Another Brick in the Cell Wall: Biosynthesis Dependent Growth Model

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Abstract
Expansive growth of plant cell is conditioned by the cell wall ability to extend irreversibly. This process is possible if (i) a tensile stress is developed in the cell wall due to the coupling effect between turgor pressure and the modulation of its mechanical properties through enzymatic and physicochemical reactions and if (ii) new cell wall elements can be synthesized and assembled to the existing wall. In other words, expansive growth is the result of coupling effects between mechanical, thermal and chemical energy. To have a better understanding of this process, models must describe the interplay between physical or mechanical variable with biological events. In this paper we propose a general unified and theoretical framework to model growth in function of energy forms and their coupling. This framework is based on irreversible thermodynamics. It is then applied to model growth of the internodal cell of Chara corallina modulated by changes in pressure and temperature. The results describe accurately cell growth in term of length increment but also in term of cell pectate biosynthesis and incorporation to the expanding wall. Moreover, the classical growth model based on Lockhart’s equation such as the one proposed by Ortega, appears as a particular and restrictive case of the more general growth equation developed in this paper.

Introduction
Plant growth implies cell divisions and irreversible expansion of cell wall s. For the latter to occur, two concomitant conditions are required. The first one is the cell wall mechanical deformation in response to the cell turgor pressure build up. The latter results from the aquaporins regulated flow of water in the vacuole driven by osmotic gradient [1,2]. The second condition is the cell capacity to synthesize, export and incorporate other bricks at the inner face of the cell wall to maintain its integrity [3,4] and to cause its mechanical relaxation. This chemically mediated deformation consists in the reorganization of load-bearing cross-links between the different bricks in the cell wall and in the creation of new ones by the incorporation of new elements.

The structure, organization and dynamics of the load-bearing network remain open questions. The constitutive bricks in the growing plant cell wall consist mainly in three groups of polysaccharides: cellulose microfibrils embedded in a matrix of hemicelluloses and pectins and some structural proteins. Cellulose microfibrils orientation regulates the expansion anisotropy by promoting cell growth along the perpendicular fibers direction [5] in relation with local stress field [6,7]. Since the 199’s and the “tethered network” [8], xyloglucan (XG), the main growing cell wall hemicellulose hydrogen bounded to cellulose is considered as the main load-bearing network. The extensibility of the cell wall is promoted by proteins (expansins) and wall-bound enzymes (xyloglucan endotransglycosylase) which dissociate and reorganize the load-bearing cross-links. This chemorheological process [9,10] promotes the creep of the cell wall. Apart from hemicellulose, the cell wall matrix is composed of pectin. It is made of homogalacturonan (HG), rhamnogalacturonan I (RG I) and II (RG II) rich polysaccharides which ionic interactions and hydrogen bonds play roles in cell-cell adhesion, in the regulation of cell wall porosity and mechanical properties [11]. The major pectic polysaccharide, HG, is secreted in a highly methylsterified form that is later on selectively de-esterification by pectin-methylsterase (PME) [12]. Such modification determines HG cross-links formation via calcium ions that were recently revealed to play an equivalent load-bearing role in substitution of the hemicellulose-carbohydrate network in Arabidopsis cell wall [13] depleted in XG [14]. Such results reactivated debates on the “tethered network” model [3,14,15].

Usually, plant cell growth is investigated from a biophysical point of view in the light of models based on Lockhart’s equation [16] (equivalent to Bingham’s model) written:

\[
\dot{e} = \begin{cases} 
0 & \forall P \leq Y \\
m(P - Y) & \forall P > Y
\end{cases}
\]

with \(\dot{e}\) the length, \(\dot{e}\) the time derivative of the length (i.e. the growth rate), \(m\) a factor controlling the longitudinal irreversible wall extensibility, \(P\) the turgor pressure and \(Y\) the yield threshold. This equation describes the cell as a non-newtonian fluid, which
irreversibly flows when a pressure is applied above a critical yield value. Lockhart’s equation describes only the irreversible deformation of the cell and cannot model stress/pressure relaxation and elastic deformation [17]. Thus Ortega [18] proposed a model similar to Maxwell-Bingham equation to account for reversible deformation:

\[
\dot{\epsilon} = \begin{cases} 
\frac{P}{E} & \text{reversible} \\
\frac{m(P-Y)}{E} + \frac{P}{E} & \text{irreversible}
\end{cases} \quad \forall P \leq Y
\]

with \( E \) the longitudinal volumetric Young modulus and \( P \) the time derivative of the turgor pressure.

The Lockhart/Ortega’s model describes growth rate as a function of cell wall properties modelled initially by the empirical parameters \( m, Y, E \). Experiments demonstrated that none of the parameters of Ortega’s equation are constant (e.g. [17,19,20]). At least, the time dependency of each empirical parameter \( m, Y, E \) need to be considered. This means that some physical, chemical and biological mechanisms are not described by this equation.

