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Protein Biostimulant Foliar Uptake Modeling: The Impact of Climatic Conditions

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Biostimulants are substances which promote plant metabolism and are able to increase yields of various crops. However, their efficiency at field can be affected by climatic conditions. A novel mathematical model based on diffusion transport mechanism is proposed to predict the biostimulant uptake at different climatic conditions. The main input model parameter is experimentally measured effective diffusion coefficient of the biostimulant. The model is applied to a biostimulant prepared from leather waste by enzymatic hydrolysis. Simulations show that climatic conditions have significant impact on biostimulant penetration and should not be neglected in biostimulant application and further research. The suggested model is able to explain observed differences between laboratory and field biostimulant investigations, as well as draw recommendations for protein biostimulant application. The model also shows that the theoretical tools of chemical engineering can be used for optimization of biostimulant performance. © 2011 American Institute of Chemical Engineers
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Introduction

The term ''biostimulant'' or ''biostimulator'' usually refers to a substance which is able to promote growth of plants or to enhance their metabolism.[1,2] Even though biostimulants are commonly applied in small quantities, they are able to increase plant tolerance to abiotic stresses,[1] improve nitrogen use efficiency,[2] induce defense responces,[3] and also raise yields of various crops.[4] As a result, biostimulants can lower the amount of necessary mineral fertilizers through more efficient nutrient utilization and, consequently, also decrease costs of the production and environmental pollution caused by extensive fertilizer application.[2,5] Their recent investigation was, therefore, motivated by the effort to develop an environmentally friendly system which is able to produce quality crops with competitive yields and ensure plant health even in stress conditions.[1,2]

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Biostimulants are generally divided into three groups: humic substances, seaweed extracts and amino acid-containing products.[5] The last group includes protein hydrolysates which are prepared by hydrolysis of a suitable protein substrate, and consequently these hydrolysates can be considered ''natural biostimulants''.[4] Obviously, the efficiency of different hydrolysates is influenced by the choice of the protein substrate and by the specific conditions of the hydrolysis procedure and must be, thus, assessed individually. Several articles on this topic appeared recently. Ertani et al.[5] and Schiavon et al.[2] found that protein hydrolysates were able to improve conventional fertilizer efficiency. Apone et al.[3] concluded that a mixture of peptides and sugars derived from plant cell walls induced defense mechanisms to stresses not only in plants, but also in cultured skin cells, which makes it interesting as a potential cosmeceutical. Positive effects on plant metabolic system were reported by Kaufmann et al.[1] and overall improvement of plant health and crop yields were reviewed in the article by Maini.[4] In addition, protein hydrolysates can also be prepared from byproducts of leather and food industry, which makes their

production interesting also from the economical and waste management points of view.[6]

However, while results obtained in a highly controlled and repeatable laboratory environment are promising, performance of biostimulants in the field conditions may be inconclusive.[1] Unfortunately, field and laboratory conditions as well as the entire experimental design differ in many aspects which may contribute to this phenomenon. Nevertheless, according to the available biostimulant investigations, one can conclude that biostimulants are systemic agents. As such, they must penetrate into the plant body and reach active sites to affect the plant. Hence, their successful penetration into the plant tissue is necessary condition for a reliable biostimulant efficiency evaluation. Moreover, if the uptake of the biostimulant can be assessed and the crucial factors of this process evaluated, it is possible to optimize its penetration and, thus, enhance the biostimulant efficiency and, consequently, lower the overall costs. The importance of this task is well recognized namely in the area of pesticide science in which the xenobiotics uptake has been studied for the past decades.[7] Since biostimulants are usually foliarly applied, we will deal with this route of uptake. Foliar uptake is essentially a diffusion process.[8] A substance which is to penetrate the leaf tissue to reach an active site in the plant body must diffuse through the leaf prime barrier—the cuticle.[9] The cuticle of higher plants is composed of lipophilic substances—cuticular waxes and the polymers cutin and cutane.[7,10] Diffusion across this hydrophobic barrier is considered to be the transport-limiting step during the whole transport process of a substance applied onto the leaf surface.[8,11] That is the reason why many studies of foliar uptake are focused mainly on investigation and modeling of cuticular penetration.

Various mathematical models of foliar uptake were proposed in the literature (e.g., see [10,12–15]), and for most of them results of their validation were also presented. The majority of suggested models are based either on the modification of the Fick's laws of diffusion[10,13] or employ empirical and semiempirical expressions.[12,14] Even though predictions of empirically based models are usually well correlated with the experimental data (e.g., see[12]), their use is limited to the specific measurement conditions. In addition, the published models were mostly designed for penetration of lipophilic compounds, which makes their application for the modeling of protein hydrolysate foliar uptake problematic because these protein compounds are mainly mixtures of polar and ionic molecules of various lengths. (The biostimulant consists of small molecules of free amino acids as well as larger molecules—mainly oligopeptides and a small fraction of long proteins.) To be more specific, the questionable feature of the aforementioned cited models lies mainly in their assumption that small and mainly lipophilic molecules may penetrate across the plant cuticle also after the solvent (which carries the active ingredient) has evaporated. Of course, this assumption should be valid since the cuticle consists of hydrophobic materials where lipophilic substances can be solubilized. However, using the same approach for hydrophilic and high-molecular protein biostimulants uptake modeling does not seem appropriate. More precisely, zero penetration of the active ingredient should be assumed after the solvent complete evaporation. This approach is strongly supported by the work of Schönherr et al.[16,17] who observed that penetration of potassium[16] and calcium[17] salts across the pear leaf cuticles practically stopped when the outer humidity decreased under the point of deliquescence of these salts.

For the sake of completeness, we report that also very complex models were published, which assume different routes of uptake (not only foliar).[18] However, such models usually need many input parameters and, moreover, some of them are also difficult to measure. The results predicted by such models with some of the precise input data unavailable may differ by several orders of magnitude from the experimentally measured uptake.[18]

The objective of our study is to develop a mathematical model suitable for comparison of protein biostimulant uptake at miscellaneous types of climatic conditions (especially at various levels of air humidity, temperature, and wind velocity). The model is based on the chemical engineering approach, mass transfer of the biostimulant into the plant tissue is modeled according to the known mechanisms of the physical processes involved (Fick's law and evaporation modeling).

