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# Modeling the bioconversion of polysaccharides in a continuous reactor: A case study of the production of oligo-galacturonates by *Dickeya dadantii*

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Running title: *Production of UGA by Dickeya dadantii*

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**Keywords:** Continuous reactor, pectin, pectate lyases, galacturonate, unsaturated oligo-galacturonate, galacturonic acid, *Dickeya dadantii*, gene regulation, mathematical model, dimensionless parameter

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## ABSTRACT

In the quest for a sustainable economy of the earth resources and for renewable sources of energy, a promising avenue is to exploit the vast quantity of polysaccharide molecules contained in green wastes. To that end, the decomposition of pectin appears to be an interesting target because this polymeric carbohydrate is abundant in many fruit pulps and soft vegetables. To quantitatively study this degradation process, here we designed a bioreactor that is continuously fed with de-esterified pectin (PGA). Thanks to the pectate lyases produced by bacteria cultivated in the vessel, the PGA is depolymerized into oligo-galacturonates (UGA) which is continuously extracted from the tank. A mathematical model of our system predicted that the conversion efficiency of PGA into UGA increases in a range of coefficient of dilution until reaching an upper limit where the fraction of UGA that is extracted from the bioreactor is maximized. Results from experiments with a continuous reactor hosting a strain of the plant pathogenic bacterium *Dickeya dadantii* and in which the dilution coefficients were varied, quantitatively validated the predictions of our model. A further theoretical analysis of the system enabled an *a priori* comparison of the efficiency of eight other

pectate lyases-producing microorganisms with that of *D. dadantii*. Our findings suggest that *D. dadantii* is as the most efficient microorganism and therefore the best candidate for a practical implementation of our scheme for the bioproduction of UGA from PGA.

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The exploitation of biological resources, especially plant waste, is an important issue in economic and ecological terms. For instance, various studies have been initiated during the last decades in order to generate energy from renewable resources (1). This requires an improvement of knowledge on the degradation of plant complex polymers. The general idea is that these wastes contain a huge quantity of polysaccharides that could be recycled in valuable products, as their components are sugars possessing some economical value. One obstacle, however, to exploit polysaccharides is their recalcitrance to an effective degradation (2). For instance, although the lignocellulosic resources are the most abundant in biomass wastes, the difficulty to degrade them in elementary components hinders its practical use. Therefore one strategy is to exploit plants possessing a substantial amount of more easily digestible material than lignin

or cellulose, like starch or pectin (2). For example, pectin-rich materials are abundant in some biomass wastes like sugar beet pulp, citrus wastes or in apple pomace (3). In that respect, targeting the transformation of pectin-rich feedstocks offers a perfect resource to improve the efficiency to convert plant cell walls to high value-added products. Pectin has traditionally found applications in food industry, mainly as gelling and thickening agents (4). In the last years, however, there has been an increasing interest on the production and uses of pectin-derived oligosaccharides (POS) and galacturonic acid obtained by depolymerizing the native molecules in small chains of hetero- or homodimers (4–6). Galacturonic acid is used in food, chemical and pharmaceutical industries (5, 7, 8) whereas promising applications of the POS are their probiotic potential and their antitumor activities. For example POS have been shown to selectively promote the proliferation of beneficial gut bacteria like *Bifidobacteria* spp. and *Lactobacillus* spp., thus improving immunity and metabolism (9). Moreover, recent studies point out towards the capacity of POS to bind galectin-3 (Gal3), a molecule which is recognized as a key protein enhancing metastatic cancers, e.g. in lung, stomach or colons (9, 10). In this perspective POS is currently under study as a potential inhibitor of Gal3, with the great advantage, contrary to anticancer drugs, to be not cytotoxic for normal cells (10). Thus a wealth of benefits can potentially emerge from bioprocesses able to decompose pectin in oligomers and in monomers. Pectins are complex polysaccharides mainly present in the middle lamella of plant cell wall. Despite its complexity, the structure of pectin is mainly arranged around backbones formed by  $\alpha$ -1,4-linked galacturonic acid residues. Therefore one key target to an effective exploitation of pectic substances is to use efficient enzymes to degradate the polygalacturonic acids (PGA). The depolymerases, i.e. the class of enzymes acting in the depolymerization of polygalacturonic acids (PGA), are divided in 3 groups according to their mode of action and substrates they prefer : (i) the polygalacturonases [PG, E.C. 3.2.1.15], (ii) the pectin lyases [PNL, E.C. 4.2.2.10] and (iii) the pectate lyases [Pel, E.C. 4.2.2.2] . Amid the 3 groups, although these enzymes attack different parts of the pectins, the catalytic efficiency of the pectin lyase is relatively low in comparison

with other degradating enzymes (11). On the other hand, pectate lyases (Pels) are pectinases forming the most efficient enzymes of these classes of depolymerases (12). In their review Payasi *et al* report 32 bacteria able to produce pectate lyases (13). Among them 9 different microorganisms are considered in this article and are characterized with some details in table tab:cazy referring to the Carbohydrate-Active enZYmes Database (14). In this table, one of the most performant bacterium is *Dickeya dadantii* (previously called *Erwinia chrysanthemi*). The phytopathogenicity of *D. dadantii* is primarily associated with the production of a set of pectinases, mainly several pectate lyases like PelA, PelB, PelC, PelD, PelE, PelZ (15).

