SCIENTIFIC NOTE

Water Sorption Isotherms of *Beauveria bassiana* (Bals.) Vuill. Conidia

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Isotermas de Sorção de Água de Conídios de *Beauveria bassiana* (Bals.) Vuill.

RESUMO - Um dos requisitos para o sucesso na produção comercial de agentes de controle biológico é manter sua viabilidade por longos períodos, em condições não controladas, aumentando a vida de prateleira. A atividade de água (A_w) é um parâmetro importante no estudo da estabilidade no armazenamento de produtos desidratados, mas sua determinação direta exige equipamentos nem sempre disponíveis. As isotermas de sorção de água correlacionam A_w com a umidade de um produto; a isoterma de adsorção é importante para definir o sistema de embalagem e a de dessorção o processo de secagem. O objetivo deste trabalho foi determinar as isotermas de sorção de conídios de *Beauveria bassiana* e ajustá-las utilizando um modelo matemático. A adsorção e a dessorção foram determinadas por técnica gravimétrica a 25ºC e os dados ajustados pelo modelo de BET. Na dessorção, inicialmente os conídios apresentaram alto conteúdo de umidade (98% em base seca) e A_w (0,93); de 98% a 50% foi observada uma baixa redução na A_w, pois a água estava na forma livre. Quando a umidade estava em torno de 505, uma pequena redução na umidade resultou em grande depressão na A_w, pois a água estava na forma ligada. A curva de adsorção mostrou que quando a umidade variou de 9% a 50% um pequeno aumento na umidade dos conídios resultou num grande acréscimo na A_w. O modelo de BET representou adequadamente as isotermas de sorção dos conídios de *B. bassiana,* sendo útil no estudo do armazenamento do patógeno.

PALAVRAS-CHAVE: Entomopatógeno, controle biológico, armazenamento, atividade de água, umidade

ABSTRACT - One of the requirements for successful commercial production of biological control agent is the maintenance of viability for long periods at room temperature, increasing shelf life. The water activity (A_w) is an important parameter to study the stability in the storage of dehydrated products but its determination demands equipments not always available. The water sorption isotherms correlate the A_w with the moisture of a product; adsorption isotherm is important to define the packaging system, and desorption isotherm the drying process. The objective of this study was to determine the sorption isotherms of *Beauveria bassiana* conidia and adjust them using a mathematical model. The adsorption and desorption were determined by gravimetric technique at 25°C and data were adjusted by the BET model. During desorption, conidia initially presented high moisture content (98% in dry basis) and A_w (0,93); from 98% to 50% a low reduction in A_w was observed, because the water was in a free form. When moisture was around 50%, a small reduction on moisture resulted in a great decrease in A_w, because the water was in a bound form. The adsorption curve showed that when moisture varied from 9% to 50% a small increment in conidia moisture resulted in a high increment in A_w. The BET model adequately represented the sorption isotherms of *B. bassiana* conidia, being useful to define the ideal moisture to storage this biological control agent.

KEY WORDS: Entomopathogenic, biological control, storage, water activity, moisture

Commercial mycoinsecticides should be formulated with the following main objectives: easy field application to target the pest, increased shelf life and environmental persistence after application (Feng *et al*. 1994). One of the limiting factors for the mass use of the entomopathogenic fungus *Beauveria bassiana* (Balls. Vuill.) is the maintenance of conidial viability for long periods under environmental storage conditions, with no control of temperature and humidity.

Several studies on storage of entomopathogenic fungi focus on the temperature influence on shelf life, viability of the formulated conidia (Marques & Alves 1999, Pereira & Roberts 1991, Smith *et al*. 1999) and formulation moisture to extend the shelf life (Hedgecock *et al*. 1995, Moore *et al*. 1996, Hong *et al*.1997, Sanyang 2000).

In food science it has long been recognized that a relationship exists between the water content of food and its perishability. However, it has also been observed that various foods with the same water content differ significantly in perishability. This inadequacy can be attributed, in part, to differences in the intensity with which water associates with non-aqueous constituents. The term "water activity" (A_w) was developed to take this factor into account and it is a better indicator of food perishability than water content (moisture). So this concept can be adopted to study the water dynamic on dry process and storage of entomopathogenic fungi.