This work shows that the theoretical tools of chemical engineering are of value not only in the area of biostimulant production itself (i.e., in the engineering and process design of the hydrolysis reaction), but also in the application of the final product at field conditions. Our aim is to obtain qualitative results (based on sound physical principles), which help us to understand how the weather may influence the complex process of biostimulant penetration into the plant tissue. The model enables us to define the optimal climatic conditions to maximize the biostimulant uptake and, consequently, maximize its positive effect and minimize costs of the application. Moreover, the model can explain why the performance of various biostimulants at field conditions may fluctuate without any apparent cause.

Uptake mathematical model

Biostimulant is supposed to enter the inner volume of the plant mainly through the leaves, let us, therefore, concentrate on the modeling of this route. Consider the surface of a leaf covered (from both sides) by a water solution of the biostimulant. The thickness of the leaf is considered uniform, much smaller than the other two dimensions. This allows us to come to one-dimensional (1-D) setting where the space variable denotes the thickness of the leaf. Even though the solution of an active ingredient is in practice often applied in the form of droplets on the leaf surface, the assumption of a film formation is justified by several facts: A similar approach was used for modeling of cuticular penetration by an ionic compound,[10] and the model was satisfactorily able to quantitatively predict experimentally measured uptake from droplets, i.e., from the finite-dose diffusion system. Moreover, the applied solution usually contains surfactants which enhance droplet spread area and, therefore, it is possible to obtain also thin surface film.[19] In addition, certain microorganisms which were observed after repeated foliar application of nutrients can contribute to increased wettability of the leaf surface,[20] which can also result in a formation of a surface film. We further assume that the leaf is isotropic so that axisymmetry can be exploited. Both sides of the leaf (upper and lower) are assumed to absorb the biostimulant in the same way. All these assumptions neglect some details of the complex process of biostimulant uptake; nevertheless, they allow us to capture the possible discrepancy between

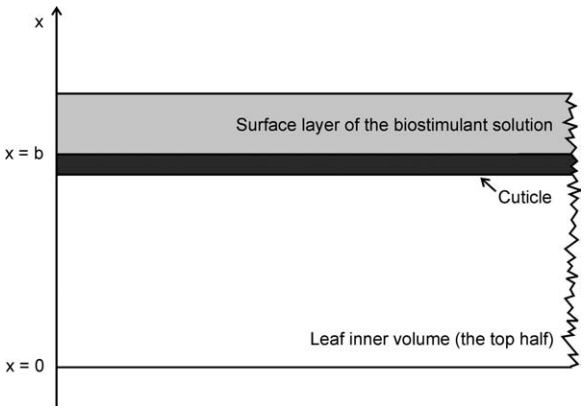

Figure 1. Scheme of leaf geometry used in the uptake model.

laboratory and field conditions, and also to estimate the impact of weather on the process, without interfering with other factors.

Let us denote $2b$ by the thickness of the leaf, the independent space variable x stands for the distance from the center of the leaf (thus $x = 0$ is at the center, $x = b$ on the upper surface, and $x = -b$ on the lower surface, see Figure 1). The time variable is denoted by τ.

Concentration of the biostimulant inside the leaf $c(\tau, x)$ is then a function of space and time. On the surface of the leaf, there is a layer of the biostimulant solution of the volume V_0. Let us denote by $c_0(\tau)$ the concentration of the biostimulant in this layer and let us suppose that the solution is homogeneous, thus, C_0 is a function of time only. Furthermore, it is necessary to account for the possible evaporation of water from the solution, which decreases the volume V_0, and, thereby possibly increasing the concentration c_0.

Let us first model the concentration of the biostimulant in the solution layer. Two processes act one against the other. First, the concentration decreases due to the infiltration of the biostimulant into the inner space of the leaf. Second, the concentration increases due to the evaporation of the water from the solution. By balancing the mass $m_0(\tau)$ of the biostimulant in the layer during an infinitesimal time $d\tau$ we obtain

$$m_0(\tau + d\tau) = m_0(\tau) - SD\partial_x c(b, \tau)d\tau \tag{1}$$

The last term in the equation stands for the decrease of the mass due to penetration into the leaf. Here, S denotes the surface of the leaf, and D the effective diffusion coefficient. In Eq. 1, evaporation is not present, since it is only water which evaporates. Writing Eq. 1 in terms of concentration, we get

$$V_0(\tau + d\tau)c_0(\tau + d\tau) = V_0(\tau)c_0(\tau) - SD\partial_x c(b, \tau)d\tau$$

which, after taking the limit $d\tau \to 0$ results in the balance equation

$$\partial_t[V_0(\tau)c_0(\tau)] = -SD\partial_x c(b, \tau) \tag{2}$$

The initial concentration of the biostimulant $c_0(0)$ is equal to a known concentration of prepared biostimulant solution c_{0p}.

The volume of the surface layer decreases due to evaporation. The evaporated amount of water can be estimated by physically-based relationship (Dalton's equation) in which the transferred amount is directly proportional to the difference between actual water-vapor pressure in the air and saturated water vapor pressure p_s with the proportionality constant k (i.e., mass-transfer coefficient). Saturated water-vapor pressure of pure water is used (at the temperature of air), the effect of biostimulant on water-vapor pressure p_s is, thus, omitted. If an air relative humidity is employed instead of water-vapor pressure, the whole surface-layer volume function can be expressed as

$$V_0(\tau) = V_0(0) - \frac{k(U) \cdot p_s(T) \cdot (1 - \varphi)S}{\rho}\tau \tag{3}$$

where ρ is the density of the water, and φ relative humidity of the air. Since the partial pressure of saturated water vapor p_s is a function of temperature, we shall use the empirical relation published by Tetens,[21] which offers very good interpolation of real experimental data in temperature range convenient for our simulation

