and wheat bran (18.8% *w*/*w*)	PROPHYTA® L05 fixed-bed fermenter (16 L): 4 bed layers with cooling coils between layers. h_{bed} values of 5, 7 and 8 cm tested. Square base 25 cm × 25 cm Incubation temperatures (temperature of water supplied to the cooling coils) of 23, 25 and 28 °C Air at 15, 50 and 95% RH V_Z = 0.19, 0.34 and 0.50 cm s^{-1}	Temperature gradients reported, but not useful because the air gaps between layers are included in the calculation, but the sizes of the air gaps between layers are not specified
Aspergillus niger Zhang and Jiang [39]	Woody part of corn cobs (6–8 mm) (1) 4.5 kg woody part of corn cobs + 4.5 kg of water (2) 3.2 kg woody part of corn cobs + 2.56 kg of water	Vehicular rotary solid-state bioreactor (VRSBR). Column h = 40 cm, ∅ = 35 cm Water jacketed. T_{water} possibly 30 or 35 °C V_Z depends on necessity for bed cooling. Varied from 0.07 to 0.90 cm s^{-1} During static operation, the 40 cm column is vertical. For intermittent mixing, bioreactor rotated 90° (such that the 40 cm column is horizontal); rotated like a rotating-bed bioreactor (20 rotations for each mixing event) (1) h_{bed} = 22 cm. Mixing events at 10, 12, 19, 24, 30, 48, 72, 98, 145 and 192 h (2) h_{bed} = 15 cm. Mixing events at 18, 24, 38, 48, 72, 96, 120, 144 and 168 h	Interesting design for intermittent mixing. Spatiotemporal temperature profiles indicate uniform airflow through the bed (1) Temperature control not good, with gradients as large as 20 °C at time of peak heat generation (26 °C at bottom of bed and 46 °C at top of bed) (2) Temperature control better, but still with gradients as large as 9 °C at time of peak heat generation (earlier, 26 °C at bottom of bed and 35 °C at top of bed; later, 28 °C at bottom of bed and 37 °C at top of bed)

Table 4. *Cont.*

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Yarrowia lipolytica Nascimento et al. [40]	Soybean hulls (400 g) supplemented with a nutrient solution containing either glucose or soybean oil IMC = 55% (presumably on a wet basis)	Column h = 36 cm, ∅ = 10 cm, h_{bed} = 28 cm Side walls insulated by a vacuum-sealed jacket to mimic the situation at a large scale in a wide packed bed. Moist air at ~26.5–28 °C (not specified clearly) V_Z = 1.06 cm s^{-1}	Temperature control not good, with temperatures as high as 35 °C being reached Radial temperature gradients of up to 2 °C detected in the upper sections of the bioreactor, even though the side walls were insulated (which should eliminate such gradients). No visual evidence of the formation of preferential flow paths
Irpex lacteus (basidiomycete) Álvarez Pallín et al. [41]	Chopped wheat straw (5.35 kg, presumably wet basis) IMC = 82% (presumably wet basis)	Column h = 28 cm, ∅ = 14 cm T_{air} switched between lower and higher values (not stated) to maintain the bed temperature at 30 °C. Inlet air humidity modified manually by controlling the airflow through the humidifier. V_Z = 0.35 cm s^{-1}	Although *Irpex lacteus* grows slowly, T_{bed} peaked at 36 °C. Moreover, T_{bed} was consistently higher than the outlet air temperature, suggesting that air was flowing around the bed rather than through it
Aspergillus niger Bhattacharya et al. [42]	Substrate loadings of (i) 100 g, (ii) 150 g, (iii) 200 g, (iv) 300 g, (v) 400 g IMC = 66% (presumably wet basis)	Column ∅ = 15 cm. h_{bed} = (i) 0.5 cm, (ii) 1 cm, (iii) 2 cm, (iv) 3 cm, (v) 4 cm Air at 30 °C V_Z = 0.07 cm s^{-1}	Poor temperature control, with T_{bed} reaching 35 °C No visual evidence of bed compaction nor formation of preferential flow paths

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if it was specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor. RH is the relative humidity of the inlet air.

Sala et al. [27,28] also used a "bed porosity modifier" in experiments performed in their 22 L bioreactor with beer draff (brewer's spent grain) (Table 3). The medium was 4 kg of a 60:40 mixture of beer draff with wood chips, with an initial porosity around 0.8. However, unlike Biz et al. [29] and Pitol et al. [30], they used low nominal superficial air velocities of around 0.1 cm s^{-1}, or even less. As a result, the temperature control in the bed was not good: radial temperature differences of up to 10 °C occurred during the production of conidia of *Trichoderma harzianum* [27].

2.4. Intermittent Agitation of Packed-Bed Bioreactors Can Improve Product Uniformity

Although intermittent agitation has long been used in SSF bioreactors, it was only recently that the effects of infrequent agitation on product uniformity in a packed-bed bioreactor were investigated (Table 5) [43–46]. The investigation of Finkler et al. [43] was motivated by an earlier study, in which *Aspergillus niger* was used to produce pectinases on 30 kg (dry mass) of a 90:10 mixture of wheat bran and sugarcane bagasse in an unmixed pilot-scale bioreactor, with a bed height of 40 cm [10]. In this earlier study, the bed shrank, and a gap appeared between the bed and the wall; preferential flow of air through the gap led to bed temperatures as high as 43 °C, compared to an inlet air temperature of 30 °C. At the end of the fermentation, the pectinase activity in the bed was highly non-uniform, with a 2.5-fold difference in pectinase activity obtained in samples removed from different parts of the bed (from 11 to 28 U g^{-1}) [10]. Finkler et al. [43] used the same substrate–microorganism–bioreactor combination, but applied one to five mixing events during the 15 to 20 h that it took to reach peak pectinase levels. The fermentations were denominated MIX-1 (10 h), MIX-3 (8, 10 and 12 h) and MIX-5 (8, 10, 12, 14 and 16 h), where the times of

the intermittent mixing events are indicated within the parentheses. Mixing was performed by rotating the whole bioreactor and then leveling the top of the bed before resuming the fermentation. The best results were obtained with MIX-3. Good uniformity of pectinase activity was obtained at the time of harvesting, with the sample standard deviation of activities at 15 different locations in the bed being 2 U g^{-1}, which is less than 10% of the mean activity of 22 U g^{-1}.

Table 5. Studies of the use of infrequent mixing in packed-bed bioreactors.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Aspergillus niger Finkler et al. [43]	27 kg of wheat bran and 3 kg of sugarcane bagasse IMC = 62%	Rectangular box, base 70 cm × 60 cm, h = 50 cm. h_{bed} = 40 cm Air saturated at 30 °C V_Z = 9.9 cm s^{-1} Intermittently mixed by rotating whole bioreactor	In absence of mixing, T_{bed} had reached 43 °C, and there was evidence of non-uniform airflow [10]. With intermittent mixing, maximum T_{bed} < 40 °C, airflow more uniform
Rhizopus oryzae Arora et al. [44]	200 g of a 1:1 mixture of wheat bran and linseed oil cake IMC = 34.5% (corresponds to 55% dry basis)	Column ∅ = 15 cm. h_{bed} = 3.5 cm Agitated intermittently Compared (1) unmixed (2) mixing every 6 h (3) mixing every 10 min Air at 30 °C and 80% RH V_Z = 0.38 cm s^{-1}	Poor temperature control, despite small scale. The unmixed bed compacted, causing non-uniform airflow, with T_{bed} reaching ~41 °C. With mixing, T_{bed} reached from 38 to 40 °C
Rhizopus oryzae Arora et al. [45]	1:1 mixture of wheat bran and linseed oil cake (presumably 200 g)	Column ∅ = 15 cm. h_{bed} = 3.5 cm Mixing events every 1 h Air at 30 °C, in different experiments, 60, 70 and 80% RH. In different experiments V_Z = 0.28, 0.38 and 0.47 cm s^{-1}	Poor temperature control, despite small scale. In different experiments, T_{bed} peaked at values ranging from 34 to 38.5 °C
"*Trichoderma Brev T069*" [46]	Cassava peel. Values used were (i) 250 kg, (ii) 500 kg, (iii) 750 kg (presumably wet masses) IMC = 56% (presumably wet basis)	Rectangular perforated stainless-steel plates 900 cm × 200 cm. h_{bed} values of (i) 7 cm, (ii) 14 cm, (iii) 21 cm. Agitator moves from one end of the bed to the other, with motors mounted on a carriage. Design of agitator blades not specified. Agitator used intermittently. Air temperature and RH unclear. Airflow adjusted based on temperatures measured in bed, reaching 50 m^3 min^{-1}, which corresponds to V_Z = 4.6 cm s^{-1}	Temperature control poor, considering the small bed heights. For the 7 cm bed, T_{bed} increased from 28 to 35 °C. For the 14 cm bed, T_{bed} increased from 28 to 39 °C. The fermentation failed with the 21 cm bed height (ammonia odor was produced)

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if it was specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor. RH is the relative humidity of the inlet air.