Regarding these enzymes PelB and PelE have the highest specific activities in comparison with other microbial sources (13). Furthermore the affinities of the *D. dadantii* Pel enzymes for their polymeric substrates are among the highest compared with other bacteria. Therefore *D. dadantii* appears *a priori* as being one of the most effective bacteria that could be considered for degradation of pectin compounds. The main objective of the present study is to test this hypothesis through a modeling approach and a quantitative analysis of a *continuous stirred-tank reactor*, called simply *continuous reactor* in the sequel. In this system, bacteria are cultivated by continuously feeding a bioreactor with a source of PGA. The latter is enzymatically decomposed into unsaturated oligogalacturonates (UGA) by the action of the pectate lyases secreted by the bacteria. A small fraction of UGA is directly consumed by the microbes for their growth, and the other part of UGA is withdrawn from the tank to be exploited. Based on a previous study (16), we develop a simple mathematical model to predict how evolve the variables of interest of this system, namely the bacterial biomass, the secreted pectate lyases (Pel) and the produced UGA. The aim of our analysis is to design the optimal dilution coefficient in order to maximize the efficiency of conversion of PGA into UGA. Our model predictions are confronted with data obtained from experiments performed in a continuous reactor inoculated with *D. dadantii* in presence of PGA. On the other hand our theoretical model enables one to express the conversion efficiency of PGA into UGA in function of a dimensionless parameter that

lumps several biochemical parameters of the system (namely, the enzyme-substrate affinity, the  $V_{max}$  of the enzyme and its degradation rate, the growth rate and the yield of bacteria, and their Monod parameter). Then, combining our analysis with data of the review (13), we are able to compare, at least on basis of the available parameters, the performance of nine micro-organisms in what concern their conversion efficiency of PGA into UGA. The results of our study strengthen the idea that *D. dadantii* should be the best candidate to perform this task.

In the next Section we report the main analysis done on the mathematical model and compare the predictions with direct measurements taken from growth of *D. dadantii* in a continuous reactor fed with PGA. In the same Section we explain how the optimal productivity and conversion efficiency of PGA into UGA can be quantified from the model by considering a key dimensionless parameter characterizing the system. Then in the Section Discussion, we apply these results to compare the potential efficiencies of PGA to UGA conversion of nine different microorganisms able to produce pectate lyases. Finally in the last Section we present the experimental procedures and the modeling method for the considered continuous reactor.

## RESULTS

In this section predictions provided by our mathematical model are presented and experimental results are reported to validate the theoretical analysis. A cartoon representation of that system based on the regulation of *pel* genes is depicted on Fig. 1. The corresponding system of equations (6) is detailed in the Material and Methods Section. The main conclusion will be that the bacterium *D. dadantii* could be quite efficient in the perspective of production of oligomers UGA from pectin.

*Analysis of continuous reactor variables and comparison with experiments.* The first interesting step to consider for a continuous reactor is the behavior of its stationary regime. Theoretically, the steady states of the reactor are obtained by solving the system of equations (6) where the time derivatives are replaced by 0. The sought solutions are functions of the dilution coefficient  $D$  defined as the inflow debit feeding the reactor tank, divided by its vol-

ume : thus  $D = F_{in}/V$  has a physical dimension of the inverse of time and  $1/D$  can be thought as the residence time of the chemical species in the continuous reactor. On Fig. 2, the stable steady states of system variables have been plotted in function of  $D$  (solid black lines). The correspondence between the variable names used in eqs. (6) and the biochemical names on Fig. 2 is detailed below. In particular the panel (B) of Fig. 2 shows the output variable of interest, namely the mass concentration of UGA. In the following we will call the mass concentration simply the mass.

The mathematical analysis of system (6) establishes that the non-zero solution branch of UGA is increasing in function of  $D$ . Moreover, it predicts that a bifurcation point is reached for some limit value  $D = D_c$ , beyond which the system jumps to the trivial steady state with no longer bacteria in the medium. Indeed, when  $D$  is increased, the influx of polymeric substrate augments also in the culture vessel. Then the degradation of PGA into UGA increases also thanks to the Pels produced by the bacteria. In the same time, however, the net growth rate of the bacterial population decreases, due to the lost of the bacteria through the outlet of the culture vessel. This net growth rate becomes negative when  $D > D_c$  and so appears the washout phenomenon of the continuous reactor. For the considered set of parameters, the predicted value of  $D_c$  is  $0.54 \text{ h}^{-1}$ . Experimentally, the washout was already observed for  $D = 0.53 \text{ h}^{-1}$ . Therefore one can conclude that in practise  $0.4 \text{ h}^{-1} < D_c \leq 0.53 \text{ h}^{-1}$ .