$$p_s = 611 \exp\left(17.27\frac{T - 273.2}{T - 36}\right) \tag{4}$$

We further presume that mass-transfer coefficient k is a function of wind speed only. Although the evaporation is influenced also by radiation and, more specifically, it is a process of simultaneous heat and mass transfer, models limited to mass-transfer only are able to calculate the evaporated amount adequately.[22,23] Moreover, the experimental evaporation rates may significantly fluctuate even in the highly controlled laboratory experiments,[24] and, thus, more complicated mass-transfer coefficient function does not necessarily increase the accuracy of the model. As a result, we employ formula (5) presented by Rohwer[23] which is based on large experimental data obtained at different measuring sites. Note that we do not include the expression which corrects the influence of altitude on the process

$$k(U) = \rho(g + hU) \tag{5}$$

where g and h are coefficients of linear wind function, and U is the wind speed. Equation 3 makes sense for $\tau < \tau_k$ where τ_k stands for the "terminal time" when all the water evaporates. At the terminal time, the process of uptake into the leaf terminates. In modeling of the decrease of the surface layer volume (Eq. 3), only the decreasing volume of the solvent (water) is taken into account (i.e., the decrease of the volume of the biostimulant itself is neglected). This simplification is justified by the low-initial biostimulant concetration (usually in the order of 10^{-3} w/w), and the fact that the dependence of diffusion coefficient on concentration is neglected.

Inside the leaf, the concentration obeys the molecular diffusion equation

$$\partial_\tau c(x, \tau) = D\partial_x^2 c(x, \tau) \tag{6}$$

for $0 < x < b$ and $0 < \tau < \tau_k$ with the symmetry boundary condition

$$\partial_x c(0, \tau) = 0 \tag{7}$$

The boundary condition on the surface of the leaf is expressed by

$$c(b, \tau) = \varepsilon c_0(\tau) \tag{8}$$

where ε stands for the porosity of the leaf, i.e., the leaf is viewed as a porous material in which the biostimulant may diffuse only through the pores. This limitation of disposable leaf surface (and also its volume) was experimentally observed by confocal laser scanning microscopy,[25] and it is also hypothesized from many indirect observations.[7,20] However, the biostimulant most probably diffuses through stomatal pores of living plants as well. Their actual surface and permeability is influenced by factors like humidity, light radiation and solution surface tension.[16,26,27] Hence, a more rigorous approach may assume "epsilon" to be a function rather than a constant. On the other hand, the ideal case of constant surface porosity is convenient for our (mainly qualitative) purposes, makes the model simpler and reduces the need of experimental input parameters. The initial concentration of the biostimulant inside the leaf is equal to zero.

It is convenient to transform the model into dimensionless variables. This transformation simplifies and generalizes the model solution and limits number of process key parameters. Let us, therefore, define the dimensionless quantities in the following way

$$X := \frac{x}{b}$$

$$Fo := \frac{D\tau}{b^2}$$

$$C(X,Fo) := \frac{c(x,\tau)}{c_{0p}} = \frac{c\left(bX, \frac{b^2 Fo}{D}\right)}{c_{0p}}$$

$$C_0(Fo) := \frac{\varepsilon c_0(\tau)}{c_{0p}} = \frac{\varepsilon c_0\left(\frac{b^2 Fo}{D}\right)}{c_{0p}}.$$

Consequently, the domains transform to $X \in (0,1)$ and $Fo \in (0, Fo_k)$ with $Fo_k = \frac{D\tau_k}{b^2}$.

Equations 6–8 describing the diffusion inside the leaf transform to

$$\partial_{Fo} C(X,Fo) = \partial_x^2 C(X,Fo) \quad \text{for } Fo \in (0,Fo_k) \text{ and } X \in (0,1) \tag{9}$$

$$\partial_X C(0,Fo) = 0 \qquad \text{for } Fo \in (0,Fo_k) \tag{10}$$

$$C(1,Fo) = C_0(Fo) \qquad \text{for } Fo \in (0,Fo_k) \tag{11}$$

$$C(X,0) = 0 \qquad \text{for } X \in (0,1) \tag{12}$$

$$C_0(0) = \varepsilon \tag{13}$$

Equations 2 and 3 can be combined together and dedimensionalized to obtain

$$-\frac{Ev(1-\varphi)}{Na} C_0(Fo) + \left[1 - \frac{Ev(1-\varphi)}{Na} Fo\right] \partial_{Fo} C_0(Fo) + \frac{\varepsilon}{Na} \partial_X C(1,Fo) = 0 \tag{14}$$

with dimensionless parameter $Na = \frac{V_0(0)}{Sb}$ being the so-called soaking number (i.e., the ration of the initial volume of the liquid layer to the volume of the leaf), and other dimensionless parameter Ev

$$Ev = \frac{k(u) p_s b}{\rho D}$$

Materials and Methods

Materials

Chrome leather shavings were provided by TAREX, s.r.o. (Otrokovice, Czech Republic), enzyme ALCALASE 2, 5LDX was provided by Novo Nordisk, Denmark. Magnesium oxide, sodium potassium tartrate tetrahydrate, copper sulfate pentahydrate, potassium iodide, sodium hydroxide and phosphoric acid, were purchased from IPL (Uherský Brod, Czech Republic). Isopropylamine was purchased from Sigma-Aldrich (Prague, Czech Republic). Chemicals used for protein biostimulant amino acids determination, i.e., hydrochloric acid, citric acid monohydrate, sodium citrate dihydrate, sodium chloride, thiodiglycol, boric acid, sodium azide, sodium hydroxide, ninhydrine, methylcellosolve, acetate buffer and hydrindantin were supplied by Ingos (Prague, Czech Republic). Polysaccharide pullulan standards used for GPC calibration were purchased from Polymer Laboratories (Church Stretton, U.K). All chemicals purchased for experiments were of analytical grade. Demineralized water with conductivity lower than 1 μS was used in all experiments.