The work of Finkler et al. [43] confirmed the suggestion of Schutyser et al. [47] that, in intermittently agitated bioreactors, an early agitation event should be applied to prevent the developing aerial hyphae from binding the substrate particles into strong agglomerates that do not break apart upon mixing. However, Finkler et al. [43] showed that a single mixing event does not ensure good uniformity at the end of the fermentation. In MIX-1, average pectinase activity was 25 U g^{-1} for samples removed from the bottom of the bed and 17 U g^{-1} for samples removed from the top of the bed. In fact, at times greater than 10 h since the last mixing event, the pectinase activity in samples removed from different locations in the bed became less uniform [43]. Importantly, the infrequent mixing events did not help with temperature control, as the axial temperature gradients in the bed were reestablished within a few minutes after mixing.

Finkler et al. [43] identified a design flaw in their pilot bioreactor, which has thermocouple sleeves that cross the bed at heights of 5, 18 and 33 cm, but at different horizontal positions. The sleeve at 5 cm height is only 5 cm from the side wall of the bioreactor. During some of the fermentations, there was compaction of the substrate held in the region between this sleeve, the base of the bioreactor and the side wall of the bioreactor. This compacted mass of substrate was not dislodged during mixing. It was also poorly aerated, and the temperatures registered at the bed height of 5 cm exceeded the temperatures registered at the higher bed heights of 18 and 33 cm.

Arora et al. [44] also studied the effect of intermittent mixing on bioreactor performance. They used *Rhizopus oryzae* to produce phytase in a small packed-bed bioreactor, with a diameter of 15 cm and a bed height of only 3.5 cm, with a total of 200 g of substrate. They compared three different modes of operation with forced aeration: (i) an unmixed packed bed; (ii) an infrequently mixed packed bed (mixed for 3 min at 15 rpm every 6 h); and (iii) a frequently mixed packed bed (mixed for 1 min at 20 rpm every 10 min). The mixing was performed with blades that protruded downwards from a revolving disk above the bed. In the unmixed packed bed, air channeling occurred. This was prevented by the mixing of the bed. However, despite the very small bed height, the temperature in the bed was not well controlled in any of the three bioreactors. With an inlet air temperature of 30 °C, maximum bed temperature was 40.7 °C in the unmixed packed bed, 38.7 °C in the infrequently mixed packed bed and 39.6 °C in the frequently mixed packed bed. Even at bed heights of only 1 cm, temperatures of 36 °C or more were recorded. These results are not surprising, as *Rhizopus oryzae* grows relatively quickly, and the superficial air velocity was only 0.38 cm s^{-1}. With this poor temperature control, the results of Arora et al. [44] do not give any useful insight into operation of packed-bed bioreactors at a large scale.

A further study with intermittent agitation was conducted by Zhang and Jiang [39] with *Aspergillus niger* (Table 4). The bed was mixed nine to ten times during a fermentation lasting up to 192 h, with four to six of the mixing events occurring during the first 48 h, when growth was fastest. Mixing was performed by laying the cylindrical packed bed on its side and rotating it around the central axis, in the manner of a rotating drum, before returning to upright packed-bed operation. During various fermentations with bed heights ranging from 15 to 22 cm, the temperature rose monotonically with bed height, indicating that the intermittent mixing prevented the formation of preferential flow paths. However, the bed temperature was not well controlled, with a 17 °C difference in temperature between the top and bottom of the 22 cm high bed at the time of peak heat production. Once again, this is likely due to the low superficial velocity used, only 0.90 cm s^{-1}.

In conclusion, although intermittent agitation promotes product uniformity, studies of intermittent agitation in packed-bed bioreactors have been conducted with few fungi. Different fungal species have different sensitivities to agitation, so the optimum intermittent agitation regime will need to be determined empirically for each species. It must also be remembered that the production of some products requires the undisturbed growth of aerial hyphae. One example is the production of fungal spores for use in biocontrol. In such cases, it may not be possible to agitate the bed without greatly decreasing yields.

2.5. The Structured (Multi-Layered) Packed Bed Has Received a Revival of Interest

Recent years have seen a revival of the multi-layered packed-bed bioreactor. This design was proposed by Lu et al. [48], with a multi-layered packed-bed bioreactor denominated "Prophyta" being patented soon afterwards by Lüth and Eiben [49]. The design involves several substrate layers resting on perforated bases, typically one above the other within a column (Figure 3). The motivation for using multi-layered beds has often been to prevent compaction of the substrate at the bottom of the bed [6,7], although it is important to note that the forces in a static bed of solids are not simply exerted vertically downwards: there are both vertical and horizontal forces, such that the resulting force vector is oriented diagonally downwards to the bioreactor wall [50]. Table 6 lists several studies with multi-layered packed-bed bioreactors [51–55].

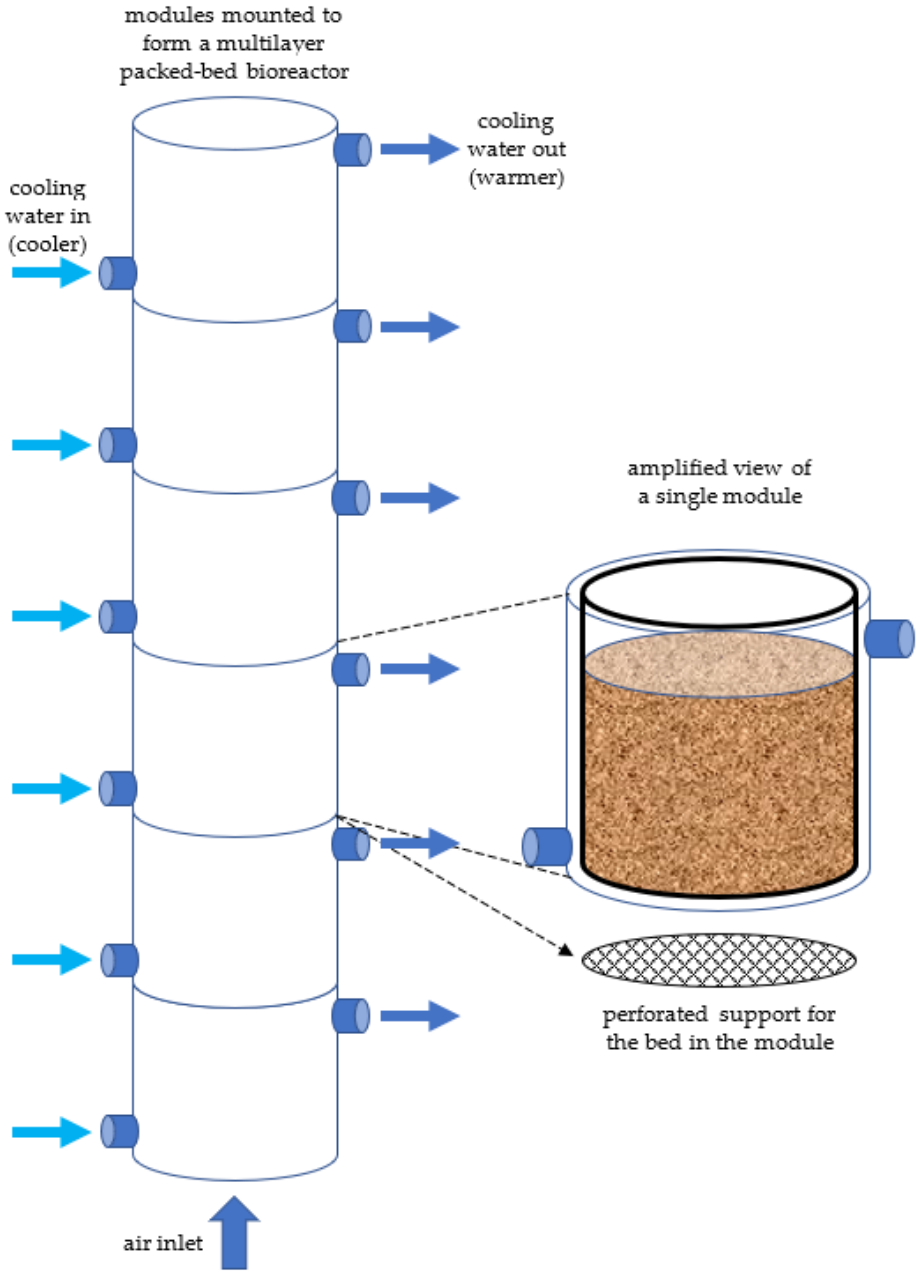

Figure 3. Basic features of a multi-layered packed-bed bioreactor made with identical water-jacketed modules stacked vertically.