Numerous models have been developed to relate the latter missing mechanisms and to balance the almighty role of turgor pressure. Relationships between load-bearing network and cell wall mechanical properties received specific attention. The Wall-gen software [21] allows the computation of the mechanical wall mechanical properties and spatial dependency in Lockhart’s parameters. Dyson et al. [25] proposed a dynamical model of hemicellulose cross-links in an expanding wall incorporating strain enhanced breakage and enzyme mediated cross-links kinetics. According to this vision, the yield threshold in Lockhart’s equation appeared as dependent of the rate of cross-links breakage over cross-links elongation. The effect of proteins such expansin and other enzymes activities was addressed by Pietruszka [24] who introduced time dependency of each empirical parameter \( m, Y, E \).

In light of these efforts, the aim of this paper is to propose a general and unified theoretical framework to model growth considering the deformation due to turgor pressure and the chemorheological process occurring in the cell wall. The idea is to consider growth as a the result of the coupling effect between different forms of energy: mechanical, chemical and thermal (Fig. 1). Mechanical energy is provided by turgor pressure and by stored energy in the load-bearing network regardless of its nature [28]. Chemical energy, refers to the synthesis of new polymers and their possible modifications by enzymes. Thermal energy particularly regulates all enzymatic mechanisms involved in cell wall biosynthesis and modifications. The coupling effect between mechanical and chemical energy reflects chemorheological processes and describes the chemically mediated load-bearing changes, the incorporation of new bricks of polymer at the inner face of the cell wall, the effect of the amorphous matrix on cellulose-hemicellulose network accessibility and enzymes or polymers diffusion [29], the cell wall mechanical relaxation. The coupling effect between chemical and thermal energy account for the enzyme activity whereas the coupling between thermal and mechanical energy for the thermal dilation.

In the next section, we present the general theoretical approach based on an axiomatic thermodynamics initially developed by Callen [30] extended in the case of non-equilibrium by Cunat [31]. This theoretical approach is then declined to model the internodal cell growth of Chara corallina. This Charophycean alga is a member of the closest relatives of land plant [32]. Its cell wall peculiarity is the synthesis and incorporation of pectic HG as mostly non-methylesterified structures [33] that readily cross link via calcium [32,34]. For this model, experimental data were extracted from the work of Proseus and Boyer [34] emphasizing the coupling effects between the mechanical, thermal and chemical energy by changing manually turgor pressure and temperature while measuring their effects on growth.

**General Theoretical Framework**

**Steady state equations.** The starting point of the present approach is the axiomatic thermodynamics initially developed by Callen [30]. In his contribution, Callen assumes the existence of a potential function called internal energy (noted \( \Psi \)), containing all the information of a system and depending \( a \) priori on all independent extensive variables of the system. Let us recall that a variable is called extensive if, in a composite system, the value of the variable for the whole system is equal to the sum of this variable on every sub-system. In the general case the extensive variables involved in the growth process are: the volume of the representative element

![Figure 1. Summary of the theoretical framework.](https://example.com/figure1.png)
describes the interactions between the different biochemical reactions relative to the synthesis, the assembly of polymers, enzymatic activities...

A very important property of the Tisza matrix is that its components are interrelated via the extensity property of the energy potential. Indeed, Callen [30] demonstrated that if we suppose the internal energy $\Psi$ extensive, then Gibbs-Duhem’s relationships exist:

$$\lambda \Psi(V,S,N) = \Psi(\lambda V, \lambda S, \lambda N)$$

by differentiation of Eq. 6 with respect to $\lambda$ and by taking $\lambda = 1$, we get:

$$\Psi(V,S,N) = PV + T S + N \ N.$$

Hence,

$$d\Psi(V,S,N) = VdP + PdV + TdS + SdT + N \ dN + N \ d\mu.$$ 

By identification with Eq. 2, Gibbs-Duhem relation is deduced:

$$VdP + SdT + N \ d\mu = 0.$$  

By injecting the relation Eq. 5 into Eq. 9 and collecting the terms in $dV$, $dS$ and $dN$ we obtain Gibbs-Duhem relationships written in matrix form

$$\begin{pmatrix}
\gamma \\
\alpha \\
\beta
\end{pmatrix}
\begin{pmatrix}
\vec{E} \\
\vec{D} \\
\vec{G}
\end{pmatrix}
\begin{pmatrix}
V \\
S \\
N
\end{pmatrix} = 0.
$$

In practice, these relationships may be useful to express unknown components of Tisza’s matrix from known ones.

The key aspect of the present framework is to extend the validity of Eq. 1 outside equilibrium. This assumption has already been formulated by Cunat [31] and gave rise to several successful studies on polymers (e.g. [32]). However, Eq. 5 revealing coupling effects of the different forms of energy does not contain information on the relaxation kinetic of the system back to its equilibrium after a mechanical or thermal solicitation. Thus, an integration of kinetic equations is necessary to supplement the previous thermodynamic equations.

Kinetic equations. Internal reorganizations of the cell wall can be modeled by chemical reactions involving the chemical species $\mathcal{N}$ and $\mathcal{z}$, the chemical fluxes (or degrees of reactions) associated with chemical reactions. As an illustration, we consider the chemical reaction presented in Table 1 linking 4 species denoted $\mathcal{S}_{i}$ with quantities $\mathcal{N}_{i} = \{N_{i}, N_{2}, N_{3}, N_{4}\}$. If the species are weighted by stoichiometric ratios $\tau = \{v_{1}, v_{2}, v_{3}, v_{4}\}$ then for the initial time $t_{0}$ and the time $t$, the following quantities $\mathcal{N}$ are obtained in function of $\tau$ (Table 1).

