Effect of water activity on gibberellic acid production by *Gibberella fujikuroi* under solid-state fermentation conditions

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Abstract

The evolution of water activity during solid-state cultivation of *Gibberella fujikuroi* was followed. A typical organic substrate, wheat bran and soluble starch, was used. Culture sorption isotherms were determined verifying that, as culture evolves, higher moisture contents were necessary to maintain the same water activity level. Optimal values for *Gibberella fujikuroi* growth and gibberellic acid production rates and yields were established, around a_w 0.99. A non-linear model, based on neural networks, is proposed to represent the sorption curves of the substrate during the fermentation process.

Keywords: *Gibberella fujikuroi*; Gibberellic acid; Neural network; Solid-state fermentation; Water activity

1. Introduction

The filamentous fungus *Gibberella fujikuroi* is the main producer of gibberellins. These hormones are widely used to optimise growth in several products, particularly seedless grapes [1]. Among the gibberellins, the most important from an industrial perspective is gibberellic acid (GA_3), which can be produced by fermentation at relatively high concentrations [2].

Solid-state fermentation (SSF) has been traditionally employed to produce a broad range of microbial products [3]. SSF technology is not widely used, however, because of the need for accurate know-how and control of process variables that determine microbial growth and metabolite production ratios [4,5]. Also, given the complexity and heterogeneity of the solid medium, environmental parameters are not easily accessible and measurable [6].

In general, ad hoc SSF reactors have been developed and operational conditions are determined empirically for a specific product [7,8], while some other studies have attempted new ways to determine optimum culture conditions in these complex media [9]. Besides, scale-up of the process requires accurate modelling [10–12]. Predictive modelling techniques, such as neural networks, are currently being used for obtaining those models [13,14].

Several papers describe moisture content and water activity (a_w), for both SSF and submerged culture, as critical variables that limit microbial growth, metabolite production and product efficacy [15–17]. Moreover, the effect of a_w on metabolite production and growth can differ [18]. For example, in *Penicillium roqueforti* a_w is a critical variable for growth and spore production in SSF [19,20]. Some studies report that the optimum a_w for growth of *Trichoderma viride* ranges between 0.99 and 0.992, while spore production is maximised at a_w 0.98 [21,22]. On the other hand, optimum a_w for cyclopeptide production in *Metarhizium anisopliae* is 0.921, although variations according to media composition were observed [23].

In this work, humidity and water activity are studied to assess their importance, for both growth and GA_3 production, in *Gibberella fujikuroi* grown on wheat bran/starch solid culture medium. Non-linear modelling is used for determining the complex substrate sorption curves during the SSF process.

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2. Material and methods

2.1. Microorganism and growth

Gibberella fujikuroi ATCC 12616, an asporogenic and hyperproducing strain of gibberellic acid, was maintained at 4 °C and periodically subcultured on malt-yeast extract agar slant tubes at 28 °C.

A propagation medium consisted of 80 g/l anhydrous glucose, 0.45 g/l magnesium sulphate, 5 g/l KH_2PO_4, 1.85 g/l NH_4NO_3, and 10 ml/l salt solution (in g/l: 0.2 $FeSO_4{\cdot}7H_2O$, 0.2 $ZnSO_4{\cdot}7H_2O$, 0.1 $CaCl_2{\cdot}2H_2O$, 0.02 $CuSO_4{\cdot}5H_2O$, 0.02 $CoCl_2$, 0.02 $Na_2B_4O_7{\cdot}10H_2O$, 0.02 $Na_2MoO_4{\cdot}2H_2O$, 0.02 $MnSO_4{\cdot}H_2O$ and 0.6 EDTA). Two milliliter of homogenised hyphae were used to inoculate 50 ml of the propagation medium, and culture flasks were incubated on a shaker at 180 rpm, 30 °C, for 64 h.

For solid-state cultures, two laboratory systems were used: 10 g columns and scaled up 100 g columns. Small columns were prepared by mixing 8 g wheat bran and 2 g soluble starch on a dry basis [24]. The substrate was complemented with 0.5 ml linseed oil, 1.3 ml urea solution (2.5 g/l), and 3 ml of a 1:10 solution, containing 1.49 g/l $ZnSO_4{\cdot}7H_2O$, 1.49 g/l $CuSO_4{\cdot}5H_2O$, 1.49 $MgSO_4{\cdot}7H_2O$ and 169 ml/l HCl. When 100 g columns were used, the mixture consisted of 80 g wheat bran, 20 g soluble starch (dry basis), 13 ml urea solution (2.5 g/l), 5 ml linseed oil and 15.7 ml of the 1:10 salt solution described above.

2.2. a_w Regulation

To obtain constant a_w levels, water–glycerol solutions were prepared to generate known and fixed relative humidity conditions. Concentrations were calculated according to UNIFAC [25].