Biostimulant preparation

Biostimulant preparation was based on a procedure of leather scraps enzymatic hydrolysis described previously,[28] the model biostimulant was prepared by one-step enzymatic process. Briefly, glass reactor was filled with 500 g of leather scraps, 1670 g of demineralized water, 8.95 g of isopropylamine and 10 g of magnesium oxide, and this mixture was agitated and heated to 70°C. After reaching this temperature, 0.25 g of proteolytic enzyme Alcalase was added to the reaction mixture. The temperature was kept at a constant level and pH was maintained by additions of the isopropylamine at a level of 9. After 4 h of the heating the resultant reaction mixture was filtered by standard grade of qualitative filter paper. The obtained filtrate was neutralized by phosphoric acid to pH approximately 7 and used as a model biostimulant.

Biostimulant characterization

Dry matter was determined by drying biostimulant samples to constant weight at 105°C. Amino acids were determined according to the following procedure: For quantification of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine, the samples were subjected to acid hydrolysis with 6 mol·l-1 HCl at 110 $\pm$ 1°C for 23 h. Amino acids containing sulfur (methionine and cysteine) was hydrolyzed separately after oxidizing using performic acid. Amino acids were determined by using an AAA 400 amino acid analyzer (INGOS, Czech Republic), with ion exchange chromatography with post column ninhydrin-based detection by using sodium citrate buffers. The sample preparation procedure and chromatographic conditions were used as described in[29]. Tryptophan was not analyzed. Average molecular weights and polydispersity index were determined by gel permeation chromatography, instrument PLGPC-50 (Polymer Laboratories, Church Stretton, U.K.) equipped with PL differential refractometer and online viscometer detectors. Analyses were performed on two columns connected to series —TSK GMPWXL column (Tosoh Bioscience, Stuttgart, Germany) and Ultrahydrogel 250 column (Waters, Milford MA, USA). Analytical conditions and sample preparation procedure described in detail in[6] were used during all analytical runs.

Biostimulant quantification

Biuret method was employed for estimation of biostimulant concentration. At first, Biuret reagent was prepared by dissolving of 9 g of sodium potassium tartrate tetrahydrate, 3 g of copper sulfate pentahydrate and 5 g of potassium iodide in 400 mL of 0.2 M sodium hydroxide solution. Finally, the solution was diluted to the volume of 1 liter with the 0.2 M sodium hydroxide solution. A biostimulant sample of volume 0.85 mL was precipitated with 3 M trichloroacetic acid and centrifuged at 5,000 rpm. for 10 min. The supernatant was discarded and 5 mL of biuret reagent was added to the precipitate. Obtained solution was left in dark for 30 min, and was then diluted with demineralized water to the final volume of 10 mL. The solution absorbance against the blank sample was measured at 546 nm with Helios alpha UV–vis spectrometer (ThermoFisher Scienfitic, Waltham MA, USA). The quantification of samples was done according to the calibration curve measured with prepared biostimulant solutions of known concentrations.

Effective diffusion coefficient estimation

Freshly cut off leaves of rapeseed (*Brassica napus var. napus*) were immersed in 7.7% (w/w) biostimulant solution. The soaking number was equal to 1.5, and the average thickness measured with a calliper was 0.45 mm. Samples of the solution were taken at prescribed time intervals, and the biostimulant content was quantified by the biuret method described earlier. The measured concentrations were plotted against square root of time and fitted with linear regression. The total biostimulant uptake is equal to the decrease of biostimulant amount in the solution and the effective diffusion coefficient can be then estimated from the linear function slope of the following expression based on "Sorption method" by Crank[30]

$$\frac{c_{0p} - c_0(\tau)}{c_{0p} - c_0(\infty)} = \frac{2}{\sqrt{\pi}} \cdot \frac{1 + Na}{Na} \cdot \sqrt{\frac{D\tau}{b^2}} \tag{15}$$

where $c_0(\infty)$ denotes equilibrium concentration of biostimulant solution.

Model solution

The proposed mathematical model is a particular case of a rather a wider class of models considered (see, e.g.,[31]) where the authors prove the existence and uniqueness of a solution. Here, we restrict ourselves to a numerical solution of the model. We use a simple finite difference method, where the second derivative with respect to the space variable is approximated by the second-order central difference and the time derivative is approximated by a forward difference. The resulting numerical scheme is an explicit one, which makes it very easy to implement. The discretization has to meet the well-known requirement of $dF0 \leq 1/2dX^2$ in order to be stable. Here dFo denotes the dimensionless time discretization step, and denotes dX the dimensionless space discretization step.

The computation proceeds as follows: First, the boundary conditions (10) and (11) are set. Next, one time step of the diffusion process is performed updating the values of the biostimulant concentration field. Then the flux of the biostimulant accross the leaf surface is used to update the concentration of the biostimulant in the surface layer. These three steps are repeated in a time loop until the terminal time is reached (thus $\tau = \tau_k$). See the Appendix for a pseudo-code of the algorithm.

From the mathematical point of view, system of Eqs. 9–14 represents a nonstandard model, because it consists of a (standard) partial differential equation whose boundary condition is governed by an ordinary differetial equation. Standard software packages for numerical solution of PDEs are not adapted to solving such systems, we, therefore, developed a simple routine in the MatLab® environment to solve the problem.

Table 1. Prepared Biostimulant Amino Acid Composition

Amino acid	Content (% w/w ± S.D.)
Asp	6.72 ± 0.14
Thr	1.74 ± 0.06
Ser	3.01 ± 0.12
Glu	8.41 ± 0.07
Pro	16.47 ± 0.22
Gly	26.38 ± 0.84
Ala	10.05 ± 0.50
Val	2.43 ± 0.03
Ile	1.60 ± 0.05
Leu	3.14 ± 0.01
Tyr	0.47 ± 0.02
Phe	2.06 ± 0.07
His	1.67 ± 0.07
Lys	3.85 ± 0.11
Arg	10.39 ± 0.16
Cys	0.23 ± 0.01
Met	1.37 ± 0.03

Results and Discussion

Prepared model biostimulant was characterized by its amino acid composition and average molecular weight. These data are necessary for the estimation of biostimulant quality[2] and its transport properties. Amino acid composition is presented in Table 1, the measured weight-average molecular weight was 18 200 g/mol, and the polydispersity index was equal to 12.1. Despite the molecular weight is quite high, the polydispersity index shows that the prepared model biostimulant consists of wide range of macromolecules with significantly different chain lengths, i.e., the biostimulant molecular weight distribution is not uniform. Since protein biostimulants usually contain fractions of large macromolecules and oligomers as well as free amino acids, and it is still not clear which fraction carries the desired biostimulant properties, the prepared hydrolysate is suitable for investigation of average biostimulant transport parameters.