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and highlights the definition of the characteristic times given by:

\[ \tau = \{ \tau_1, \ldots, \tau_p \} \]

and the affinities \( \mathcal{A} = \{ A_1, \ldots, A_p \} \) driving the process (also called generalized non-equilibrium forces) given by:

\[ N_i = N_i(t_0) + \sum_{\text{reaction } j} v_j \zeta_j, \quad A_j = - \sum_{\text{species } i} v_i \mu_i \quad (11) \]

or in matrix form:

\[ \mathbf{N} = N(t_0) + \mathbf{V} \zeta, \quad \mathcal{A} = -\mathbf{V} \mu. \quad (12) \]

In Eq. 11, \( v_j \) stands for the stoichiometric coefficient of the \( i \)th reaction of the \( j \)th species, taken negative if the species is a reagent and positive otherwise.

Rewriting Eq. 5 accounting for Eq. 11 yields:

\[ \begin{pmatrix} P \\ T \end{pmatrix} = \begin{pmatrix} \gamma & \alpha & \mathbf{B} \tau \\ \alpha & \beta & \mathbf{D} \tau \\ -\mathcal{A} \end{pmatrix} \begin{pmatrix} V \\ S \end{pmatrix}. \quad (13) \]

In general, in the context of irreversible thermodynamics the relation of chemical fluxes can be written in the form [30, 36]:

\[ \zeta = \mathcal{L}(P, T, \mathcal{A}) \quad (14) \]

with \( \mathcal{L} \) a vector of functions of the intensities (pressure, temperature and affinities) that must be experimentally determined.

As we assume that \( \mathcal{L} \) is only a linear function of affinities \( \mathcal{A} \) (i.e. \( \mathcal{L} = \mathcal{L} \mathcal{A} \) with \( \mathcal{L} \) a constant matrix) then the third vectorial equation of Eq. 13 rewrites:

\[ \mathcal{A} + (\mathcal{V}^T \mathcal{A} \tau) \mathcal{L} = -(\mathcal{V}^T \mathcal{B}) V - (\mathcal{V}^T \mathcal{D}) S \quad (15) \]

and highlights the definition of the characteristic times \( \tau = (\mathcal{V}^T \mathcal{A} \tau)^{-1} \) for the system to reach back equilibrium after a mechanical or thermal solicitation, the latter being driven by the right hand side of Eq. 15. In this sense, it can be seen that the submatrix \( \mathcal{L} \) of the Tisza’s matrix partially governs the damping behavior described by the constitutive equations (Eq. 13 and Eq. 14). In the more general case where \( \mathcal{L} \) may depend on \( P \) and \( T \) (Eq. 14), non linearities of mechanical or thermal kind can be taken into account, symbolically \( \tau = \tau(P, T) \).

In the next section, a realistic case of growth is treated with the general framework presented in this paragraph. More precisely, Eq. 13 and Eq. 14 were used to model the growth of the internodal cell of \( C. corallina \).

### Results and Discussion

**Modeling Growth of Chara Corallina**

Proseus and Boyer have experimentally demonstrated, by supplying externally pectate, that a chemical mechanism, called “pectate cycle”, controls the growth rate of the internodal cell of \( C. corallina \). By changing externally the turgor pressure and the temperature while measuring growth increment [34], the authors also highlighted that the “pectate cycle” is pressure dependent and not only controlled growth but account for an efficient mechanism allowing to “store” growth for a while during low pressure period. Data used below to model the cell growth of \( C. corallina \), have been extracted from the latter work [34].

**Phenomenological aspects.**  \( C. corallina \) cell wall is composed of a high amount of mainly non-methylesterified HG pectin (pectate) and a small amount of XG and cellulose [33, 34, 38, 39]. The Ca\(^{2+}\) -pectate links constitute the main load-bearing network in the wall during growth. Incorporation of new pectate results in the cell wall mechanical relaxation. In this particular case, wall relaxation is a non-enzymatic process and is linked to a temperature and pressure dependent “pectate cycle” (Fig. 2 adapted from [34]). At a given turgor pressure, the created tensile stress in the cell wall increases the calcium mobility (Fig. 2 left) by enlarging bound distances and thus, weakening calcium and pectate bonds. The temperature affects the quantity of free pectate synthesized (Fig. 2 center in which “U” denotes an unknown reagent introduced in the next section). Thus, when temperature and turgor pressure are not limiting factors, free pectates can be incorporated in the extending cell wall and chelate calcium to create new bonds (Fig. 2 right). The creation and the breakage of Ca\(^{2+}\) -pectate bounds occurs simultaneously providing a wall loosening mechanism [10] driving the growth.

Despite the non-enzymatic bonding process, the extension and the renewal of the cell wall is similar to terrestrial plant such as pollen tubes [10, 32]. Growth of the alga is diffuse, anisotropic (Fig. 3) and not restricted to the cell tip. The direction of growth is perpendicular to the direction of the cellulose microfibrils axis in the cell wall i.e. along the longitudinal direction of the cell. The cell wall thickness and the transverse section area (noted \( A \)) are considered constant with time (which is true at least on the time range of the experiment).