2.3. Sorption curves

Sorption curves were obtained by gravimetric methods. A 10 g column system was used for this purpose [26]. Four cultures were run in duplicate, samples were harvested every 24 h. All samples were irradiated for 15 min using a Mineralight UVG-11 UV lamp (UV 254 nm), to eliminate fungal growth during the study. Samples were left overnight at 30 °C and then introduced in dessicators, containing defined a_w obtained by placing beakers with the water–glycerol solutions, described above. Samples were weighed every 12 h, until the weight remained constant. Constant weight indicates chemical equilibrium between relative humidity in the system and the sample, i.e. an equal a_w. Equilibrium was generally reached after three or four days. Finally, the sample was dried at 80 °C for 16 h, and the dry weight was calculated. Humidity was determined as follows:

$$\text{Humidity}(\%) = \frac{\text{weight}_{\text{initial}} - \text{weight}_{\text{final}}}{\text{weight}_{\text{initial}}} \quad (1)$$

The same protocol was employed to determine sorption curves from the pilot plant. For this purpose, samples were taken at 0, 50 and 120 h of cultivation. As described before, for each sampling point four values were obtained and a linear average and standard deviation was calculated.

2.4. Effect of a_w on fungal growth and GA_3 production

The effect of a_w on *Gibberella fujikuroi* growth was studied in malt-yeast Petri dish cultures. The initial a_w level was set using glycerol water solution, instead of pure water, for the preparation of the agar medium. The inoculated Petri dishes were introduced in sealed containers with controlled gaseous atmosphere, using the same glycerol–water solution, at 28 °C. Growth rate was determined by periodically linear measurements in three axes of the dish. Four samples were run for each condition.

For studying GA_3 generation at different a_w levels, three ranges (''high'' 1–0.98, ''medium'' 0.98–0.96 and ''low'' 0.96–0.94) and three sampling times (24, 72 and 144 h) were used. A 100 g column system was set, where a_w range was indirectly controlled using humidity level as the manipulated variable. Humidity values were obtained using the sorption curves from the initial experiments. Every twelve hours, and in a sterile environment, columns were agitated, samples taken and a_w determined. Humidity level was adjusted by addition of the appropriate amount of water. GA_3 concentration was determined fluorometrically [27].

2.5. Data modelling

Sorption curves in complex substrates generally show non-linear behaviour; therefore, a single pattern to describe any curve for any culture could not be stated. Neural network models are universal approximators of non-linear functions and they are being used for modelling non-linear biotechnology processes [28]. In this work, they were used for fitting the sorption curves of substrate–microorganism complexes during the fermentation process of *Giberella fujikuroi*. The artificial neural networks were implemented using MATLAB® software.

Humidity was represented as a non-linear function of a_w and time. Fifty-four experimental data were used to adjust the neural network parameters. A neural network with five hidden neurons was chosen after structural optimisation. A hyperbolic tangent sigmoid transfer function is used at the hidden layer and a linear transfer function for the output layer. The neural net model obtained, is described in Fig. 1, where w_{ij} is the weight from ith input to jth hidden neuron, b_j the bias of jth hidden neuron, v_j the weight from jth hidden neuron to the output neuron and b_o is the bias of the output neuron.

Model error was obtained using Eq. (2).

$$\text{Error}(\%) = \frac{\text{humidity}_{\text{real}} - \text{humidity}_{\text{estimted}}}{\text{humidity}_{\text{real}}} \quad (2)$$

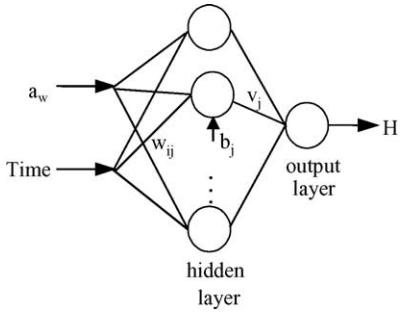

Fig. 1. Neural net model.

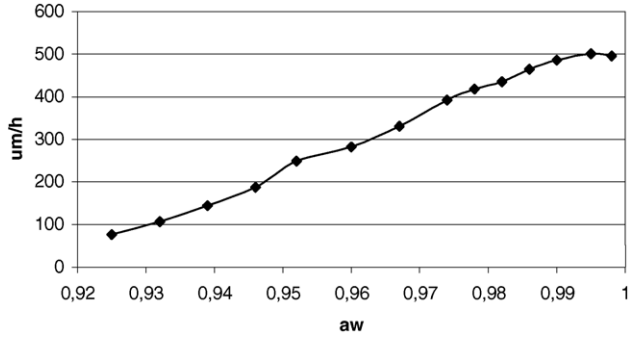

Fig. 3. *Gibberella fujikuroi* growth rate (μm/h) at different a_w.

3. Results and discussion

3.1. Laboratory and pilot plant results

Laboratory level sorption results of *Gibberella fujikuroi* fermented samples show that water availability strongly decreases as the process advances, reaching a constant level after 144 h (Fig. 2). Therefore, as the microorganism grows on the culture medium, an increasingly higher humidity of the substrate is required to achieve a similar a_w. The standard deviation of each sampling data (four experimental values each) is negligible.