The effective diffusion coefficient was estimated from the total hydrolysate uptake and computed from the expression (15) to be $9.1 \cdot 10^{-14}$ m^2/s. Figure 2 shows the measured experimental data used for the diffusion coefficient estimation according to Eq.15. The calculated value corresponds to data published elsewhere[10]; however, the measured coefficient is low and corresponds to the reported value of the effective cuticular diffusion coefficient (which is the lowest one in the leaf environment). This finding confirms our initial assumption since the rate of penetration is dependent on the size of the penetrating molecule,[26] and, thus, large biostimulant molecules should diffuse slowly. It should be noted that the selected analytical method does not distinguish between different lengths of molecules, the calculated value is, therefore, an average coefficient among all peptides in the investigated mixture. This is convenient for our purpose of uptake

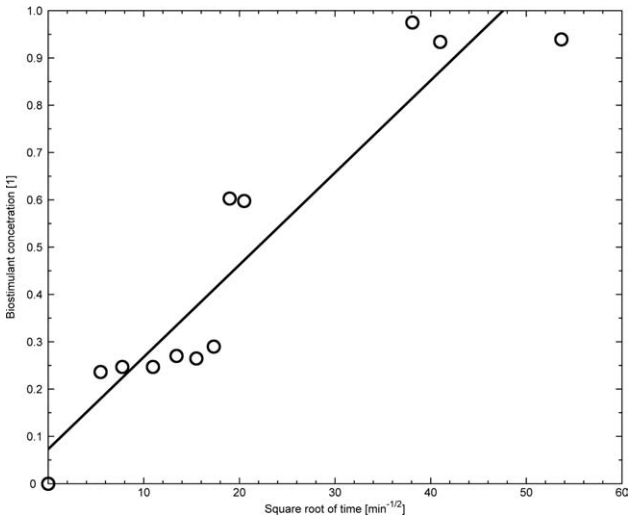

Figure 2. Measured biostimulant uptake used for effective diffusion coefficient estimation.

Biostimulant concentration was calculated according the expression (15).

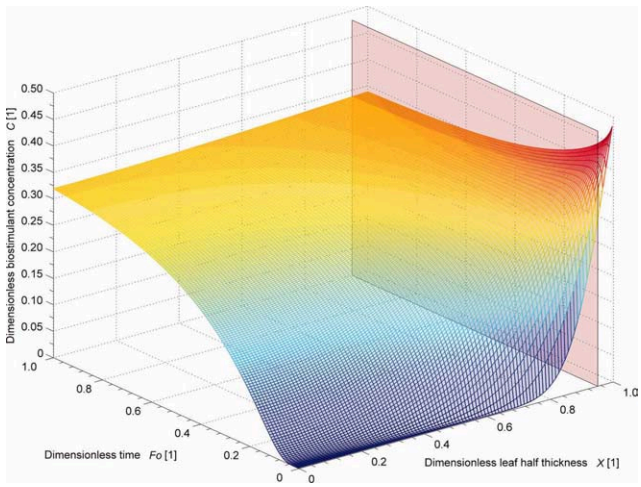

Figure 3. Scenario I: Evolution of the biostimulant concentration field inside the leaf.

The vertical plane represents the bottom of the leaf cuticle. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

simulation because virtually all the protein compounds of the biostimulant are taken into account. The effective diffusion coefficient is a cornerstone of the model, since it determines the amount of biostimulant capable of reaching the active sites of the leaf before the terminal time is reached. This, in turn, enables us to select optimal conditions for the biostimulant application (i.e., optimal temperature, humidity, wind speed).

The proposed uptake model takes into account solvent (i.e., water) evaporation from biostimulant solution and its simultaneous diffusion into the leaf. Most experimental studies concentrate on the effect of climatic conditions on the plant biological response (i.e., stomata opening or closing, cuticle permeability, etc.). The model presented here shows that climatic conditions also affect the physical nature of the mass-transport process by changing the concentration gradients driving the uptake of the biostimulant into the plant body. We have compared four different scenarios in order to investigate this physical effect. The input parameters used for the simulations are summarized in Table 2.

The first situation— marked I— describes conditions of an ideal greenhouse. The relative humidity is 100% so that no evaporation occurs, the diffusion process has enough time to reach an equilibrium. The evolution of the simulated biostimulant concentration field inside the leaf is captured in Figure 3. The transparent plane shown in the image (placed beneath the surface layer where $X = 1$) represents the inner boundary of the cuticle. As can be seen, the concentration in the surface layer of the leaf decreases in time and practically constant biostimulant concentration is reached inside the whole leaf when the dimensionless time reaches the unity ($Fo = 1$).

A completely different situation was simulated in scenario II. The temperature is high, air humidity low, and the wind is strong enough to move small branches (corresponds to Beaufort number 4)—the conditions are selected to match a hot summer day. The calculated concentration field is shown in Figure 4. The figure shows that the uptake process is terminated very quickly (the value of Fo_k is as low as 0.0014), and practically no biostimulant reaches the inner volume of the leaf (beneath the cuticle). However, the concentration of biostimulant in the cuticle surface layer becomes very high, especially close to the termination time where its value rockets up. This phenomenon is caused by evaporation which, in

Table 2. Input Parameters of Biostimulant Uptake Simulation

Parameter	Unit	I	II	III	IV
Na	[1]	1	1	1	1
D	[$m^2 \cdot s^{-1}$]	$9.1 \cdot 10^{-14}$	$9.1 \cdot 10^{-14}$	$9.1 \cdot 10^{-14}$	$9.1 \cdot 10^{-14}$
φ	[%]	100	30	91	91
T	[°C]	25	30	12	12
U	[$m \cdot s^{-1}$]	0.0	7.5	0.2	13.5
ε	[1]	0.5	0.5	0.5	0.5
b	[m]	10^{-4}	10^{-4}	10^{-4}	10^{-4}
c_{0p}	[$kg \cdot m^{-3}$]	10	10	10	10
δ	[m]	$5 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	$5 \cdot 10^{-6}$
Ev	[1]	532	980	66	536