**Representative volume.**  On a thermodynamic point of view, the representative volume that need to be considered in order to use the proposed framework is that of the cell wall. The latter is assumed to be under an homogeneous stress state so that the Cauchy stress tensor may be decoupled into two contributions, as it is classically done for porous media (see e.g. [40]):

### Table 1. Example of a chemical reaction linking 4 species \( S_p \), weighted by stoichiometric ratios \( v_i \).

| \( t = t_0 \) | \( v_1 S_{p1} \) | \( + \) | \( v_2 S_{p2} \) | \( \rightarrow \) | \( v_3 S_{p3} \) | \( + \) | \( v_4 S_{p4} \) | \( \text{moles} \) | \( \text{moles} \) |
|---|---|---|---|---|---|---|---|---|
| \( N_{i} \) | \( N_{i}(t_0) \) | \( + \) | \( \sum_{\text{reaction } j} v_j \zeta_j \) | \( - \) | \( \sum_{\text{species } i} v_i \mu_i \) | | | |

The degree of reaction \( \zeta(t) \) describes the entire state of the reaction if the quantity of reagent at \( t_0 \) and stoichiometric ratios are known.

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**Another Brick in the Cell Wall**

Growth of the alga is diffuse, anisotropic (Fig. 3) and not restricted to the cell tip. The direction of growth is perpendicular to the direction of the cellulose microfibrils axis in the cell wall i.e. along the longitudinal direction of the cell. The cell wall thickness and the transverse section area (noted \( A \)) are considered constant with time (which is true at least on the time range of the experiment).

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Another Brick in the Cell Wall

Figure 2. "Pectate cycle" (adapted from [34]). Turgor pressure $P$ (left) creates a tensile stress in the cell wall. The bounding distance between calcium and pectate increases with pressure inducing a higher mobility of calcium. A non-optimal temperature $T$ (centre) can reduce the number of free pectate produced by the alga ("U" denotes an unknown reagent introduced in section). When the temperature is optimal for pectate synthesis then free pectate are produced. When $P$ and $T$ allow growth (right), free pectate are bound with calcium to form new cell wall and involve its mechanical relaxation.

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Figure 3. Diffuse and anisotropic growth of the internodal cell of C. corallina. Growth occurs in the longitudinal direction at every point of the cell wall whereas the thickness ($e$) and the internal $R_1$ and external $R_2$ radii of the cell remain constant in time ($t_1 > t_2$).

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Constitutive equations. Based on the previous section, extensive variables describing the system are the volume of the cell wall $V$ proportional to the length of the cell $\ell$ (the transverse section $k$ of the cell wall $k = \pi(R_2^2 - R_1^2)$ remains constant Fig. 3), the entropy $S$, the quantity of free pectate $N_f$, bound pectate $N_b$ i.e. pectate in the cell wall and $N_u$ reagents involved in the synthesis of free pectate. $N_a$ is an arbitrary variable used to describe the complex synthesis processes of pectate. The internal energy then reads:

$$\Psi = \Psi(V, S, N_f, N_b, N_u).$$  \hspace{1cm} (16)

The differentiation of Eq. 16 leads to a similar equation to Eq. 2 and to a similar form of constitutive equation presented in Eq. 5.

In the specific case of C. corallina cell growth, Tisza’s matrix is simplified thanks to the latter experimental evidences. We assumed that the elongation rate was only function of bound pectate $\{B_0, B_b, 0\}$, that the assembling process was not function of temperature $\{D_f, 0, D_u\}$ and that the number of free pectate synthesized is not dependent on the amount of bound pectate $\{G_{ba} = 0 \text{ in } T\}$. The thermal dilation $\chi$ is neglected because of the small range of variation of the temperature. Hence, the final form of the constitutive equations for the growing C. corallina cell is:

$$\begin{pmatrix}
\frac{dP}{dV} \\
\frac{dT}{dV} \\
\frac{d\mu_f}{dV} \\
\frac{d\mu_b}{dV} \\
\frac{d\mu_u}{dV}
\end{pmatrix} =
\begin{pmatrix}
\gamma & 0 & 0 & B_0 & 0 \\
0 & \beta & D_f & 0 & D_u \\
0 & D_f & G_{bf} & G_{bb} & G_{bu} \\
0 & 0 & G_{bf} & G_{bb} & 0 \\
0 & 0 & G_{bf} & G_{bb} & G_{uu}
\end{pmatrix}
\begin{pmatrix}
\frac{dV}{dS} \\
\frac{dS}{dV} \\
\frac{dN_f}{dV} \\
\frac{dN_b}{dV} \\
\frac{dN_u}{dV}
\end{pmatrix}. \hspace{1cm} (17)
$$

The Gibbs-Duhem relationships (Eq. 10) allow expressing $\gamma$, $B_0$, $\beta$ and $D_f$ in function of the chemical coupling matrix $G$:

$$\gamma = \frac{N_b \left( G_{bb} N_b + G_{fu} N_f \right)}{V^2}. \hspace{1cm} (18)$$
\[ B_h = -\frac{G_{hb} N_h + G_{jh} N_j}{V}, \]  

(19)

\[ \beta = D_e^2 \left( \frac{(G_{hb} N_h + G_{jh} N_j)^2 + 2G_{hb} N_h N_j + G_{ff} N_f^2}{(G_{au} N_u + G_{hu} N_h)^2} \right), \]  

(20)

\[ D_j = \frac{D_e (G_{hb} N_h + G_{hu} N_h + G_{ff} N_f)}{G_{au} N_u + G_{hu} N_h}. \]  

(21)

Thus, the number of unknown parameter of Tisza’s matrix decreases from 10 to 6.