Next, the steady states predicted by the model are experimentally validated. As represented on Figure 2, experimental data points are obtained by considering the continuous reactor working with 5 values of the dilution coefficient, namely (0.1, 0.2, 0.3, 0.4, 0.53). For each of these  $D$  values, and at each time point during 14 hours, a sample is taken from the medium and 3 independent physical variables are recorded, with the following relations between measurements and variables of eqs. (6) : firstly, the absorbance at 600 nm, denoted as  $A_{600}$  is related to the bacterial density by :  $A_{600} = 10^3 \rho$ . The second measurement is the absorbance at 230 nm,  $A_{230}$ , which is related to mass of UGA by the phenomenological relation (UGA mass =  $a A_{230} + b$ ) as described in the Methods Section. Finally, the en-

zymatic activity of the pectate lyases (Pel), which is expressed as units of  $mmol\ min^{-1}$ , can be related to the concentration  $p$  of Pel by the relation :  $Act\ Pel = k_{cat}p$ . These 3 independent measurements recorded after 14h enable one to match with the predicted steady states curves drawn on the left panels (A,B,C) of Fig. 2. On the other hand, the same measurements enable to plot experimental points on the 3 right-panels (D,E,F) of Fig. 2. The latter are constructed indirectly from the previous measurements, through 3 *observers* of respectively the mass of PGA, the molar concentration of repressor KdgR (variable  $r$  in equations) and of the inductor KDG (variable  $i$ ).

These experimental data are concerned with the (quasi) stationary data collected at the final time  $t = 14$  h. Another test to validate the model is to compare model and experiments during the transient dynamics. This is performed on Fig. 3, showing the transient dynamics of the 3 directly measured variables, for two fixed value of the dilution coefficient, namely  $D = 0.4\ h^{-1}$  (continuous lines) and  $D = 0.4\ h^{-1}$  (dashed lines).

As a whole, the obtained experimental points obtained at steady states and during the transients are well placed with respect to the predicted curves (with some spreading for the enzymatic activities). Regarding the steady states in function of  $D$ , the match is still better when one compares on Fig. 2 the experimental measurements with the numerical simulations of the *pre-equilibrium* curves, that is the curves obtained at  $t = 14$  h (dashed curve). One observes, however, that for  $D = 0.53\ h^{-1}$ , which is very close to the critical  $D_c$ , the washout-state is already observed in the experimental data at  $t = 14h$ , whereas the model predicts for the same time a state which has yet a larger bacterial density. This means that for  $D \sim D_c$  our model is not capturing the right time-scale but well the stationary behavior.

*The use of dimensionless parameters in designing the optimal functioning point.* The efficiency of the bioreactor functioning can be evaluated by two quantities; first its *productivity*, which can be defined as the flux of mass of UGA leaving the reactor (expressed in g/l/h). At the stationary state of the system, and regarding the simple mathematical model (6), the flux of mass going out of the reactor can be

written as:

$$\phi = Du^* \mathcal{M}_{UGA} \quad (1)$$

where  $u^*$  is the steady state molar concentration of UGA and  $\mathcal{M}_{UGA}$  is its molar mass. Secondly, the efficiency of the depolymerization can be quantified by the *conversion efficiency* of the polymeric substrate into oligomers. This dimensionless parameter is defined as:

$$\eta = \frac{\text{Outgoing mass of UGA}}{\text{Incoming mass of PGA}} \quad (2)$$

Thus  $\eta$  gives the fraction of incoming PGA that is decomposed into UGA and extracted at the reactor outlet. By definition  $\eta$  is strictly bounded by 1. Indeed, there is always a fraction of incoming PGA which is used for the growth of the bacteria. It can be shown that  $\eta = u^*/(ns_{in})$ , where  $s_{in}$  is the concentration of the polymer substrate feeding the continuous reactor. Moreover, one can prove that by varying only the dilution coefficient for a given set of system parameters, the *optimal* conversion efficiency,  $\eta_{opt}$ , and the maximal flux,  $\phi_{opt}$ , are attained when the dilution coefficient reaches its limit value  $D = D_c$  (17). Finally, the optimal productivity and optimal conversion efficiency are related by the equation :  $\phi_{opt} = \eta_{opt} D_c \sigma_{in}$ .

There is no closed mathematical expression which formulates  $\eta_{opt}$  or  $\phi_{opt}$  in function of the system parameters. (These are defined on Table 2). *A priori*  $\eta_{opt}$  depends on all the system parameters and it would be a heavy task to numerically compute the ranges of values which maximize the conversion efficiency by systematically scanning all the parameter space. In the case of our minimal model (6) the mathematical analysis of its steady states shows that the best value of  $\eta_{opt}$  can be reached by decreasing as much as possible the following dimensionless parameter:

$$\epsilon = \frac{\gamma\alpha_1 K_m \bar{\mu}}{V_{max} \mathcal{M}_{Pel} \beta_1 (K_2 + ns_{in})} \quad (3)$$

where  $V_{max} \mathcal{M}_{Pel} = k_{cat}$  (See Supporting Information). In other words the analysis shows that  $\eta_{opt}(\epsilon) \rightarrow 1$  if  $\epsilon \rightarrow 0$ . To see it intuitively, let us note that when  $K_2$  is larger than  $ns_{in}$  – a situation which holds for the considered parameters – then  $\epsilon$



defined in eq.(3) can be reformulated as:

$$\frac{(\gamma_{\bar{u}}/K_2)}{(k_{cat}\beta_1/\alpha_1K_m)} = \frac{\text{rate of UGA bacteria consumption}}{\text{rate of UGA enzymatic production}} \quad (4)$$