Table 6. Studies of the use of multi-layered packed-bed bioreactors.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Aspergillus niger Chysirichote [51]	Copra waste, 2.0 kg IMC = 60%	Column. h = 80 cm and ∅ = 45 cm. Total h_{bed} = 36 cm. In different experiments: • 1 layer, 36 cm high • 2 layers, each 18 cm high • 3 layers, each 12 cm high Moist air at 30 °C V_Z = 0.12 cm s^{-1}	V_Z of 0.12 cm s^{-1} is low for a fast-growing organism like *A. niger*. Peak T_{bed} values (~50 °C) were higher than the outlet air temperature, suggesting non-uniform airflow occurred
Myceliophthora sp. Casciatori et al. [52]	Sugarcane bagasse and wheat bran. Proportions unclear IMC = 75%	Column: 8 water-jacketed modules, each h = 10 cm and ∅ = 7.62 cm. Total h_{bed} = 80 cm Water jacket presumably at 45 °C Air at 45 °C and 95% RH V_Z values of 0.73 and 1.46 cm s^{-1}	With V_Z = 0.73 cm s^{-1}, T_{bed} peaked ~52 °C in the bottom section of the bed, at which time T_{bed} was lower in the middle and higher sections, suggesting non-uniform airflow. With V_Z = 1.46 cm s^{-1}, T_{bed} peaked at ~50 °C at all three bed heights, suggesting non-uniform airflow. Significant drying occurred, with final bed moisture contents <15% at all bed heights below 60 cm.
(1) *Aspergillus awamori* (2) *Aspergillus oryzae* Manan and Webb [53]	Wheat bran (12 g per module) IMC ranging from 50 to 75% (in different modules)	Column with six modules with perforated bases, each h = 5 cm and ∅ = 10 cm, tightly stacked one above the other (total height of 33.5 cm). h_{bed} in each module = 1.5 cm Moist air at 30 °C V_Z = 0.42 cm s^{-1}.	Temperature profiles for individual modules not reported, only averages over the 6 modules. Bed temperatures over 40 °C occurred, despite the small amount of substrate in the bioreactor
Myceliophthora thermophila Oliveira et al. [54]	Sugarcane bagasse (70%) and wheat bran (30%) IMC = 75%	Both cases: Column with 4 water-jacketed modules, each h = 10 cm and ∅ = 13 cm. Perforated metal at base of each module Water jacket at 45 °C Air saturated at 45 °C V_Z = 0.73 cm s^{-1} (1) Batch operation: Same 4 modules throughout, in the same positions (2) "Descending" semicontinuous operation (Figure 4): • initial transient period—1 inoculated module added daily at top • semicontinuous period—1 module removed daily from bottom, modules moved down, and 1 inoculated module added at top. Each module stays in bioreactor for 4 days • final transient period—1 module removed daily from bottom	Batch operation: Spatiotemporal temperature profiles at time of peak heat production suggest non-uniform airflow occurred. Maximum T_{bed} was 47.9 °C for batch operation and 47.7 °C for "descending" semicontinuous operation.
Myceliophthora thermophila Rodrigues et al. [55]	Sugarcane bagasse (70%) and wheat bran (30%) IMC = 75%	Column with 8 water-jacketed modules, each h = 10 cm and ∅ = 13 cm. Perforated metal at base of each module Water jacket at 45 °C Air saturated at 45 °C V_Z = 0.73 cm s^{-1} (1) Batch operation: Same 8 modules throughout, in the same positions (2) Cyclic operation (Figure 4). Same 8 modules throughout. Every 24 h, 2 modules moved from the top to the bottom, with the other 6 modules moved upwards (3) "Ascending" semicontinuous operation (Figure 4): • initial transient period—2 inoculated modules added daily at bottom • semicontinuous period—2 inoculated modules added daily at bottom and 2 fermented modules removed from top • transient period—2 fermented modules removed daily from top	Cyclic operation: bottom-to-top axial temperature gradient quickly reestablished after movement of modules. Peak T_{bed} of 48.2 °C "Ascending" semi-continuous operation: Peak T_{bed} of 48.3 °C

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if it was specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor. RH is the relative humidity of the inlet air.

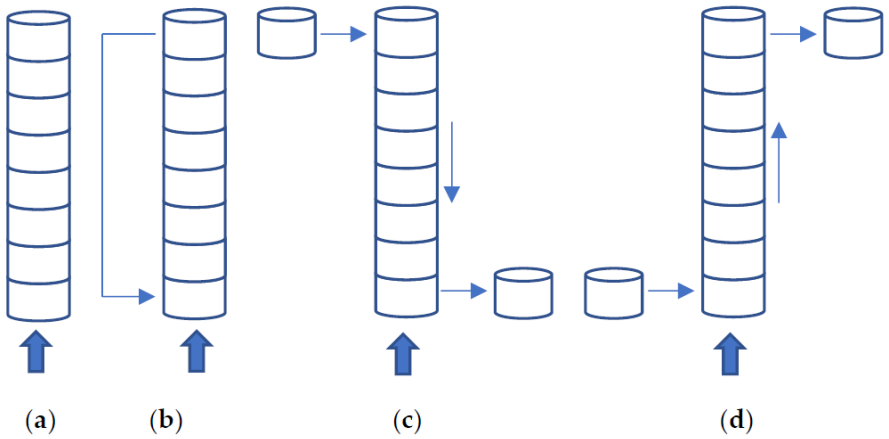

Figure 4. Different modes of operation that are possible with a modular multi-layered packed-bed bioreactor. (**a**) Batch operation; (**b**) Cyclic operation; (**c**) "Descending" semi-continuous operation; (**d**) "Ascending" semi-continuous operation. In all cases, the aeration is bottom-to-top, as indicated by the broad arrow. The thin arrows indicate the movement of modules. The diagrams represent the general principle of operation; details of how many modules were moved and with what frequency are given in the text and in Table 6.

In several recent studies with multi-layered packed beds, temperature control was poor; additionally, the temperature did not rise monotonically between the air inlet and outlet, which indicates that air was flowing through preferential flow paths in the bioreactor, rather than flowing uniformly through each layer in sequence [6,7,51,52]. Chysirichote [51] grew *Aspergillus niger* on copra waste, comparing three configurations with the same total bed height of 36 cm: a single-layer bed (36 cm high), a two-layer bed (each 18 cm high, separated by a 15 cm air gap) and a three-layer bed (each 12 cm high, separated by 10 cm air gaps). Temperature control was poor for all three configurations, with peak bed temperatures reaching around 50 °C in all layers of all three configurations, compared to an inlet air temperature of 30 °C. Importantly, at the times of peak heat generation, these bed temperatures were higher than the outlet air temperature. Likewise, in the 20 cm diameter packed-bed bioreactor of Perez et al. [6], the bed temperature reached 54 °C, compared to an inlet air temperature of 45 °C, even though the fermentations were performed with a relatively slow-growing fungus, *Myceliophtora thermophila* (Table 1). During long periods, the axial bed temperatures did not increase monotonically with bed height, with $T_{40cm} < T_{20cm} < T_{80cm} < T_{60cm}$. In the similar 20 cm diameter packed-bed bioreactor of Cunha et al. [7], similar non-monotonic axial temperature profiles occurred, with $T_{46cm} < T_{0cm} < T_{69cm} < T_{23cm}$ during most of a fermentation performed with *Metarhizium anisopliae* (Table 1). In the 7.62 cm diameter bioreactor of Casciatori et al. [52], during the first 60 h of the fermentation performed with *Myceliophtora thermophila* using a superficial air velocity of 0.73 cm s^{-1}, the temperature profile was the inverse of what would be expected, with $T_{30cm} > T_{60cm} > T_{90cm}$ (Table 6).