A_w is defined as (Fennema 1996):

$$A_w = \frac{p}{p_0} = \frac{ERH}{100}$$

where p is the partial pressure of water above the sample, p_0 is the vapor pressure of pure water at the same temperature (must be specified), and *ERH* is the equilibrium relative humidity (%) surrounding the product. A_w is an intrinsic property of the sample whereas ERH is a property of the atmosphere in equilibrium with the sample.

Indirect determination of A_w is a time-consuming process and the direct determination requires equipment not always available. Plots interrelating food water content (expressed as mass of water per unit mass of dry material) with A_w, at constant temperature, are known as sorption isotherms (adsorption and desorption) and this isotherms are useful in the dehydration process and for assessing stability of food. A moisture sorption isotherm prepared by the addition of water (resorption or adsorption) to a dry sample will not necessarily overlap the desorption isotherm (water losses). This overlap lack is referred as to a *hysteresis* (Fennema 1996).

The knowledge and understanding of sorption isotherms for food science is of great importance. Many problems can be solved by drying processes optimization for assessing storage and packaging, and contributing for estimation of physical, chemical and microbiological stability of the food (Spiess & Wolf 1983). This concept can be applied to entomopathogenic fungal formulation and storage processes.

The aim of this study was to determine the sorption isotherms of *B. bassiana* conidia and to adjust them, using a mathematical model.

B. bassiana conidia (strain CG425) were produced in rice using the tray method (Alves & Pereira 1989). The adsorption and desorption isotherms were determined by gravimetric technique at 25ºC, according to the method standardized by Cost 90 program (Spiess & Wolf 1983).

Equilibrium relative humidity (ERH) was obtained with saturated saline solutions, in 100 ml plastic tight air containers. Inside the containers, a support of polyvinyl carbonate (PVC) was adapted to sustain the conidia samples (three replications). Each container received about 20 ml of saturated saline solution. Salts were chosen to provide an ERH range from 10% to 100% for adsorption and from 100% to 10% for desorption [lithium chloride (11.3%), magnesium nitrate (55.4%), sodium chloride (75.3%), potassium sulfate (97.0%) and distilled water (100%)]. The samples were weighted every three days until constant weight, when moisture and A_w were determined. The moisture (dry basis) was determined at 105ºC until constant weight (about 4h), according to AOAC (1995), and A_w was determined directly by Aqualab Decagon equipment, Model CX-2, at 25°C.

The data obtained in the adsorption and desorption processes were better adjusted by the BET model (Iglesias & Chirife 1982):

$$X = \frac{X_m . C . A_w}{[1-A_w].[1+(C-1).A_w]}$$

Where:
X = water content (dry basis)
X_m = water content in the monomolecular layer (dry basis)
A_w = water activity
C = BET constant

The desorption (water loss) isotherm indicates that the conidia presented high water content (X = 98%) with A_w (0.93) (Fig. 1). From 98% to 50% moisture, a low reduction was observed in A_w(0,93 a 0,90), because the water was in a free form, therefore easily removed. This initial high moisture value (98%) can be explained because the moisture was determinate in dry basis, probably on wet basis the value obtained would be lower (< 100%). However, the sorption isotherms have to be determinate using dry basis moisture *versus* A_w. Below 50% moisture a small reduction on moisture resulted in a relatively high decrease in A_w, because the water was in a bound form. These results are useful in providing the moisture level that *B. bassiana* conidia must reach in order to determine water activity. This study will give support to define conidia drying process and formulation because a low water activity is important in conidial stability and viability.