**Kinetics.** To model growth of *C. corallina* internodal cell, two chemical reactions are considered in a simple manner because the exact reactions are unknown. Such chemical model and approximation have already been used to model the mechanical expansion of pollen tubes cell wall [10].

The first one describes the synthesis of new pectate. A simple way to model the reaction is to suppose that an unknown reagent (noted \( X_u \)) produces free pectate:

\[ X_u \rightarrow \text{(Pectate)}_j \]  

(22)

The sign of the affinity of the reaction gives the direction of the reaction \( A_s = \mu_k - \mu_j \). If \( A_s > 0 \) then free pectate are produced and unknown reagents are consumed. *A priori*, \( A_s \) is positive. The second reaction describes the cell wall elongation i.e. the incorporation of new pectate within the existing cell wall by creation of \( Ca^{2+} \)-pectate load-bearing cross links.

\[ \text{(Pectate)}_j \rightleftharpoons \text{(Pectate)}_h \]  

(23)

The direction of the reaction is still determined by the sign of the reaction affinity \( A_{cw} = \mu_k - \mu_j \). *A priori*, \( A_{cw} \) is positive. Considering Eq. 22 and Eq. 23 the quantity of chemical components of the growing cell wall is defined as follows.

\[
\begin{align*}
N_u &= N_{u0} - \frac{\xi}{\xi_1}, \\
N_j &= N_{j0} - \frac{\xi}{\xi_2} + \frac{\xi}{\xi_3}, \\
N_h &= N_{h0} + \frac{\xi}{\xi_4}
\end{align*}
\]  

(24)

with \( N_{u0}, N_{j0}, N_{h0} \) the initial quantities of unknown reagents, free and bound pectate. In addition \( \xi_1 \) and \( \xi_4 \) are the degrees of the two reactions i.e. the number of mole of synthesized and bound pectate. In the context of irreversible thermodynamics such as treated by Prigogine and Kondepudi [36], the relation between chemical fluxes and intensities (Eq. 14) can be written

\[ \dot{\xi} = \mathcal{L} \mathcal{A}. \]  

(25)

This form depends only on the affinity of reactions and allows exploring the chemical part of the energy and its coupling. As concerns kinetics laws, we consider, in this first approach, a first order Taylor’s development of the general relations presented in Eq. 25, viz.

\[
\begin{align*}
\frac{\dot{\xi}}{\xi} &= \mathcal{L}_{1,\text{cw}} \mathcal{A}_{\text{cw}} \\
\frac{\dot{\xi}}{\xi} &= \mathcal{L}_{1,1} \mathcal{A}_s
\end{align*}
\]  

(26)

in which \( \mathcal{L}_{1,\text{cw}} \) and \( \mathcal{L}_{1,1} \) are constant coefficients. Eq. 17 then becomes:

\[
\begin{pmatrix}
\dot{P} \\
\dot{T}
\end{pmatrix} =
\begin{pmatrix}
\gamma & 0 & 0 & B_b \\
0 & \beta & D' & -D_f \\
-A_s & 0 & G_{11}' & G_{12}' \\
-A_{cw} & B_b & -D_f & G_{12}' & G_{22}'
\end{pmatrix}
\begin{pmatrix}
\dot{\xi} \\
\dot{\xi}_s
\end{pmatrix}
\]  

(27)

with

\[ D' = D_j - D_{u0} G_{11}' = G_{uu} + G_{ff} - 2 G_{ju} \]

\[ G_{12}' = G_{ju} + G_{ff} G_{22}' = G_{ff} + G_{bb} - 2 G_{fb}. \]

Note that the matrix of Eq. 27 is still symmetrical.

**Growth equation.** By expressing the rate of entropy in Eq. 27.

\[ \dot{S} = \frac{1}{\beta} (\dot{P} - B_b \dot{\xi}) \]  

Eq. 27 becomes.

\[
\begin{align*}
\dot{\xi} &= (1/\beta) \left( \frac{\dot{P} - B_b \dot{\xi}}{\xi} \right) \\
-A_s &= (D'/\beta) \dot{T} + (G_{11}' - D'^2/\beta) \dot{\xi}_s \\
+ (G_{12}' + D'D_f/\beta) \dot{\xi}_{cw} \\
-A_{cw} &= B_b \dot{\xi} - (D_f/\beta) \dot{T} + (G_{12}' + D_f D_f/\beta) \dot{\xi}_f \\
+ (G_{22}' - D_f^2/\beta) \dot{\xi}_{cw}
\end{align*}
\]  

(28)