In the case of Manan and Webb [53], who cultivated *Aspergillus oryzae* and *Aspergillus awamori* on wheat bran in a multi-layered packed-bed bioreactor with six substrate layers, it is not possible to judge the uniformity of the airflow, because the authors did not provide time-course profiles for the temperatures of each of the layers; rather, they plotted the average of the 6 layers. However, temperature control was poor, with the maximum average temperatures reaching 39 °C for *A. awamori* and 41 °C for *A. oryzae*, compared to an inlet air temperature of 30 °C.

Due to the poor temperature control, these recent investigations do not give useful insights into the design and operation of multi-layered packed-bed bioreactors. However, special attention obviously needs to be given to sealing the various modules or layers of the bioreactor, thereby forcing the air to flow through the layers and not allowing it to flow around them. Moreover, better temperature control would require either higher superficial

air velocities than those used by the authors [6,7,51–53] or the cooling of the air between the bed layers. In fact, interlayer cooling was incorporated into the Prophyta bioreactor over two decades ago [49]. However, cooling surfaces in the interlayer air spaces are likely to promote condensation, so it is necessary to design the bioreactor to prevent the condensate from dripping onto the substrate layer below.

If each layer of the bed is designed as a separate module, then the position of the layers in the bed can be changed during the fermentation (Figure 4). Recently, Oliveira et al. [54] investigated the use of a multi-layered packed-bed bioreactor in a "descending" semi-continuous mode (Figure 4c). In this mode, four "fermenting modules" were in the bioreactor at any one time. A new module was added at the top of the bioreactor every 24 h, with the existing fermenting modules descending one position, and the fermenting module at the bottom being harvested, after having spent 96 h in the bioreactor. During the cultivation of *Myceliophthora thermophila* in a bed aerated with air at 45 °C, the maximum bed temperatures during batch operation (47.9 °C) and during the descending semi-continuous mode (47.7 °C) were very close [54]. In fact, the temporal temperature profiles experienced by the microorganism in the two modes of operation were similar. In batch operation, most of the layers increased in temperature from 43 °C to almost 48 °C, over the first 24 h; the temperature then decreased to around 45 °C at 72 h, remaining near this value for the next 24 h. In descending semi-continuous operation, a module that was introduced at the top of the bed was quickly heated to around 48 °C, due to the hot air arriving from the lower modules; over the 96 h period that it spent in the bioreactor, the temperature slowly decreased to around 43 °C.

Rodrigues et al. [55] conducted a similar study of semi-continuous operation with the same fungus and substrate, with the main difference being that they added fermenting modules at the bottom and removed them from the top; this will be referred to as "ascending" semi-continuous operation (Figure 4d). They also studied cyclic operation, in which modules were cycled from the top to the bottom of the bioreactor (Figure 4b). In cyclic operation, the module at the bottom at any particular time had the lowest temperature, and the module at the top at this time had a higher temperature [55], showing that the bottom-to-top axial temperature gradient was quickly reestablished after the modules were moved. The temporal temperature profile obtained for cyclic operation was similar to that of the batch results of Oliveira et al. [54] described above, with a peak temperature of 48.2 °C. In ascending semi-continuous operation, a module that was introduced at the bottom of the bioreactor increased in temperature from around 43 °C to 46–47 °C over about 16 h; the temperature decreased to between 44 and 45 °C over the next 48 h or so, and it then remained at this temperature until it was removed from the bioreactor after a residence time of 96 h. The peak temperature Rodrigues et al. [55] reported for ascending semi-continuous operation was 48.3 °C.

An earlier modeling investigation by Mitchell et al. [56] had suggested that the descending semi-continuous mode of operation could reduce the maximum temperature reached in the bed by several degrees in relation to a packed-bed bioreactor operating in batch mode. The studies of Oliveira et al. [54] and Rodrigues et al. [55] did not confirm this: in both of their studies, batch and semi-continuous operation gave peak bed temperatures close to 48 °C. However, Mitchell et al. [56] modeled large-scale packed beds that were wide enough for heat transfer through the side walls to be negligible, whereas both Oliveira et al. [54] and Rodrigues et al. [55] used water-jacketed bioreactors with 10 cm diameter beds. With the possibility of significant heat transfer through the side walls, their results do not give good insight into behavior at a large scale.

Even though semi-continuous operation did not decrease the maximum temperature reached in the bed in the studies of Oliveira et al. [54] and Rodrigues et al. [55], this mode of operation still has two key advantages [56]. First, in semi-continuous mode, all modules experience the same temperature profile during their time in the bioreactor; this should increase product uniformity compared to batch operation, in which different zones of the bed can experience quite different temperature profiles. Second, downstream processing

equipment can be smaller. For the systems of Oliveira et al. [54] and Rodrigues et al. [55], in semi-continuous mode, the desired product, cellulases, would be extracted from one or two modules each day; in batch mode, the cellulases would be extracted from four modules once every four days.

3. Packed-Bed Bioreactors with Novel Features

Several authors have presented designs for packed-bed bioreactors with some novel features (Table 7) [57–61]. These bioreactors are analyzed in the following subsections.

Table 7. Performance of new designs of packed-bed bioreactors.

Organism [Ref.]	Substrate [a]	Bioreactor Design and Operation [b]	Comments
Trichoderma asperellum Maïga et al. [57]	Grapevine shoots (33.3%), sugarcane bagasse (10%), wheat bran (33.3%), olive pomace (13.3%) and potato flakes (10%) Total mass = 6 kg IMC = 66%	Disposable plastic bag reactor. Rectangular base 52 cm × 40 cm, working height up to 19 cm. h_{bed} = 15 cm Air at 25 °C. V_Z values from 0.08 to 0.36 cm s^{-1}	Not intended for scale-up to larger scales. The intention is for the bioreactor to be portable and disposable. V_Z < 1 cm s^{-1} Peak T_{bed} = 48 °C
None (but would presumably be *Saccharomyces cerevisiae*) Chen et al. [58]	Chopped sweet sorghum stalks Feed moisture content = 80% Feed and discharge at 2.5 kg wet substrate h^{-1}	Continuous packed-bed bioreactor. Column with h_{bed} = 120 cm, ∅ = 40 cm. Working volume 150 L Inoculated new substrate added at top. Spent solids removed from bottom. Residence time of solids = 24 h CO_2 circulated through bed to strip ethanol; ethanol condensed externally and then CO_2 reheated and returned to bed	Plug-flow continuous system Focus on ethanol stripping in the absence of growth. However, with aeration with air, could be adapted for aerobic SSF
Aspergillus ficuum Shahryari et al. [59]	Wheat straw (particle size 0.70–0.14 mm)	Column h = 30 cm and ∅ = 10 cm. h_{bed} = 19.5 cm (1) Trickle bed. Intermittently agitated with helical screw. After day 3, 195 mL of liquid (usually water) trickled into the bed daily over 1-min period (2) Static bed, no helical screw. No trickling of liquid Both bioreactors held in a 30 °C chamber Aeration for 2 h, twice a day. Air temperature presumably 30 °C. V_Z = 0.21 cm s^{-1} (during aeration period)	V_Z < 1 cm s^{-1} and aeration infrequent (1) Good temperature control, peak T_{bed} ~31 °C (2) Reasonable temperature control, peak T_{bed} ~34.5 °C Aeration intermittent, so, for most of the time, did not operate as a true packed bed. However, could be adapted for continuous aeration
mixed culture of *Trichoderma reesei* and *Aspergillus oryzae* Brijwani et al. [60]	Soybean hulls (1) 2.51 kg (2) 3.51 kg IMC = 70%	Cube 30 cm × 30 cm × 30 cm, with wire-mesh walls. (1) h_{bed} = 15 cm; (2) h_{bed} = 25 cm Central perforated tube with perforated horizontal branches at 3 heights Cube incubated in chamber with air at 30 °C and 95% RH Air saturated at 25 °C. Aeration of 50 L min^{-1} equivalent to V_Z = 0.93 cm s^{-1} if aeration were end-to-end.	Temperature control poor, with T_{bed} reaching 44 °C Aeration rate low (equivalent to V_Z < 1 cm s^{-1})
Myceliophthora thermophila Perez et al. [61]	Sugarcane bagasse (70%) and wheat bran (30%) (1) 45 g of substrate per module. (2) 620 g of substrate per module IMC = 75% Porosity = 0.75	Column with water-jacketed modules, each module with a perforated grid at bottom (1) 8 modules, each h = 10 cm and ∅ = 7.62 cm. Total h_{bed} = 80 cm. V_Z = 0.43 cm s^{-1} for "Normal" aeration, also operated with "Split" aeration (2) 4 water-jacketed modules, each h = 20 cm and ∅ = 20 cm. Total h_{bed} = 80 cm. Operated with split and radial aeration. Information insufficient to calculate V_Z Water jacket at 45 °C Saturated air at 45 °C Aeration strategies: (i) Normal: All air introduced at the bottom of the column. Performed only in the narrow column (ii) Split: Half the air enters bottom of column and half through a perforated ring near the wall at half bed height. Performed in bioreactors (1) and (2) (iii) Radial: Air introduced through perforated tube at central vertical axis. Performed only in bioreactor (2)	(1) Good temperature control (T_{bed} < 48 °C) with both Normal and Split aeration (2) Temperature control not good. T_{bed} reached ~55 °C for both Split and Radial aeration In both cases, spatiotemporal temperature profiles indicate non-uniform airflow