Therefore the best conversion efficiency of the polymeric substrate will be promoted when  $\varepsilon$  is as small as possible since in this case the production of UGA dominates its consumption by the bacteria. The lumped parameter  $\varepsilon$  allows to compare the optimal conversion efficiency in various systems by ranking their  $\varepsilon$ . An example of application of this method is reported in the Discussion Section below.

A plot of the graph of  $\eta_{opt}$  in function of  $\log(\varepsilon)$  is illustrated on Figs.4. As a matter of fact our model predicts that  $\eta_{opt}$  possesses a simple linear-logarithmic behavior. This means that in natural scale, the fall of the curve  $\eta_{opt}(\varepsilon)$  is quite sharp. In the case of *D. dadantii*, using the parameters of Table 2, one can compute that  $\varepsilon = 1.7 \cdot 10^{-3}$ , leading to an optimal conversion efficiency of  $\eta_{opt} = 0.75$ . Let us note that this conversion efficiency depends on the mass concentration of the incoming PGA from the feeding vessel, which here is fixed to 1 g/l. For instance, if we double  $s_{in}$ , the optimal conversion efficiency diminishes to  $\eta_{opt} = 0.69$ . Nevertheless, this will be shown to be quite efficient regarding comparative estimates of  $\eta_{opt}$  for other micro-organisms (see Discussion Section).

## DISCUSSION

The first objective of our study was to design a continuous reactor setting to transform polygalacturonate (PGA) into unsaturated oligogalacturonates (UGA). This is performed thanks to the enzymatic activity of pectate lyases (Pel) produced by the bacterium *Dickeya dadantii* cultivated in a bioreactor. The goal is to collect at the outlet of the vessel as much as possible UGA which possess a greater economical value than the PGA<sup>1</sup>. The main result is that this process can be achieved by increasing the dilution coefficient of the reactor as close as possible of a limit value  $D_c$ , but not above, to avoid the washout of the bioreactor.

Let us note that this bioprocess is quite different in a continuous reactor than what would be ob-

served in batch or in fed-batch reactors. To discuss this point quantitatively, Fig. 6 compares the numerical simulations of the mass production of UGA in 3 different reactor schemes, assuming that the same amount of PGA is consumed in each case. Taking our experiments done with a continuous reactor as the reference, with a dilution coefficient  $D$  and a vessel reactor of volume  $V$ , the total volume of consumed PGA is  $V_f = DVt_f$ , where  $t_f$  is final time of functioning (i.e. 14 h). In batch condition with such a volume, the simulation shows that the PGA is fastly decomposed into UGA but the latter is next completely metabolized by the bacteria (cf. dotted line on Fig. 6, vanishing to 0 after 4 to 5 hours). Interestingly, this numerical computation reveals the existence of a peak of the production of UGA after one hour, suggesting that an alternative process to produce UGA would be to stop the batch at an appropriate time predicted by our model, in fact before the growth of the bacterial density. On the other hand, a continuously fed reactor creates a permanent dilution effect which enables to downregulate the bacterial density to its minimal value, while the produced UGA is extracted at the outlet of the reactor tank. In the stationary regime of the continuous reactor, the quantity of produced UGA increases linearly in time (cf. continuous curve on Fig. 6). Now, in principle, controlling a low bacterial density can also be reached by using a *fed-batch* reactor, where a vessel of volume  $V_f$  is gradually filled in by an inflow of debit  $F_{in}$ . If the fed-batch is performed with a constant volume debit  $F_{in}$ , the volume  $V(t)$  of the vessel increases so that the dilution rate  $D = \frac{F_{in}}{V}$  decreases (see caption of Fig. 6 for numerical details). The resulting production of UGA is illustrated by the dashed line on Fig. 6. One observes that the initial growth of the production of UGA follows the same behavior as in the continuous reactor, but next the curve stagnates and falls back to smaller values.

Thus, building on a previous mathematical model describing the synthesis of Pel by *D. dadantii* in batch conditions, our minimal system of equations was adapted to the continuous reactor conditions. Keeping the same parameters as previously, the predictions of the new model were observed in a se-

<sup>1</sup>For instance, the commercial value of purified unsaturated di-galacturonate sold by our provider (Sigma Chemical Co., St. Louis, MO, USA) is currently 2000 higher than the one of PGA.

ries of experiments, therefore validating the present model. The comparison with experimental data was concerned with the stationary states of the system with various values of the dilution coefficients  $D$ , as well as with the dynamical evolution of the system variables for a fixed value of  $D$ .