By replacing expressions of \( \gamma, B_b, \beta, D_f \) by Eq. 18, Eq. 19, Eq. 20, Eq. 21 and \( V \) by \( \kappa \ell \) we obtain the growth equation system written in matrix form:

\[
\begin{pmatrix}
\dot{\xi} \\
\dot{A}_s \\
\dot{A}_{cw}
\end{pmatrix} =
\begin{pmatrix}
-\chi_1/\kappa & 0 & 0 \chi_4/\kappa \\
\chi_5 & \chi_6 & \chi_7 & 0 \\
\chi_9 & \chi_{10} & \chi_{11} & \chi_{12}
\end{pmatrix}
\begin{pmatrix}
\dot{\xi} \\
\dot{\xi}_s \\
\dot{\xi}_{cw}
\end{pmatrix}
\]  

(29)

with \( \chi_i \) function of Tisza’s matrix coefficients and extensitites (expressions of \( \chi_i \) are given in Table 2) and rates of reactions \( \dot{\xi}_{cw} \), \( \dot{\xi}_s \) expressed in kinetic equations (Eq. 26).

The growth equation system (Eq. 29) is constituted by nonlinear differential equations. In broad outlines, it describes growth increment \( \dot{\xi} \) as always dependent on the synthesis rate of \( Ca^{2+} \)-pectate cross link \( \dot{\xi}_{cw} \) and, when \( P \) is not constant also dependant
Table 2. Expressions and signs obtained after fit of $c_i$ involved in growth equation (Eq. 28).

| $c_1$          | $-\frac{\beta_0}{\gamma} > 0$ |
|----------------|--------------------------------|
| $c_2$          | $\frac{1}{\gamma} > 0$        |
| $c_3$          | $-\beta G_0 + \frac{\beta G_0 - D_I D_A D_D}{\beta} > 0$ |
| $c_4$          | $-\beta G_0 - 2(\beta G_0 - D_I D_A D_D)^2 + 2 D_I D_A D_D - D_D > 0$ |
| $c_5$          | $-\beta G_0 - 2\beta G_0 - D_I D_A D_D - \beta D_D > 0$ |
| $c_6$          | $\frac{D_I - D_A}{\beta} > 0$ |
| $c_7$          | $-\beta G_0 - 2\beta G_0 - D_I D_A D_D - \beta D_D > 0$ |
| $c_8$          | $-\beta G_0 - 2\beta G_0 - D_I D_A D_D - \beta D_D > 0$ |
| $c_9$          | $\frac{D_I - D_A}{\beta} > 0$ |
| $c_{10}$       | $\frac{D_I - D_A}{\beta} > 0$ |

$\gamma$, $\beta_0$, $\beta$, and $D_I$ known and expressed by Eq. 18 to Eq. 21.

of a deformation induced by the turgor pressure change. In other word, the two conditions needed for cell growth (a tensile stress due to pressure and the capacity to incorporate new bricks at the inner face of cell wall to lengthen it and to relax the tensile stress) are modeled naturally by such thermodynamics framework. A rational of the system is presented on Fig. 4. The core of the model is composed by the two reactions relative to the synthesis of free pectate and their assembly in the cell wall. The rate of pectate incorporation $\xi_{cw}$ drives directly the growth $\dot{t}$ ($c_1 \neq 0$), influences the synthesis of free pectate $\xi_0$ ($c_5 \neq 0$) and its future evolution $A_{cw}$ ($c_6 \neq 0$). The kinetics of chemical reactions are dependant on changes of turgor pressure and temperature ($c_7, c_1, c_12 \neq 0$). Thus, when temperature and pression are not limiting factors and do not vary ($P = 0$ and $T = 0$), growth occurs depending on the rate of pectate incorporation. This incorporation of new bricks in the wall involves its mechanical relaxation [10]. Pressure rate has a double role. It can influence growth rate directly by deforming the wall ($c_4$ in Eq. 28 and on Fig. 4) but is also a regulator of growth since it influences directly ($c_12$ in Eq. 28) and implicitly ($c_5$ in Eq. 28) the kinetics of chemical reaction of the equation system (loops in core graph Fig. 4). This complex role of pressure which is a necessary and not sufficient growth condition, has already been observed and studied on pollen tubes [10,26].

Result of the fit. The value obtained for each parameter is presented in Table 3 and the result of the fit is depicted on Fig. 5 (depicted in orange in the Fig. 5, data extracted from Proseus and Boyer [34] are depicted in black). The agreement between length increment modeled and experimental data is quite good ($R^2 > 0.998$), Tisza’s matrix obtained with the set of parameters is positive definite since all the eigenvalues are positive $\lambda = \{2 \times 10^6, 1 \times 10^4, 5 \times 10^6, 8 \times 10^6, 1 \times 10^{-3}\}$.

The amount of pectate presents in the cell wall and synthesized is globally growing (Fig. 5 $\xi_{cw}$ $\neq 0$).