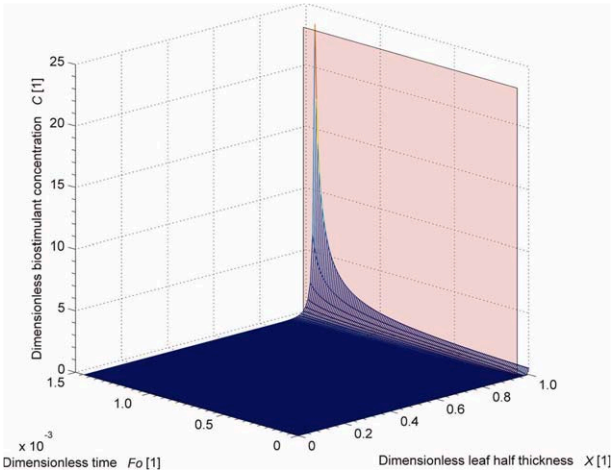

Figure 4. Scenario II: Evolution of the biostimulant concentration field inside the leaf.

The vertical plane represents the bottom of the leaf cuticle. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

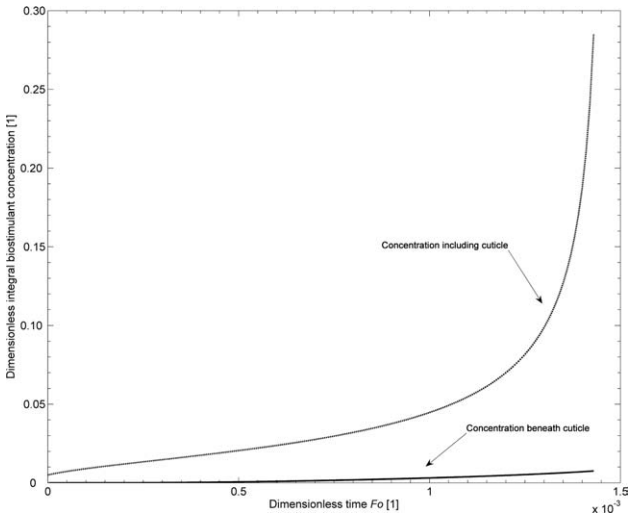

Figure 5. Difference between the integral biostimulant concentration inside the leaf including and excluding the cuticle.

this case, proceeds much faster than the biostimulant diffusion. As a result, the biostimulant concentration in the surface film increases which speeds up the uptake because the difference in concentrations across the leaf boundary is the driving force of the uptake process. Nevertheless, the biostimulant present on the leaf surface at the termination time takes the form of of a solid residue which cannot further diffuse. The model clearly overestimates the diffusion rate of highly concentrated biostimulant shortly before the termination time, because it uses an average value of effective diffusion coefficient and terminates when all the water has evaporated. Therefore, we conjecture that only biostimulant present beneath the cuticle should be considered capable of affecting the plant, it is improbable that biostimulant trapped within the cuticle is able to penetrate further below when all the solvent on the leaf surface has evaporated. In addition, cuticle permeability itself is also dependent on air humidity,[32] which further decreases the diffusion coefficient. It is common to measure penetration of substances into leaves by methods that quantify only those molecules that penetrate below the cuticle (e.g., see [10,16,17]). These considerations led us to use the average integral biostimulant concentration beneath the cuticle as a convenient measure of biostimulant amount capable of reaching active sites of a plant. This quantity is defined by

$$\bar{c}(\tau) = \int_{x=0}^{b-\delta} c(\tau, x)dx \tag{16}$$

where δ is the thickness of the cuticle. There is a substantial difference between integral concentration calculated under the cuticle only ($\bar{c}(\tau)$), and the total integral concentration defined by

$$\bar{c}_{\text{tot}}(\tau) = \int_{x=0}^{b} c(\tau, x)dx \tag{17}$$

Figure 5 compares the evolution of both these integral averages. The figure reveals that in scope of Scenario II, only a negligible amount of biostimulant is able to penetrate below the cuticle (cf. Figure 4). Therefore $\bar{c}(\tau)$, the quantity will be used for assessing the total effective concentration of the biostimulant inside the leaf. Thus, in Scenario II, the total amount of biostimulant capable of reaching the active sites of a plant is substantially smaller than in Scenario I.

The next Scenario III, is related to the previous one. The summer day in scenario II is hot but in the night, the temperature falls down which causes the relative humidity to increase significantly (as calculated accurately by means of expression (4)). There is practically no wind and, consequently, the evaporation parameter *Ev* is low. However, if wind starts to blow vigorously (Beaufort number 6), the parameter *Ev* is changed substantially— such situation is simulated in scenario IV. All four simulations are summarized in Table 3, the results are expressed in terms of the final integral biostimulant concentrations beneath the cuticle. The overall level of penetration of the biostimulant into the plant can be very low if the climatic conditions are not suitable, as presented by Scenario II. It can be calculated that the biostimulant application would have to be repeated approximately 39 times to reach the same level of penetration as in the greenhouse case (Scenario I). However, when slow evaporation occurs, the biostimulant concentration in the solution steadily increases which promotes its diffusion into the leaf. The final integral concentration in such a case (Scenario III) can even surpass the greenhouse case level. On the other hand, even at a suitable humidity level, evaporation can be accelerated, e.g., by wind (Scenario IV) so that the overall level of penetration is reduced (ca. $^2/_3$) compared to the greenhouse scenario.

The simulations performed by means of the suggested model show a considerable impact of climatic conditions on the process of protein biostimulant uptake. This is in agreement with published results.[26] These results advocate for a study of the parameter sensitivity of the model in order to assess the influence of the respective parameters on the biostimulant diffusion more deeply, especially from the point of view of the physical processes involved. The dimensionless formulation of the aforementioned model introduced is particularly suitable for this analysis because it restricts the quantity of parameters to the smallest possible number in a physically reasonable way. The basic input data used for the parameter sensitivity study are summarized in Table 4, only one parameter at a time was varied in the simulations in the following description.