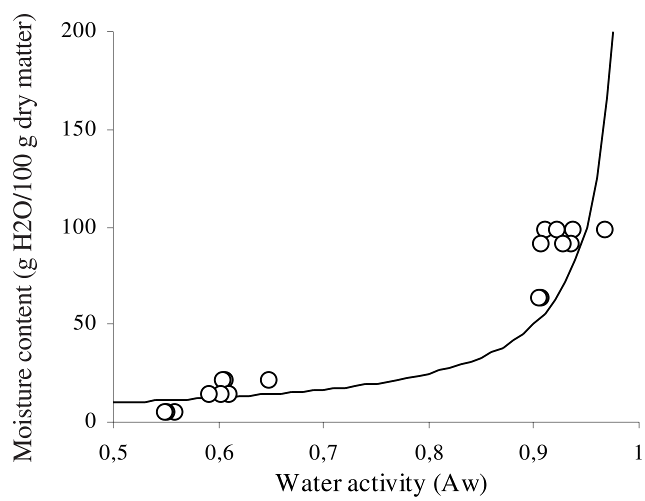

Figure 1. Desorption isotherm of *B. bassiana* conidia formulation at 25 ± 1.5°C.

The adsorption isotherm (Fig. 2) shows that from 9% to 50% of moisture a small increment of the conidia water content resulted in a relatively high increment in A_w. This result emphasizes the importance of an appropriated packaging system to store fungus, because only a small gain in water is enough to raise the A_w to inadequate levels, probably with viability losses.

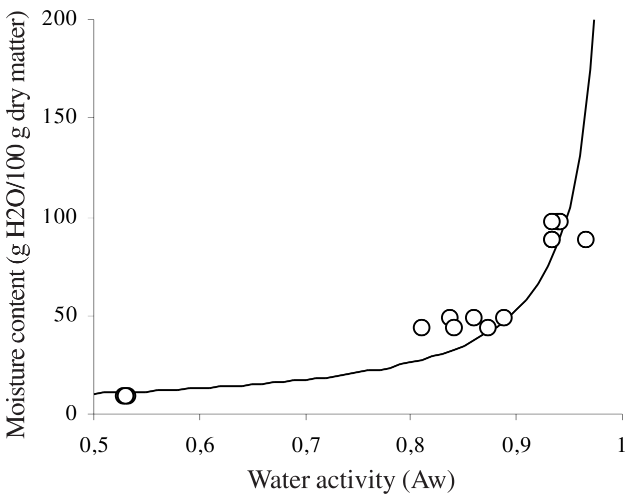

Figure 2. Adsorption isotherm of *B. bassiana* conidia formulation at 25 ± 1.5°C.

If we overlap desorption and adsorption isotherms we can observe that conidia did not gain and loss water in the same way. This difference characterized a *hysteresis*, i.e., we cannot use a single isotherm to describe adsorption and desorption processes.

According to Yin (1983), cited by Feng *et al*. (1994), the viability of a *B. bassiana* conidia preparation with 8% water content was 81% after 12 months of storage at 4°C, but decreased to 4.5% after 46 months. When the water content was increased to 15%, the same preparation could be stored for only six months, at the same temperature. When conidia were formulated with an inert (Attaclay X-250) and stored at 26°C during 12 months, there was no significant reduction in the virulence (Chen *et al*., 1990, cited by Feng *et al*., 1994).

Sorption isotherms data, adjusted to BET model parameters, are presented in Table 1. The BET model adequately represented the sorption isotherms of *B. bassiana* conidia and will be useful to study the storage and drying processes of this BCA, increasing its packaging life.

Table 1. BET model parameters adjusted to the desorption and adsorption isotherms of *B. bassiana* conidia.

Parameters	Desorption	Adsorption
X_m [1/]	5.24 [6/]	4.99
C [2/]	232,086.0	227,957.8
R^2 [3/]	0.66	0.67
$(obs\text{-}pred.)^2$ [4/]	9184,14	5060,76
N [5/]	18	15

[1/] X_m = water content in the monomolecular layer (dry basis)
[2/] C = BET constant
[3/] R^2 = determination coefficient
[4/] $(obs\text{-}pred.)^2$ = loss function
[5/] N = samples number
[6/] All levels of significance < 0.001

These results contribute to drying processes studies for large-scale storage of *B. bassiana* conidia considering factors time, cost and type of formulation.

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