[a] Masses and percentages of substrates are dry mass, unless otherwise stated. IMC is initial moisture content (by mass on a wet basis, unless otherwise stated). Bed porosity is cm^3-voids cm^{-3}-bed. [b] Details are specifically for the part of the bioreactor holding the fermentation bed. ∅ indicates the column diameter (internal diameter, if it was specified by the authors). V_Z is the nominal superficial velocity, calculated by dividing the informed airflow rate by the cross-sectional area of the empty bioreactor. RH is relative humidity.

3.1. A Disposable Packed-Bed Bioreactor Is an Interesting New Design for Niche Applications

An interesting new idea is the disposable packed-bed reactor of Maïga et al. [57]. This bioreactor is made of a flexible and sterilizable plastic, polyethylene terephthalate. The fermentation compartment has a base approximately 0.5 m by 0.4 m and a working height of approximately 0.2 m, giving it a working volume of 40 L. This compartment sits on top of a base compartment made of the same plastic and which contains three parallel aeration channels that run the length of the base. The tops of these channels are perforated, so that air can pass upwards into the fermentation chamber. The fermentation and base compartments are separated by a microperforated grid.

This bioreactor is suitable for producing fungal spores for use as biocontrol agents, as it minimizes the need for handling of the fermented solids [57]. When necessary, either during the fermentation or during processing after the fermentation, the contents of the bioreactor can be agitated manually or with the aid of an external mechanical device [57]. After the fermentation, dry air can be passed through the bioreactor to dry the fermented solids. The bag bioreactor can then be transported from the production facility to the site of application of the biocontrol agent. Metabolites can also be produced; they can be extracted by adding the extracting fluid to the bioreactor, mixing the contents and then draining the liquid containing the solubilized metabolites from the bag [57].

Maïga et al. [57] used the bag bioreactor to produce conidia of the biocontrol fungus *Trichoderma asperellum*. Temperature control was poor, suggesting that improvements are required: The bioreactor was aerated with air at 25 °C, but the bed temperature reached 48 °C, indicating that air was not flowing uniformly through the bed. One factor that may have contributed to the high temperatures is that Maïga et al. [57] used relatively low superficial air velocities of 0.08 to 0.36 cm s^{-1}.

3.2. A Continuous Packed-Bed Bioreactor Might Find Niche Applications in Biorefineries

Another interesting design is the continuous packed-bed bioreactor of Chen et al. [58]. In this bioreactor, chopped sorghum stalks were fed continuously by a screw feeder into the top of the bed where they were inoculated (Figure 5). At the bottom of the bed, another screw continuously removed the fermented solids, causing the bed to move downwards. Effectively, this represents plug-flow operation.

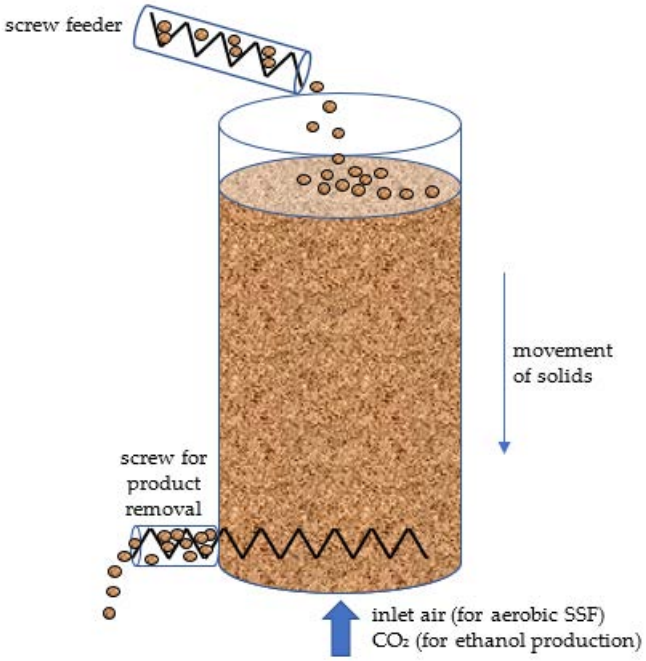

Figure 5. Principles of operation of the continuous packed-bed reactor of Chen et al. [58].

A key feature of this bioreactor is that CO_2 was recirculated through the bed, stripping ethanol from the fermented solids [58]. After leaving the bed, the gas stream was cooled to condense the ethanol and then reheated to the fermentation temperature and sent back to the bed. The cooling and heating of the gas stream were coupled in a heat pump to minimize energy expenditure.

Unfortunately, almost no information is available about the performance of the ethanol fermentation in this system. Chen et al. [58] focused on the stripping of ethanol from a substrate that was artificially imbibed with ethanol and on modeling the ethanol recovery system. However, the system could be adapted for aerobic SSF.

3.3. A Packed-Bed Bioreactor with a Helical Screw and Intermittent Trickling of Liquid

Shahryari et al. [59] built a trickle-bed reactor consisting of a 20 cm high bed in a cylindrical column 30 cm high and 10 cm in diameter (Figure 6). The bed height was approximately 20 cm. The column contained a vertical helical screw that supported the substrate bed, helping to prevent the substrate particles from compacting. During fermentations, the helical screw was intermittently rotated, either to stop agglomerates forming or to help to distribute the liquid trickled into the bioreactor and the air blown through the bottom. The aeration was only intermittent, with aeration at 1 L min^{-1} for 2 h, two times a day. It is not clear whether the bed was agitated during the whole of this 2 h aeration period. Daily, approximately 200 mL of liquid medium was trickled through the bed over a one-minute period.

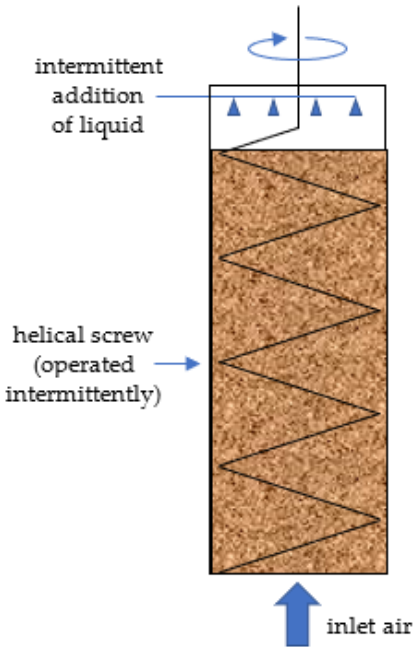

Figure 6. Principles of the design and operation of the trickle-bed reactor of Shahryari et al. [59].

With the intermittent aeration, the reactor of Shahryari et al. [59] did not operate as a true packed-bed bioreactor. Although it could be operated with continuous aeration, thereby qualifying as a packed-bed bioreactor, it is not clear how one would scale it up. A larger-scale bioreactor would be wider and taller, but it is unlikely to be practical to build a bioreactor of this type over 1 m wide with a single helical screw. Large-scale bioreactors would probably, therefore, have several helical screws. In fact, the large-scale version of this type of bioreactor would be the bioreactor described by Durand and Chereau [62]: a 2 m long, 0.8 m wide packed-bed bioreactor, with a bed height of 1 m, in which several screw augers are mounted on a carriage on top of the bioreactor, mixing the bed intermittently as they travel back and forth along the length of the bed.