The temporal evolution of the internal entropy, defined as the sum of the temporal evolution of entropy of the two reactions [36],

$$\dot{S}_i = \dot{S}_{cw} + \dot{S}_t = A_{cw} \frac{\dot{\xi}_{cw}}{T} + A_t \frac{\dot{\xi}_t}{T}.$$
Another Brick in the Cell Wall

Table 3. Parameters of the model.

| Name | Value | Unit | Dimensions | Adjusted | Sensitivity Rank |
|------|-------|------|------------|----------|-----------------|
| $\Delta_0$ | $4.81 \times 10^3$ | $J \cdot mol^{-1}$ | $[M] [L]^2 [T]^{-2} [N]$ | yes | 1 |
| $N_{00}$ | $8.35 \times 10^{-1}$ | $mol$ | $[N]$ | yes | 2 |
| $\xi_{ls}$ | $5.11 \times 10^{-9}$ | $mol^2 \cdot min^{-1} \cdot J^{-1}$ | $[M]^{-1} [L]^2 [T] [N]^2$ | yes | 3 |
| $\xi_{ls}$ | $1.14 \times 10^{-8}$ | $mol^2 \cdot min^{-1} \cdot J^{-1}$ | $[M]^{-1} [L]^2 [T] [N]^2$ | yes | 4 |
| $\Delta_{out}$ | $8.49 \times 10^2$ | $J \cdot mol^{-1}$ | $[M] [L]^2 [T]^{-2} [N]$ | yes | 5 |
| $G_{sw}$ | $7.87 \times 10^3$ | $J^{-1}$ | $[M]^{-1} [L]^2 [T]^2$ | yes | 6 |
| $D_{sw}$ | $2.65 \times 10^3$ | $K^2 \cdot J$ | $[\theta] [N]^{-1}$ | yes | 7 |
| $G_{sw}$ | $2.08 \times 10^5$ | $J^{-1}$ | $[M]^{-1} [L]^2 [T]^2$ | no | 8 |
| $G_{sb}$ | $-1.00 \times 10^6$ | $J^{-1}$ | $[M]^{-1} [L]^2 [T]^2$ | no | 9 |
| $G_{sb}$ | $1.00 \times 10^7$ | $J^{-1}$ | $[M]^{-1} [L]^2 [T]^2$ | no | 10 |
| $G_{sb}$ | $1.00 \times 10^8$ | $J^{-1}$ | $[M]^{-1} [L]^2 [T]^2$ | no | 11 |
| $N_{00}$ | $1.00 \times 10^{-20}$ | $mol$ | $[N]$ | no | 12 |
| $N_{00}$ | $1.00 \times 10^1$ | $mol$ | $[N]$ | no | 13 |

Value, unit and dimensions of the growth model parameters. $[M]$ mass dimension, $[L]$ length dimension, $[T]$ time dimension, $[\theta]$ temperature dimension, $[N]$ chemical quantity dimension, $K$ Kelvin, $J$ Joule, $mol$ mole, $min$ minute.

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pressure ($c_{12} > 0$) or temperature ($c_{11} > 0$). The more pectate are linked by chelation, the more free pectate are synthesized ($c_5 > 0$) but the synthesis of new pectate can be stopped by a reduction of temperature ($c_7 > 0$). This joint fluctuation is modified by amount of free pectate (variation of $b$ Eq. 20 and $D_f$ Eq. 21 in $c_5$ in function of $N_f$). This dependency of the rate of incorporation of free pectate with the cell wall is an important mechanism which allows the algal cell to store growth when pressure is low for a certain length of time and to resume growth when pressure reaches a non limiting value [34].

These results are in total agreement with the experimental evidences found by Proseus and Boyer [34] and sketched on Fig. 2.

Back to Lockhart/Ortega’s equation. To model growth of C. corallina cell, the form of the function describing the rate of reactions Eq. 14 was chosen as proposed by Prigogine [36] and Callen [30], in order to explore the relations between growth and cell wall remodeling and led to Eq. 25. To develop a more physical approach, focusing mostly on the mechanical part of the energy, another form of the Eq. 14 can be used. If temperature is omitted and if the cell wall synthesis and assembly are coarsely described, we can assume the rate of reactions expressed as in Lockhart’s equation.

$$\dot{c} = (c_1/k) \dot{c}_{sw} + (c_4/k) \dot{P}$$

With $K$ a positive constant, $\dot{P}$ the pressure and $Y$ a threshold pressure. The Eq. 30 models the turgor pressure as driving the growth when the pressure is over the threshold $Y$, its role of regulator disappears. This new form of chemical fluxes and the constitutive equations (Eq. 30) imply that the growth rate becomes independent of other growth equations describing the affinities (Eq. 28) since only mechanical quantities $\dot{c}$ and $\dot{P}$ remain. The growth increment is then modeled by a unique equation:

$$\xi_{sw} = K (P - Y) \quad P > Y, \quad \xi_{sw} = 0 \quad P \leq Y$$

Experimental Limits

The efficiency of this approach, despite the flexibility of the equations due to Gibbs-Duhem relationships (Eq. 10), depends on
The accessibility of measurements. In particular, some intensities or extensities such as chemical potential $\mu$ and entropy $S$ are not measurable. Therefore, predicting pressure as a function of the biochemical behaviour is impossible without assumptions on Tisza's matrix coefficients. To obtain $G$, measuring growth triggered by more complex variations of temperature and pressure would be pertinent to obtain a more robust fit. In any case, new experiments focussed on producing quantitative biochemical measurements are required to describe the initial quantity of polymers (Eq. 24) or their relative abundance and kinetics of reaction (i.e. the form of $L$ in Eq. 25).