In particular our study focusses on predicting  $\phi_{opt}$  and  $\eta_{opt}$ , i.e. respectively the optimal productivity and conversion efficiency of polysaccharides PGA into oligomers UGA. In the case of Pels produced by *D. dadantii*, and considering our parameter set, the theoretical value predicted is  $\eta_{opt} = 75\%$ . Experimentally, we could reach  $\eta_{exp} = 60\%$ . However the purpose was not to optimize this experimental value, as it would require some control setting as discussed below. The point is that we can retrieve the increasing behaviour of  $\eta_{exp}$  in function of  $D$ , and confirm that there is a limit value over which the washout phenomenon occurs.

Although the present model is designed with respect to the bacteria *D. dadantii*, it offers some generality for describing the decomposition of polymers by other micro-organisms. How the optimal conversion efficiency of PGA into UGA by *D. dadantii* can be matched with other micro-organisms able to synthesize pectate lyases? To address this question, one can rely on the study by Payasi et al (13) reviewing some enzymological properties of microbial pectate lyases. On basis of three main enzymatic parameters ( $V_{max}$ ,  $K_m$  and molar mass), the other parameters being identified to the ones of *D. dadantii*, the last two columns of Table 3 foresee a tentative comparison of the optimal PGA/UGA conversion efficiencies and productivities between *D. dadantii* and other micro-organisms reported in (13). The corresponding points of  $\eta_{opt}$  are plotted as squares on figures 4. Certainly, an accurate comparison of the bacterial performances should take into account all their biochemical parameters. Nevertheless our method gives a rational ranking of the pectinolytic efficiency of each microorganism reported on Table 3, because the considered parameters in this Table are among the most essential in the process of enzymatic degradation. On figure 4 the position corresponding to *D. dadantii*, maximizes  $\eta_{opt}$ . A relatively closed score is reached by the pectate lyase of *Amycolata*. But the latter microorganism

possesses only one type of pectate lyase (cf. Table 1), whereas the reference value considered for *D. dadantii* is actually grounded on the mean biochemical parameters of its major 5 pectate lyases, PelA, PelB, PelC, PelD and PelE, having various performances. Furthermore, *D. dadantii* possesses an arsenal of other genes that can deal with methylated or de-methylated pectin. Therefore, *Amycolata* is most likely less efficient than *D. dadantii* which appears to be the best candidate in view of configuring an actual bioreactor device to transform pectin into UGA.

Let us remark that in this work the considered substrate is not the standard pectin, but its simplest form corresponding to the polygalacturonate (PGA). Indeed, the goal of the present study was to achieve as far as possible quantitative results by combining computational modeling and biological experimentations. This would be more complicated with complete pectin because of its various sugars which involve more metabolic pathways. However, we think that our work has a practical biotechnological utility for the following reason. According to their degree of methoxylation, pectins can be classified as high methoxyl pectins, HMPs (50% esterified or higher) or low methoxyl pectins, LMPs (less than 50% esterified). Some pectins have very low degree of methylation. For example Citrus peel pectin from Sigma (Ref. P9135) has a methoxyl content of approx. 6.7%, and therefore is not so different from PGA used in our experiments. Furthermore, *Dickeya dadantii* produced two pectin methyl esterases (PemA and PemB) and two pectin acetyl esterases (PaeX and PaeY) responsible for pectin de-esterification. Therefore this bacterium can perform “real life” pectin biomass conversion.

On the other hand, the analysis of our model shows that in view of working in its optimal regime of UGA production, an issue of the current scheme is that the optimal point of functioning is unstable. Indeed, it corresponds to a bifurcation point of the dynamical system. For  $D < D_c$ , the steady state of interest coexists with the washout state, whereas just after the point  $D = D_c$ , the stable equilibrium disappears and only the washout state is stable. Thus, since the system parameters are not exactly known, and because the parameters can also fluctuate, the optimal state obtained for  $D = D_c$  is not robust with

respect to parameter uncertainty or external perturbations. A remedy to solve this issue is to consider a control feedback loop on the dilution coefficient  $D$  and make it a function of the measurement of the bacterial biomass  $\rho(t)$ . Actually, in another work, treating a simpler model than eqs.(6) we have brought the proof of principle of this control method by considering the following control law (17):

$$D(t) = \delta\rho(t)$$

where  $\delta > 0$  is a fixed parameter whose value can be chosen to stabilize any stationary point  $(s^*, u^*, \rho^*)$  of functioning compatible with the mass conservation law (cf. eq.(13)):

$$\sigma_{in} = (n s^* + u^* + \gamma\rho^*)M_{UGA} \quad (5)$$

In the current model, preliminary results based on numerical simulations show that this simple control method should also be able to stabilize the optimal point of functioning. Therefore, a straightforward extension of the present work will be to experimentally implement such a control method.