Let us first concentrate on the effect of the relative air humidity. The relative air humidity (precisely the ratio of the actual to the saturated water vapor pressure in the air) is one of the driving forces of water evaporation from the biostimulant solution. Its influence on the uptake process is shown in Figure 6. As discussed earlier, lower relative humidity promotes the hydrolysate uptake due to the increase of its concentration in the surface solution. However, the positive effect of this phenomenon has its

Table 3. Calculated Final Integral Biostimulant Concentration beneath the Cuticle

Scenario	Integral concentration [1]
I	0.309
II	0.008
III	0.614
IV	0.190

Table 4. Basic Input Parameters of Biostimulant Uptake Simulation used for Parameter Sensitivity Study

Parameter	Unit	Value
Na	[1]	1
D	$[m^2 \cdot s^{-1}]$	$9.1 \cdot 10^{-14}$
φ	[%]	80
T	[°C]	18
U	$[m \cdot s^{-1}]$	1.0
ε	[1]	0.5
b	[m]	10^{-4}
c_{0p}	$[kg \cdot m^{-3}]$	10
δ	[m]	$5 \cdot 10^{-6}$
Ev	[1]	139

maximum near the 100% humidity level and decreases rapidly with falling levels of relative humidity, because lower levels of humidity cause the termination time τ_k (of the solvent complete evaporation) to become shorter so that less biostimulant has the chance to penetrate into the leaf. For example, the surrounding relative humidity of 80% ensures a similar effect as an application at ideal greenhouse contitions. It can, therefore, be recommended that the relative humidity is kept as high as possible during the biostimulant application. Furthermore, high-relative humidity also improves the permeability of leaves to penetrating substances.[32]

Let us now deal with the model sensitivity to the dimensionless parameter Ev which entangles (among other parameters) the mass-transfer coefficient (dependent on wind speed) and the ratio of the relative diffusion coefficient to the leaf thickness. In practice, the value of Ev may vary over several orders of magnitude, especially due to the diffusion coefficient and mass-transfer coefficient. Figure 7 shows that the value of Ev should be kept below the order of 100 to ensure comparable effect to the greenhouse 100% humidity case. The so-called soaking number Na has a similar effect to Ev. It determines the thickness of the biostimulant layer relative to the volume of the leaf. It is obvious that thicker layer (with correspondingly higher Na) contains a higher amount of biostimulant and provides longer time for the diffusion process which results in an increased final integral concentration under the cuticle. However, the thickness of the surface solution layer is largely determined by other application parameters (e.g., coarseness of the spray, solution surface tension) and can, thus, be influenced to a limited extent only.

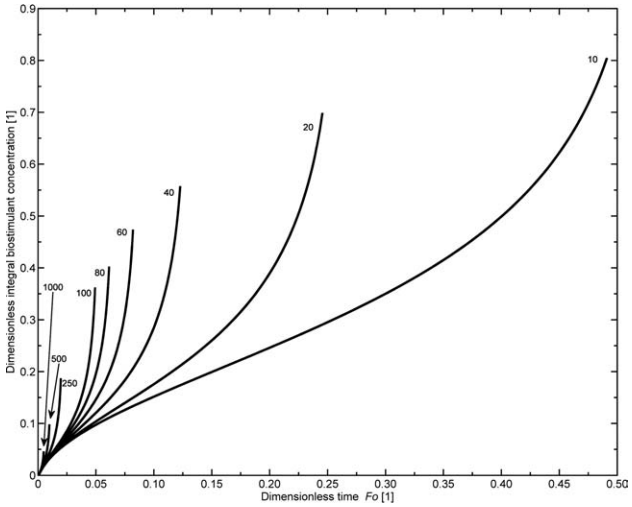

Figure 7. Influence of the parameter *Ev* on the biostimulant uptake.

The integral biostimulant concentration beneath the cuticle is shown.

Leaf porosity is the last parameter discussed. This parameter determines the surface fraction which is accessible for the biostimulant molecules, and also the volume fraction of the leaf where biostimulant diffusion may take place. Figure 8 presents the role of this parameter. It is, thus, advantageous to increase the leaf porosity to maximize the biostimulant uptake. The surface porosity itself is influenced by stomatal opening, which can considerably affect the uptake process[26] (by changing the ε value) as already discussed in the Uptake mathematical model section. However, stomatal penetration can also be enhanced by the application of a suitable surfactant.[26] On the other hand, the use of surfactants simultaneously decreases the physically achievable maximal thickness of the biostimulant solution layer,[33] thus, decreasing the

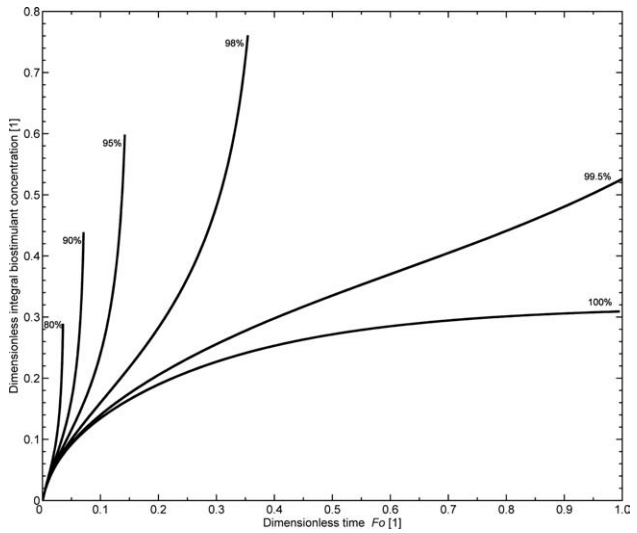

Figure 6. Influence of the air relative humidity on the biostimulant uptake.

The integral biostimulant concentration beneath the cuticle is shown.