3.4. Several Designs Have Been Proposed with Air Blown into the Bed through Perforated Tubes

Brijwani et al. [60] and Perez et al. [61] proposed packed-bed bioreactor designs in which the air, or a significant fraction of it, is introduced into the bed through perforated tubes.

Brijwani et al. [60] used a cubic bioreactor, 30 cm by 30 cm by 30 cm, with all faces of the cube made of a wire mesh. Air was introduced into the bed by a vertical perforated pipe that had cross-shaped perforated extensions that extended into the bed 7 cm horizontally at three different heights. With its perforated walls, the bioreactor is like a radial-flow packed bed, a design in which air is introduced into a cylindrical bioreactor through a perforated tube located at the central axis and leaves through the side walls [1]. Brijwani et al. [60] grew a mixed culture of *Trichoderma reesei* and *Aspergillus oryzae* on soybean hulls. When used at full capacity, the bed was 25 cm high and contained 10 kg of moist soybean hulls (corresponding to 3.5 kg of dry soybean hulls). Air at 25 °C was provided to the distributor at 3.42 kg h^{-1}, which gives a relatively low nominal superficial velocity, on the order of 1 cm s^{-1}. Temperature control was poor, with values over 40 °C measured in the bed. This is not surprising, since the design of Brijwani et al. [60] does not promote uniform airflow. Flow paths of different lengths are available, and the air will preferentially flow through the shortest path, from the ends of the cross-shaped extensions, horizontally, to the outside.

Perez et al. [61] conducted experiments in an 80 cm high multiple-layer packed bed with a water jacket, comparing two strategies for introducing the air into the bed using perforated tubes (Figure 7). Here we consider their experiments with a bioreactor 20 cm in diameter.

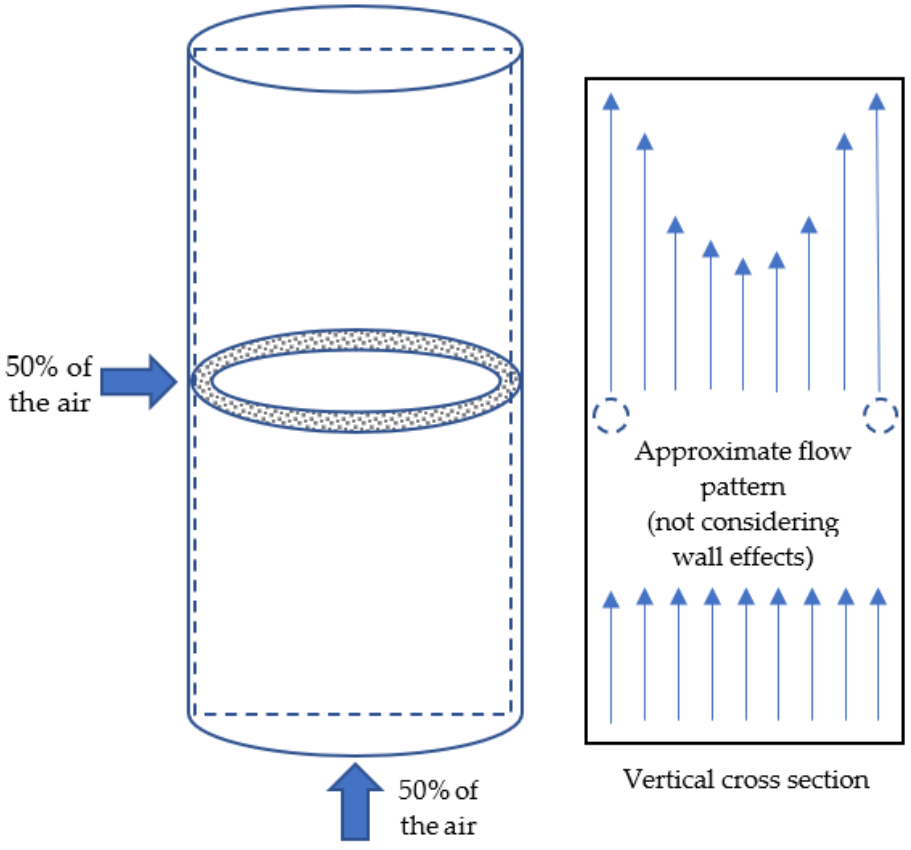

Figure 7. *Cont.*

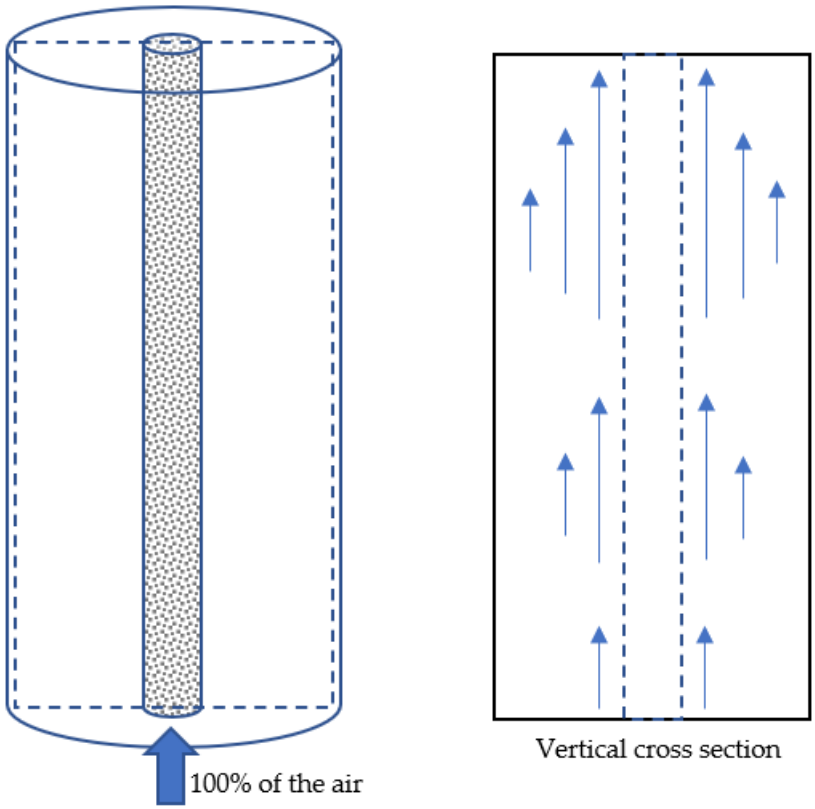

Figure 7. Strategies for aerating packed-bed bioreactors used by Perez et al. [61]. (**a**) Split aeration; (**b**) Approximate airflow patterns for split aeration; (**c**) Radial aeration; (**d**) Approximate airflow patterns for radial aeration, assuming even flow through the walls of the perforated pipe along the length (true flow patterns will depend strongly on the design of the perforations).

In one aeration strategy, there was no end-to-end aeration; rather, the air was blown into the bed through a central perforated pipe located at the vertical axis [61]. Presumably, the average superficial velocity of the air in the bed increased monotonically with height, although this would need to be confirmed experimentally. In any case, this strategy did not give good temperature control, with maximum temperatures of 52 to 55 °C occurring at different heights in the bed, compared to an inlet air temperature of 45 °C. There was evidence of poor aeration at the bottom of the bed. First, at the bed height of 20 cm, it took over 10 h for the bed temperature to increase from its initial value of around 25 °C to the temperature of 45 °C used for both the water jacket and the inlet air. Second, after the temperature peak at around 20 h, the temperature at 20 cm bed height was higher than the temperatures at the bed heights of 60 and 80 cm. The poor temperature control at the bottom of the bed is not surprising: if the air flows evenly into the bed along the height of the perforated tube, only 25% of the air has entered the bed at the height of 20 cm. This situation contrasts with what happens with normal end-to-end aeration in the absence of preferential flow paths: in this case, all the air flows through the bottom of the bed and manages to maintain the temperature in this region reasonably close to the inlet air temperature [1].