## CONCLUSION

Although the present model is designed with respect to the bacterium *D. dadantii*, it offers some generality for describing the decomposition of polymers by other micro-organisms. The main assumption is that these other organisms express one or several genes coding for pectate lyases, and that they can use a part of the decomposed substrate for their growth. As our model prediction for  $\eta_{opt}$  is based on one lumped parameter which combines the main biochemical parameters, this study can be used to compare various organisms, once their parameters are known. Doing so, we claim that the optimal conversion efficiency of *D. dadantii* is quite efficient compared to other bacteria. Therefore, in view of an actual implementation to transform pectin in UGA, the current approach provides experimental data and theoretical analysis to demonstrate the feasibility of the concept and shows that *D. dadantii* is likely to be the best natural microorganism to fulfill this purpose.

## MATERIAL AND METHODS

*Experimental Procedures.* Bacterial Strain and Culture Conditions - *Dickeya dadantii* 3937 (18)

was used for all the experiments described. Cultures were grown at 30°C in M63 minimal salt medium (19) supplemented with a carbon source: glucose at 0.2% (w/v) and various concentrations of polygalacturonate (PGA), from 0.05 to 0.2% (w/v). Liquid cultures were grown in a shaking incubator (220 r.p.m.). Polygalacturonic acid (PGA) from Sigma Chemical Co., (St. Louis, MO, USA) was obtained by de-esterification of citrus peel pectin using fungal pectin methyl esterases. Pre-culture from single colonies were grown overnight in M63 medium supplemented with glucose. Cells were re-inoculated into 500 ml of M63 medium plus glucose, CaCl<sub>2</sub> 0.1 mM and PGA 0.2% (W/V) and the resulting medium was grown at 30°C during 16 h to reach the beginning of the stationary phase. At this stage all the PGA added in the growth medium was totally degraded allowing the induction of Pel production (16). The resulting medium was used for continuous cultures as follow: briefly, the bioreactor (BiostatR B Plus, Sartorius Stedim) tank containing 900 ml of M63 medium plus CaCl<sub>2</sub> 0.1 mM and PGA 0.01 % (W/V) was supplemented by 100 ml to 250 of the batch culture in order to reach a final  $A_{600}$  of 0.35-0.5 and Pel activity of 5 U and then start the feeding pump (feeding solution: M63 medium plus CaCl<sub>2</sub> 0.1 mM and PGA 0.1% (W/V); 1 U corresponds 1  $\mu$ moles of unsaturated product liberated per min and per ml of enzymatic extract). Three aliquots were taken every hours: 1 ml for monitoring the cell growth, 1ml toluenized immediately for Pel activity quantification and a 10 ml sample for quantification of the digalu concentration. In the last case, the cells were harvested by a centrifugation of 5 min at 6000 g, and then 6 ml of the supernatant was taken, adjust to 1 mM EDTA final concentration and submitted to an incubation of 20 min at 95°C to inactivate the Pels contained in the medium. Aliquots of the media taken before inoculation with the bacteria and treated as described above were used as a reference. Enzyme Assays - Assay of pectate lyase was performed on toluenized cell extracts. Pectate lyase activity was determined by monitoring spectrophotometrically the degradation of PGA to unsaturated products that absorb at 230 nm (20). The molar extinction coefficient of unsaturated oligogalacturonates used was 5,200 (20). Pectate lyase activity is expressed as  $\mu$ moles of unsaturated products liber-



ated per min. The standard assay mixture consisted of 100 mM Tris-HCl (pH=8.5), 0.1 mM CaCl<sub>2</sub>, and 0.5 g of PGA per liter in a total volume of 1 ml. Bacterial concentration was estimated by measuring turbidity at 600 nm, given that an absorbance at 600 nm ( $A_{600}$ ) of 1.0 corresponds to 10<sup>9</sup> bacteria per ml with a volume of 0.5  $\mu\text{m}^3$  per cell. Digalu quantification was performed spectrophotometrically at 230 nm.

### Model Formulation.

The theoretical framework used in this study is a mathematical model of the growth of *Dickeya dadantii* in a bioreactor (or chemostat) fed in by polygalacturonates (PGA). This model is founded on a previous work modeling the same bacteria but in batch conditions (16). Here we extend this first study in two ways. First, the bacterial growth is described by Monod-like kinetics depending explicitly on the substrate, which is a new variable. Previously the bacterial growth was modeled by a logistic function decoupled from the intake of substrates (16). A second generalization of the initial model is to consider the setting of a continuous bioreactor, which is fed by a source of polymeric substrate with concentration  $s_{in}$  and with a coefficient of dilution  $D$ . This means that if the culture vessel has a volume  $V$ , it is submitted to a constant incoming and outgoing flow  $DV$ . Then the time-evolution of the system is described by the following set of equations:

$$\frac{d\rho}{dt} = (\mu(u) - D)\rho \quad (6)$$

$$\frac{du}{dt} = nk_{cat}p\frac{s}{K_m + s} - \rho\gamma\mu(u) - Du \quad (7)$$

$$\frac{ds}{dt} = -k_{cat}p\frac{s}{K_m + s} + D(s_{in} - s) \quad (8)$$

$$\frac{dp}{dt} = \beta_1 \left( \frac{\rho}{1 - \rho} \right) \frac{K_6}{K_6 + Y_d(r, i)} - (\alpha_1 + D)p \quad (9)$$