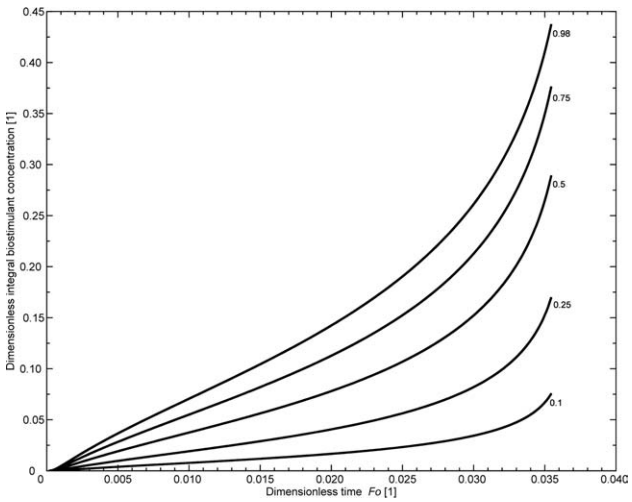

Figure 8. Influence of the leaf porosity on the biostimulant uptake.

The integral biostimulant concentration beneath the cuticle is shown.

value of *Na*. If it is possible to assess the change in ε and *Na* caused by a surfactant, the presented model can be used to decide whether the effect of the surfactant is positive or not.

Conclusions

The proposed uptake model is based on diffusion mechanism and takes into account water evaporation according to known physical relations. The model and its assumptions are appropriate for foliar uptake simulation of hydrophilic and higher molar mass substances, such as protein hydrolysates. The effective diffusion coefficient is the major experimental input parameter, which determines the rate of substance penetration into the leaf. The model is suitable for total uptake estimation for broad range of initial conditions and it is also able to assess the influence of climatic conditions. The results are in good qualitative agreement with published uptake experimental investigations (e.g., see [11,16,17,33]), they confirm the decisive role of weather on the uptake of the liquid biostimulant, and they confirm that the uptake proceeds in the order of days. In the literature,[7,26,32] the authors usually study the effect of climatic conditions on plant biological responses (stomata opening or closing, cuticle permeability, etc.). However, our model shows that it is necessary to take into account also the impact of climatic conditions on abiotic physical processes involved during the uptake (evaporation rate). From our point of view, this presents an interesting contribution to understanding the process of biostimulant foliar uptake.

The model simulations highlighted that climatic conditions have significant impact on biostimulant penetration which should not be overlooked in their application and research. For example, the overall uptake can be approximately 40 times lower at unsuitable climatic conditions compared to the recommendable ones. The suggested model, thus, may explain the observed discrepancy between laboratory and field biostimulant investigations. It can also draw recommendations for protein biostimulant application in order to maximize its penetration and consequently its positive effect:

1. High-humidity levels largely increase the total uptake, while very low-humidity leads to termination of the whole process and limits the total achievable uptake. It is, therefore, advantageous to apply the biostimulant when the air humidity is near the point of saturation—e.g., after rain, early in the morning, and in the evening (especially when dew falls). High humidity is, thus, inevitable for notable biostimulant effect.

2. Wind impedes the uptake, best results are achieved near zero wind velocities (Beaufort number 0).

3. An increase in biostimulant layer thickness and initial biostimulant concentration lead to higher total uptake.

4. High-leaf porosity values (e.g., caused by stomata opening) promote the diffusion. On the other hand, the action to incerase the porosity should not significantly affect the biostimulant layer thickness.

This article shows that the theoretical tools of chemical engineering are of value not only in the area of biostimulant production itself, but also in the application of the final product at field conditions. This approach attempts to describe and understand the mechanisms underlying the biostimulant uptake process.

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Notation

b = leaf half thickness, m
c = concentration of the biostimulant inside the leaf, $kg \cdot m^{-3}$
C = dimensionless concentration of the biostimulant inside leaf, 1
c_0 = concentration of the biostimulant in the surface solution, $kg \cdot m^{-3}$
C_0 = dimensionless concentration of the biostimulant in the surface solution, 1
c_{0p} = initial biostimulant concentration in the surface solution, $kg \cdot m^{-3}$
D = effective diffusion coefficient, $m^2 \cdot s^{-1}$
dFo = dimensionless time discretization step, 1
dX = dimensionless space discretization step, 1
δ = thickness of leaf cuticle, m
Ev = dimensionless evaporation parameter, 1
ε = leaf porosity, 1
Fo = dimensionless time, 1
g = linear wind function coefficient, $m \cdot s^{-1} \cdot Pa^{-1}$
h = linear wind function coefficient, Pa^{-1}
φ = air relative humidity, 1
k = water mass-transfer coefficient, $kg \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$
m_0 = biostimulant mass in the surface solution, kg
Na = soaking number, 1
p_s = saturated water vapor pressure, Pa
ρ = water density, $kg \cdot m^{-3}$
S = surface of the leaf, m^2
τ = time, s
τ_k = terminal time of calculation, s
T = temperature, K
U = wind speed, $m \cdot s^{-1}$
V_0 = biostimulant surface solution volume, m^3
x = space variable, m
X = dimensionless space variable, 1

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Appendix

The pseudo-code of the algorithm of the model. The algorithm was implemented in MatLab R2007b.

1. Set parameter values
2. Calculate the terminal time
3. Set discretization parameters dF and dX
4. Set initial values of the concentration field inside the leaf (variable 'con')
5. Set initial value of the concentration in the surface layer (variable 'surrcon')

for time = 0:dF:terminal time
set the boundary values:
con(1) = con(2); (*boundary condition at the center of the leaf*)
con(N) = surrcon; (*boundary condition at the surface of the leaf*)
perform one step of the diffusion process:
right = [con(1) con]; right (N +1) = []; (*shift the concentration field to the right*)
left = [con con(N)]; left (1) = []; (*shift the concentration field to the left*)
con_new = con + C* (right + left - 2*con);
compute the concentration in the surface layer:
surrcon_new = surrcon + A* surrcon + B* (con(N-1) - con(N));
update the variables:
con = con_new;
surrcon = surrcon_new;
end
Note: A, B and C are constants entangling dF, dX and the input parameters.

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