In the other aeration strategy, half of the air was blown into the bioreactor through the base of the bed, and half of the air was blown into the bioreactor through a perforated ring located at the bioreactor wall at half height (i.e., at 40 cm) [61]. With this strategy, the 20 cm bed height reached 45 °C (i.e., the temperature of the inlet air and the jacket) in just over 3 h, which is considerably faster than when the central perforated pipe was used. This is not surprising, since half of the air passes through the bottom of the bed. However, once

again, control of the bed temperature was not good. The bed height of 20 cm quickly heated to 48 °C and remained near this temperature until the end of the fermentation. The bed heights of 60 and 80 cm heated to over 50 °C, with the peak temperature of 54 °C occurring at 60 cm bed height, this being several degrees above the values of 51–52 °C measured at 80 cm bed height around the time of peak heat production. Since the nominal superficial air velocity does not change at heights above 40 cm, the temperature at 80 cm should be higher than that at 60 cm. The fact that it is not, suggests that air was following preferential flow paths. In fact, Perez et al. [61] obtained visual evidence of poor air distribution: At the end of the fermentation, the region of the bed in an annular ring 4 cm wide next to the bioreactor wall was well colonized, while the 12 cm diameter core region was very poorly colonized.

The poor temperature control and signs of uneven airflow through the bed in the studies of Brijwani et al. [60] and Perez et al. [61] are not surprising. It is difficult to ensure uniform airflow through a packed bed with the use of perforated tubes or perforated rings in the bed. It is better to use end-to-end aeration and incorporate design and operating features that promote uniform airflow. These features include (i) the design of an adequate air box, such that airflow into the bed is as uniform as possible; (ii) the use of a wide bioreactor, to minimize the importance of wall effects; (iii) the use of a highly porous medium; and (iv) the provision for intermittent mixing events, to allow the bed to be reseated if preferential flow paths develop.

4. Mathematical Modeling of Packed-Bed Bioreactors

In this section, we explore whether recent modeling work has given new insights into the design and operation of large-scale packed-bed bioreactors.

4.1. "Two-Phase" Models

Until two decades ago, models of heat and mass transfer in packed-bed bioreactors were so-called "pseudo-homogeneous" models. Such models assume that, although spatial gradients exist in the bed, at any particular height in the bed, the substrate and air phases are at the same temperature and the air is saturated [63–65]. Then, in 2002, von Meien and Mitchell [66] proposed a two-phase model, namely, a model that treats the solids and air in the bed as separate phases that are not in thermal and water equilibrium and which describes heat and water transfer between these phases using expressions composed of driving forces and heat and mass transfer coefficients.

The two-phase model of von Meien and Mitchell [66] only described heat and mass transfer in the vertical direction, in other words, parallel to the airflow between the inlet and outlet. Bück et al. [67] used a similar one-dimensional two-phase model to revisit a control strategy originally investigated by Ashley et al. [64], namely, switching the direction of aeration from one end to the other during the fermentation. Additionally, Bück et al. [67] assumed that the temperature of the water in a cooling jacket was adjusted with a simple proportional controller but provided no further details. In any case, the utility of switching the direction of aeration from one end to the other is questionable: Bück et al. [67] noted that this strategy favors high temperatures at mid height in the bed, but this conclusion had already been drawn 16 years earlier by Ashley et al. [64]. As von Meien et al. [68] had earlier showed, a better strategy would be to change the inlet air temperature in response to the temperatures measured in the bed, while maintaining the inlet air saturated.

Recently, Casciatori et al. [12] proposed a two-phase model that describes heat transfer in two directions, both vertically (i.e., parallel to the airflow) and horizontally to the side walls (i.e., normal to the airflow). Their model is useful for describing the performance of narrow laboratory-scale bioreactors, in which both axial convection and radial conduction contribute significantly to heat removal. However, as the model of Casciatori et al. [12] predicts, the contribution of heat removal through the side walls decreases as the bed diameter increases. In wide bioreactors, heat removal through the side wall only affects the bed temperature significantly in the 5–10 cm nearest to the wall. In other words, heat

removal through the side walls will be insignificant in large-scale packed-bed bioreactors that are several meters in diameter. The two-dimensional two-phase model of Casciatori et al. [12] could be used to guide the scale-up of Zymotis-type packed-bed bioreactors, although it would be necessary to change from radial to rectangular coordinates. Such a model would be preferable to the pseudo-homogeneous model describing two-dimensional heat transfer that was developed by Mitchell and von Meien [65] and used by Mitchell et al. [69] to explore the design and operation of the Zymotis bioreactor.

4.2. Models Based on Computational Fluid Dynamics (CFD)

Models based on computational fluid dynamics (CFD) are useful tools for exploring heat and mass transfer in packed-bed bioreactors. CFD can also be used to investigate the effects of particle size, shape and packing on the pressure drop across the bed [70]. Two studies have been conducted for a pilot-scale packed-bed bioreactor. The first study modeled the bed temperatures during the initial heating of the bed, immediately after bed preparation and before growth had started [24]. Such models (i.e., without equations for microbial growth) are useful for checking the heat transfer coefficients used in the model. The second study modeled the early stages of a fermentation; a problem with numerical instability meant that it was not possible to model the whole fermentation [25].

The advantage of CFD is that it can be used to investigate complex design and operating strategies that would be difficult to model with classical models of packed-bed bioreactors (which are based on partial differential equations and describe spatial domains with simple geometries). For example, CFD can be used to investigate the flow patterns in the air box underneath the bed and to investigate air-box designs that will favor uniform airflow into the bed. This would show how different designs of the air distributor within the air box can lead to different temperature patterns within the bed itself, a phenomenon that has been observed in packed-bed bioreactors [39]. CFD can also be used to investigate flow patterns in the bed when air is introduced into the bed through perforated tubes, as in the bioreactors of Brijwani et al. [60] and Perez et al. [61]. For example, in a different system, namely the drying of paddy rice, Ngu et al. [71] used CFD to simulate airflow through a packed bed in which the air was introduced through a vertical perforated tube at the central axis. Further, although CFD packages themselves cannot be used to simulate the formation of non-uniform porosity during the step of bed packing (this is a task for discrete particle models), they can be used to explore the consequences that non-uniform distributions of bed porosity have for heat and mass transfer in the bed, by attributing different initial porosities to the various cells of the CFD mesh [25]. This would allow CFD-based models to describe heat and mass transfer in thin packed-bed bioreactors in which wall effects are important, such as that used by Casciatori and Thomeo [4], or in wider packed-bed bioreactors in which non-uniform packing can cause complex porosity distributions within the bed, as observed by Finkler et al. [11]. It is also possible to model flow patterns caused by obstructions within the bed, such as the central horizontal axis of the pilot-scale packed-bed bioreactor modeled by Pessoa et al. [25].

However, CFD studies of SSF bioreactors are not simple. Although CFD has often been used to model the drying of foods and agricultural products in packed beds [72], CFD software platforms were not designed for modeling fermentation systems, so user-defined functions need to be written to describe microbial growth and its effect on the bed. The numerical instabilities in the simulations of Pessoa et al. [25] were associated with the user-defined function that was used to describe the isotherm of the solids and the role of this isotherm in determining solids–air water transfer. Further, simulating a fermentation on a desktop or laptop computer can take several weeks. Even before the CFD step itself, representing the bioreactor with mesh cells is not a simple task, but it is important: The grid that is chosen affects the rate at which the simulation converges, the accuracy of the solution of the heat and mass transfer problem and also the demand on the CPU of the computer (which affects processing time). If the available computing power is limited, then one should generate several meshes with different cell sizes and compare the results to

determine which gives the best balance between precision and processing time, as was performed by Pessoa et al. [24].

4.3. Mathematical Models as Tools for Estimating Solids–Air Heat and Mass Transfer Coefficients

Two-phase models of packed-bed bioreactors require coefficients that characterize solids–air heat transfer and solids–air mass transfer of water. The original two-phase model of von Meien and Mitchell [66] used correlations for these coefficients that had been obtained in experimental studies of the drying of corn. Casciatori et al. [12] used a totally different approach, estimating the heat and mass transfer coefficients based on dimensionless numbers: the Nusselt number, in the case of the solids–air heat transfer coefficient, and the Sherwood number, in the case of the solids–air water transfer coefficient. More recently, Finkler et al. [11] estimated these coefficients based on drying, cooling and heating experiments of a bed of solids prepared as for a fermentation, but without inoculation.