A Framework for Growth Models

The thermodynamical framework allows considering growth as the result of coupling effect between different forms of energy without restrictive hypothesis. The application of the general growth equation (Eq. 13) to the case of the $C.\ corallina$ cell growth, in spite of its qualitative aspect, can be viewed as the first step towards a more integrative biophysical modeling of growth.

Complex regulation loops, as the one involving turgor pressure and temperature in $C.\ corallina$ cell growth, seems to be naturally described by the present approach due to the strong non linearity of the growth equations. Future modeling of plant growth experiments involving complex regulations such as the ones involved in perceptive mechanisms like mechanoperception [41] or proprioception [42] will allow testing the robustness of our approach.

**Materials and Methods**

**Parameters Estimations**

The quantitative aspect of the “pectate cycle” is unknown. Consequently, to test our approach, some assumptions were made on the chemical part of the energy to determine the initial values of parameters to fit. Thus, the results of the fit will only be qualitative concerning the “pectate cycle”. The quantity of unknown reagent used by the cell to produce free pectate was supposed unlimited ($N_{u0} \gg N_{f0}$). In the same manner, the number of pectates composing the cell wall at the beginning of the experiment was supposed much higher than the number of free pectates ($N_{b0} \gg N_{f0}$). Parameters ($G_p, G_{sb}, G_{su}, L_1, L_2, A_{wb}, A_{wb}$) were chosen in order to have the number of pectate synthesized $\xi_s$ and added to the cell wall $\xi_{cw}$ in the same order of size. Signs of $A_{wb}$ and $A_{wb}$ were set positive meaning that at the initial time free pectate was synthesized and free pectate was added to the cell wall. Consequently, the set of 13 parameters to fit is composed per components of the Tisza's matrix, of initial quantities of chemical species involved in the two reactions, and of parameters used to describe the chemical kinetics called $p^{\text{pref}}$ such as

![Figure 5. Results of the fit of the Eq. 28.](https://www.plosone.org/doi/10.1371/journal.pone.0074400.g005)
In order to reduce the number of parameters to adjust, a sensitivity analysis on the simulated length was performed in the vicinity of the parameters set \( \text{pref} \). The sensitivity \( S_{p_{i}}(\ell) \) of a given parameter \( p_{i} \) around its value \( \text{pref}_i \) was computed as:

\[
S_{p_{i}}(\ell) = \frac{\partial \ell}{\partial p_{i}} \frac{1}{\ell(\text{pref})} \left( \frac{\partial \ell}{\partial p_{i}} \right)_{\text{pref}}
\]

with \( \tilde{e}_i \) the \( i \)th vector of the canonical basis of \( \mathbb{R}^{13} \) and \( \mu \) "sufficiently small" (0.01 in practice). More easily, \( S_{p_{i}} \) quantifies the relative variation of \( \ell \) for a relative variation of \( p_{i} \) equal to \( \mu \).

The results of the sensitivity test are presented in Fig. 7. Parameters with a sensitivity in the highest decade were chosen for the fit. Thus, the set of parameters to fit was reduced to 7 \( \{A_{00}, L_{1s}, N_{00}, D_{00}, G_{uu}, G_{ff}, A_{cu0}, L_{1cw}, L_{1cu}\} \) whereas others were kept constant (Table 3).

**Figure 6. Comparison with Ortega’s model.** Instantaneous time variation of \( c_1 \) and \( 1/c_4 \). The evolution of these parameters in function of growth rate agrees with results in [17].

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**Figure 7. Time evolution of the sensitivity of the model parameters (in logscale).** In dashed lines, non-sensitive parameters. On the right, names of the variables refer to parameters defined in the text (Eq. 28). \( \tilde{e}_i \)'s refer to the test-set of the experiment defined by different pressure and temperature levels (Fig. 5). Note that the sensitivity of the parameter \( N_{00} \) and \( N_{10} \) disappears.

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Computation of Observable Variables

The vector of observable variables was numerically computed from a time discretization of the following equations:

\[
\begin{pmatrix}
\Delta \ell \\
\Delta A_t \\
\Delta A_{cw}
\end{pmatrix} = \int_0^t 
\begin{pmatrix}
\dot{\ell} \\
\dot{A}_t \\
\dot{A}_{cw}
\end{pmatrix} dt
\]

(36)

The inversion of the model was carried out in the least square sense using a Levenberg-Marquardt algorithm. Measured turgor pressure and temperature (extracted from [34] Fig. 5 in purple) were used to determine length increment (Fig. 5 in black). The initial length \(L_0\) was set to 5 cm and the thickness of the cell wall \(R_1 - R_2\) to 5 \(\mu\)m and the inner radius \(R_3\) to 2.5 mm (Fig. 3).

Goodness of Fit

The adjusted \(R^2\) coefficient has been computed as

\[
R^2 = 1 - \frac{SS_{err}}{SS_{tot}}
\]

(37)

with

\[
SS_{err} = \sum_{i=1}^{N} (\ell^m - \ell^c)^2
\]

(38)

Another Brick in the Cell Wall

Author Contributions

Conceived and designed the experiments: VM AB. Performed the experiments: VM AB. Analyzed the data: AB VM ML. Contributed reagents/materials/analysis tools: AB VM ML. Wrote the paper: AB VM ML.

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