Results for *Gibberella fujikuroi* growth rates at different a_w levels are indicated in Fig. 3. Minimal a_w values that support growth was approximately 0.9. Growth rate increased continuously until a_w 0.995, and then decreases slowly. Optimum growth for *Gibberella fujikuroi* was obtained between a_w 0.985 and a_w 0.995.

GA_3 production yields are also very sensitive to water activity levels (Fig. 4). For ''high'' a_w levels (1 to 0.98), maximum GA_3 production yield was obtained; almost no GA_3 production was found for ''low'' a_w levels (0.96–0.94) (data not shown).

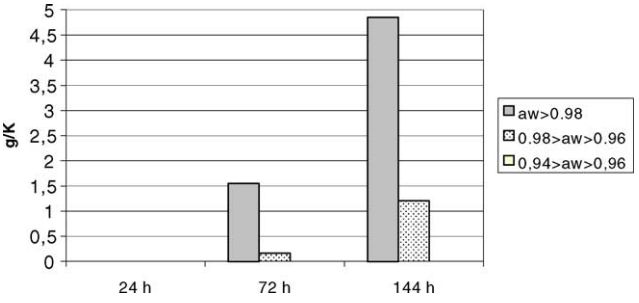

Fig. 4. GA_3 production yield (g/kg dry basis) during solid substrate cultivation of *Gibberella fujikuroi* ATCC 12616 at different a_w levels.

3.2. Sorption curve modelling

The non-linearity of experimental points was clearly shown in Fig. 1. The neural network fitting is shown in Fig. 5. Using Eq. (2), the mean error calculated for the model was 0.0114%.

Table 1 shows the parameters of the selected neural net. This table can be used to reproduce the behaviour of the sorption curves according to the neural network modelling previously defined.

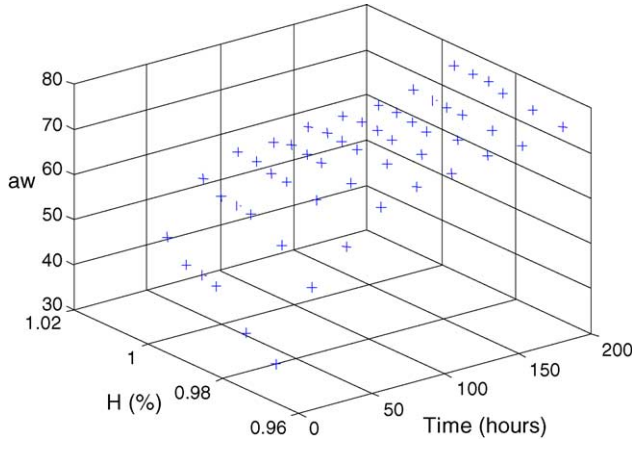

Fig. 2. Sorption data of solid medium during solid-state fermentation *by Gibberella fujikuroi* ATCC 12616 in laboratory columns (H in percent dry basis).

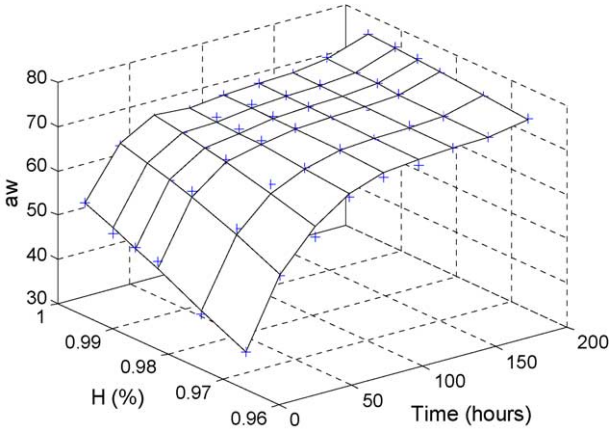

Fig. 5. Neural network model for sorption curves during SSF (+, experimental data).

Table 1
Neural network model for sorption curves during SSF

	w_{1j}	w_{2j}	b_j	v_j	b_o
$j = 1$	−0.0184	57.5781	−75.7430	13.0593	70.5184
$j = 2$	0.0217	19.9891	−19.5597	30.6576	
$j = 3$	0.0462	40.3418	−48.4118	2.7323	
$j = 4$	−240.7683	117.0850	−137.8397	11.0016	
$j = 5$	−2.0331	31.0277	66.9441	2.2345	

4. Conclusions

This work studied water availability during solid-state fermentation of *Gibberella fujikuroi* on wheat bran as organic substrate. The data obtained showed that high a_w values, −0.99 or higher—allows both optimal growth and GA_3 production rates and yields. Also, availability of water for the microorganism strongly decreased during the process.

Using experimental data, a neural network-based model was obtained for representing the non-linear behaviour of sorption curves of wheat bran during the SSF process.

This work opens some interesting future lines of study, like dynamic analysis of the agitation and hydrophobic element concentration as main process variables. Moreover, the use of the simple model developed here as a basis for optimisation of the water availability during the SSF process could be easily implemented.

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