It is convenient to use "volumetric" transfer coefficients to describe heat and mass transfer in packed-bed bioreactors. A volumetric heat transfer coefficient has units of J s^{-1} $°C^{-1}$ m^{-3}-bed, while a volumetric mass transfer coefficient has units of kg-water (kg-water kg-dry-$solids^{-1}$)$^{-1}$ s^{-1} m^{-3}-bed. These volumetric coefficients can be thought of as the product of two factors: (i) the "energy or mass transferred per unit of driving force per m^2 of contact area between the air phase and the solids phase" and (ii) the specific surface area of the bed, namely the "m^2 of air–solids contact area per m^3 of bed". The specific surface area of the bed and, therefore, the volumetric transfer coefficients will depend on how the substrate particles pack together during preparation of the bed, how the packing changes during growth and how the packing is affected by mixing events. One therefore needs to estimate the volumetric transfer coefficients, or determine them experimentally, not only for each different substrate and pretreatment method, such as different intensities of chopping or grinding, but also for different packing methods [73].

In the study of Finkler et al. [11], the volumetric heat transfer coefficient and the volumetric mass transfer coefficient were used as fitting parameters to fit the heat and mass transfer part of the two-phase model of von Meien and Mitchell [66] to temporal bed temperature profiles obtained at different bed positions in drying, cooling and heating experiments. Notably, it was impossible to fit a pseudo-homogenous model to the data obtained in the bed-cooling experiment: the pseudo-homogenous model predicted cooling fronts that were much sharper than those obtained experimentally [11]. This shows that pseudo-homogeneous models are not appropriate for describing the operation of packed-bed bioreactors, even though they are still used [5,36,45,74–79]. Additionally, it was only possible to fit the temperature curves obtained during drying of the bed by assuming that the water transfer coefficient fell as the moisture content decreased. This suggests that the substrate was already in the falling drying-rate zone at the initial moisture content of 1.5 kg-water kg-dry $solids^{-1}$ (i.e., an initial moisture content of 60%, wet basis) that was used in fermentations with this substrate [43]. However, this will not necessarily always be the case, given that substrates can have widely different water sorption properties, and quite different initial moisture contents are used in different SSF processes.

The experiments of Finkler et al. [11] were limited; they were not performed with different airflow rates. Studies over a range of airflow rates would be necessary to validate the expressions that they obtained for the volumetric heat transfer coefficient and the volumetric mass transfer coefficient. In fact, similar experiments should be used routinely in the development of models of heat and mass transfer in packed-bed bioreactors. In other words, even if dimensionless numbers are used to estimate heat and mass transfer coefficients indirectly, as performed by Casciatori et al. [12], one should undertake heating, cooling and drying experiments in the absence of growth, and check the ability of the model to describe the experimentally measured temperatures at several different points in the bed. This would give one confidence in the heat and mass transfer part of the model, which is important, since significant assumptions are involved in estimating transfer coefficients indirectly. For example, Casciatori et al. [12], in estimating the heat and water transfer

coefficients of their two-dimensional two-phase model based on the Nusselt and Sherwood numbers, assumed that the particles were cylinders with the dimensions of the major component, sugarcane bagasse, although the bed contained 30% wheat bran, which has much smaller particles. They also assumed that 70% of the cylinders received the air in cross flow, and 30% received the air in parallel flow. Further, they assumed that the Sherwood number was numerically equal to the Nusselt number, which is equivalent to assuming that intraparticle mass transfer of water was not limiting. Casciatori et al. [12] did not undertake heating, drying and cooling experiments in the absence of growth to check the heat and mass transfer part of their model, which decreases the degree of confidence that one can have in the predictions of their model about the performance of the bioreactor.

A key question about solids–air heat and mass transfer has not yet been adequately addressed, namely, how this transfer is affected by the changes to the bed properties that are caused by microbial growth. Growth can affect particle size, the nature of the solids–air interface and the rigidity of the particles, with these changes affecting the bed packing (and therefore the area available for transfer) and even the values of the heat and mass transfer coefficients themselves. It is not surprising that this question remains unanswered. To obtain reliable estimates of the heat and mass transfer coefficients in the presence of a growing microorganism, it would be necessary to have reliable estimates of the rate of metabolic heat production, and this is not a simple matter in SSF systems. Another possibility would be to find a way of inactivating the microorganism and then doing cooling, heating and drying studies like those conducted by Finkler et al. [11], but the challenge is to find a method of inactivation that does not affect the bed structure and heat and mass transfer properties.

4.4. Estimation of Other Parameters That Are Important in Heat and Mass Transfer Models

In addition to solids–air heat and mass transfer coefficients, several other properties of the substrate bed are required in mathematical models of heat and mass transfer in packed-bed bioreactors. These parameters are not covered in depth here, as the topic deserves a separate review, but comments will be made about some interesting challenges.

The water sorption isotherm of the bed is important, because mathematical models contain balances on the amount of water in the bed (which can be translated directly to water content), but key processes depend on the water activity of the bed rather than the water content itself. For example, the growth rate of the microorganism depends on the water activity, not the water content. Some attention has been given to determining the water sorption isotherms of substrates and substrate mixtures used in SSF. For example, Casciatori et al. [80] determined the water sorption isotherm of a mixture of orange pulp and peel, while Casciatori et al. [81] determined the water sorption isotherms of sugarcane bagasse and wheat bran. However, it is not sufficient to determine the water sorption isotherm of the original substrate: As Marques et al. [82] showed, the water sorption properties of the biomass can be different from those of the residual substrate, such that the isotherm of the fermenting solids can change significantly during a fermentation. This topic has not received sufficient attention, probably because it is difficult to determine the water sorption isotherm and the biomass content of the fermenting solids.

Casciatori et al. [73] investigated how the bulk density and porosity of the original substrates were affected by the moisture content and by the technique used to pack the bed. They compared “loose”, “compressed” and “vibrated” packing methods for beds composed of sugar cane bagasse, wheat bran and orange pulp and peel and two mixtures (70% bagasse + 30% wheat bran, and also 20% bagasse + 40% wheat bran + 40% orange pulp and peel). Later, Casciatori et al. [83] determined the stagnant effective thermal conductivities of these substrates over a wide range of moisture contents, showing that the true effective thermal conductivity was low for intermediate moisture contents and approached that of water at high moisture contents. Similar studies need to be conducted with a wider range of substrates.

An interesting study was recently undertaken by Canedo et al. [84], who analyzed two-dimensional digital photographs to determine the bed porosity before and after fermentation of the filamentous fungus *Myceliophthora thermophila* on a 70:30 mixture of sugarcane bagasse and wheat bran. The porosity of the original substrate was over 0.7, with the diameters of the interparticle voids varying from below 0.05 mm to as much as 5 mm. After the fermentation, the growth of the mycelium into the voids decreased the porosity to values around 0.35 [84]. Further, X-ray tomography was used to generate a three-dimensional image of the network of voids in the bed. Such studies can produce data that can increase our understanding of airflow patterns within the bed and how these patterns are affected by microbial growth.

5. Conclusions

Aerobic SSF is becoming of increasing interest for application in second- and third-generation biorefineries, especially for the production of enzymes used in lignocellulosic biomass conversion [85]. Amongst the various SSF bioreactors, significant attention has been given to packed-bed bioreactors over the last decade or so, with the intention of guiding design and operation at a large scale.

Unfortunately, some of the studies have provided little useful information, for various reasons. First, some of the studies have proposed or revisited strategies that have little chance of being effective, such as reversing the direction of the airflow or introducing air through perforated pipes within the bed. Second, many studies have used narrow water-jacketed packed-bed bioreactors: although such bioreactors can be used at laboratory scale to study the growth kinetics of the microorganism, they suffer from wall effects and also do not reflect heat removal in wide large-scale bioreactors, in which heat transfer to the side walls makes a minor contribution. Third, many studies have used superficial air velocities that are much lower than what would be required for effective heat removal by the air phase. Fourth, in many studies, the temperature profiles in the bed indicate that preferential flow paths formed, suggesting that parts of the bed were not properly aerated; this must be avoided at a large scale.

Heat and mass transfer in packed-bed bioreactors is strongly affected by the operating conditions and the physical characteristics of the substrate, such as bed porosity and tortuosity. Although some attention has been given to characterizing the porosities and volumetric heat and mass transfer coefficients of substrate beds and to other characteristics, such as water sorption isotherms, the work needs to be extended to cover a wider range of solid substrates (e.g., macroalgae biomass in 3G biorefineries). Further, more work needs to be done to characterize how these bed properties change due to microbial growth. These properties and their changes during the fermentation can then be incorporated into heat and mass models, improving their ability to describe the performance of packed-bed bioreactors and, therefore, improving the usefulness of the models for guiding scale-up. With this better understanding, packed-bed bioreactors for aerobic SSF processes will find ever more applications in biorefineries.

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