$$\frac{dr}{dt} = \beta_2 - (\alpha_2 + \mu(u))r \quad (10)$$

$$\frac{di}{dt} = (1 - \rho)\gamma\mu(u) - (k_5 + \mu(u))i \quad (11)$$

where  $\mu(u) = \bar{\mu}\frac{u}{K_2+u}$  is the growth rate of the bacte-

ria and

$$Y_d = \frac{r/2}{1 + (i/K_3)^2} \quad (12)$$

is the concentration of the main repressor of *pel* gene (see below). The state variables of this system are summarized in Table 4 and a detailed justification of each terms appearing in the set of equations (6)-(12) is provided in Supporting Information. The considered variables can be divided in two categories, either internal to the bacteria, or belonging to the extracellular medium. Firstly, the extracellular variables are the following:  $\rho$  is the fractional bacterial volume, that is the volume occupied by bacteria divided by the total volume of the vessel. Let us note that here we identify the bacterial biomass with its volume, assuming implicitly that its mass density is constant; next,  $p$  is the molar concentration of enzyme pectate lyases in the extracellular vessel; the last extracellular variables,  $u$  and  $s$ , are respectively the molar concentrations of UGA and PGA. Here the cleavage of PGA is described as a single step,  $\text{PGA} \rightarrow n \text{UGA}$ , decomposing a PGA polymer containing  $n$  di-galacturonates into  $n$  dimers. Although we considered also a more detailed model<sup>2</sup>, the experimental data could be fitted equally well with the simplest one, even by considering  $n = 2$ . Therefore we decided to stick here with the simpler formalism in the numerical simulations. Secondly, in what concerns the intracellular variables, they are on one hand  $r$ , the molar concentration of KdgR, which is the main repressor of *pel* gene, and on the other hand  $i$ , the molar concentration of 2-keto-3-deoxygluconate (KDG). This latter molecule is a catabolite product of UGA and plays the role of the *inducer* of *pel* gene, because it can derepress *pel* expression by binding to the repressor KdgR<sub>2</sub> (21).

The model parameters are listed in Table 2, keeping the same notations as the ones used in (16). The parameters are divided in three categories : in the first part, the parameters are taken from the literature and thus their values are strictly the same as the ones considered in (16). The parameters appearing in the second part of Table 2 were all identified in batch experiments, thus independently of the present work which is concerned with a continuous reactor

<sup>2</sup>A detailed model was considered where the fragmentation of PGA is described by taking into account the step-by-step decomposition of the initial polymer into oligomers of all intermediate lengths. A method to derive the kinetic equations for such extended system can be found in (17).

setting. Some of the parameters in this second part describe the Monod-like growth rate for the biomass which was not considered in (16). The third part Table 2 designates the control parameter of the experiments: the concentration of the feed bottle of the reactor, expressed either in mass concentration ( $\sigma_{in}$ ), or in molar concentration ( $s_{in}$ ). This external control parameter is kept to the fixed value  $\sigma_{in} = 1$  g/l. The only varying control parameter is the dilution coefficient  $D$  of the system. All computations concerning the system of ordinary differential equations (6)-(12) were performed in Matlab R2017b, using different ODE integrators to test the consistency of the numerical results. The stiff integrator `ode15s` was appropriate most of the time, although non-stiff procedures would work also but with longer integration times.

A frequent property of models of bacterial growth is the existence of a global variable describing the mass balance of the substrate and its consumption. Here such variable can be found to be the following:

$$w = (n s + u + \gamma \rho) \mathcal{M}_{UGA} \quad (13)$$

where  $\mathcal{M}_{UGA}$  is the molar mass of UGA dimer. The variable  $w$  can be interpreted as a record track of what becomes the mass of PGA entering the continuous reactor, counted in terms of an *equivalent* mass of UGA: the first term,  $n s \mathcal{M}_{UGA}$ , accounts for the free PGA (with a molar mass  $n$  times the one of UGA); the second term of (13),  $u \mathcal{M}_{UGA}$ , corresponds to the actual UGA produced in the bioreactor, and the last term  $\gamma \rho \mathcal{M}_{UGA}$  is the UGA metabolized by the bacteria, with a yield factor  $\gamma$ . Therefore at equilibrium, when the reactor reaches its stationary state, the mass-equivalent UGA must be equal to the injected mass of PGA, which we introduced above

as  $\sigma_{in} = 1$  g/l. This property is useful to check the consistency of the model, as reported below in the Result Section. It is also useful to make a link between the mass of UGA and the absorbance at 230 nm,  $A_{230}$ , which is measured for this purpose (see above). On the other hand, measures of  $A_{230}$  on a sample of the culture might be perturbed by the residual absorption of other molecules in the bulk. In order to simply model the relation between  $A_{230}$  and the produced mass of UGA, we first construct an *observer* of UGA, that is a dynamical system which enables to indirectly quantify UGA from the measurement of another variable (22). Indeed, by computing the derivative of  $w$  defined in eq.(13), it is readily seen that  $dw/dt = D(\sigma_{in} - w)$ . Thus the time-evolution of  $w(t)$  is given the exponential function:

$$w(t) = (w(0) - \sigma_{in}) \exp(-Dt) + \sigma_{in}$$

where  $w(0) = n s(0) + \gamma \rho(0)$  can be evaluated from the initial state of the reactor, assuming  $u(0) = 0$ . Then, from eq.(13) an estimate of the mass of UGA can be deduced from the relation  $u_{obs}(t) = w(t) - n s(t) - \gamma \rho(t)$ , where  $\rho(t)$  is *measured* via the absorbance at 600 nm and  $s(t)$  is neglected except at  $t = 0$ . Indeed the enzymatic decomposition of PGA appears to be much faster than the time-scale over which UGA is measured (i.e. hours). The points plotted on figure 5 show the *observed* UGA at all considered times of measurement (see below), versus direct measures of  $A_{230}$  at the corresponding time points. Although the resulting relationship exhibits some stochasticity, it can be approximated by an affine function (e.g., UGA mass =  $a A_{230} + b$ ) which reveals to be satisfactory to quantify the mass concentration of UGA from direct measurements of  $A_{230}$ .

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## TABLES

**Table 1:** Pectate lyases produced by 9 different microorganisms considered in this article are characterized by their origin, their Genbank accession number, and their classification in the families of polysaccharide lyases, as defined on the Carbohydrate-Active enZYmes Database (CAZy: <http://www.cazy.org/Welcome-to-the-Carbohydrate-Active.html>) (14). The more recent names of organisms have been noted in the first column, but leaving the names used in the review (13) for comparison.

Micro-organism	Characteristic and origin	Pectate Lyase	Genbank accession number	CaZY polysaccharide lyase family
<i>Dickeya dadantii</i> 3937	Plant pathogenic bacterium isolated from <i>Saint Paulia ionantha</i> in France	Pel A	ADM99550.1	PL1
		Pel B	ADN00345.1	PL1
		Pel C	ADN00346.1	PL1
		Pel D	ADM99552.1	PL1
		Pel E	ADM99551.1	PL1
<i>Amycolata</i> = <i>Pseudonocardia</i>	Nonsporulating pectinolytic actinomycetes isolated from a soil sample from Thailand	Pel	AAC38059.1	PL6
<i>Bacillus subtilis</i>	Gram-positive, spore-forming bacterium, commonly found in the rhizosphere and often in association with plant roots	Pel B	CAA52866.1	PL1
<i>Fusarium solani</i> = <i>Nectria haematococca</i> mpVI	Plant pathogenic fungi isolated from <i>Pisum sativum</i> (pea).	Pel A	AAA33339.1	PL3
<i>Pseudomonas fluorescens</i>	Bacterium, associated with spoilage of fresh fruits and vegetables	Pel	AAB46399.1	PL6
<i>Azospirillum irakense</i> = <i>Niveispirillum irakense</i>	N <sub>2</sub> -fixing plant-associated bacterium	Pel A	AAD25394.1	PL10
<i>Bacillus licheniformis</i>	Gram-positive, spore-forming bacterium, commonly found in the rhizosphere and often in association with plant roots	Pel A	CAD56882.1	PL6
<i>Bacillus sp</i> KSM-P15	Gram-positive, spore-forming bacterium, commonly found in the rhizosphere and often in association with plant roots	Pel E	BAA81752.1	PL10
		Pel H	BAA81753.1	PL9
<i>Fusarium moniliforme</i> = <i>Fusarium verticillioides</i>	Pectinolytic fungi isolated from a tropical mangrove community	Pnl	ANC98526.1	PL1



**Table 2:** List of parameters.

Parameter from literature	Description	value	unit
$\alpha_1$	degradation of enzymes Pel	$49 \cdot 10^{-3}$	$\text{h}^{-1}$
$\alpha_2$	degradation of repressor	$35 \cdot 10^{-3}$	$\text{h}^{-1}$
$\beta_2$	max. rate of repressor synthesis	$70 \cdot 10^{-9}$	$M\text{h}^{-1}$
$k_{cat}$	turnover of enzyme	$4.1 \cdot 10^6$	$\text{h}^{-1}$
$K_3$	affinity KDG-KdgR <sub>2</sub>	$0.4 \cdot 10^{-3}$	$M$
$K_M$	Michaelis Menten const.	$6.8 \cdot 10^{-3}$	$M$
Parameters identified	Description	value	unit
$\beta_1$	max. rate of Pel synthesis	$46 \cdot 10^{-6}$	$M \text{h}^{-1}$
$K_6$	dissociation const. of repressor-prom.	$10^{-7}$	$M$
$\gamma$	yield factor of substrate intake	4	$M$
$k_5$	max. rate of KDG phosphorylation	175	$\text{h}^{-1}$
$\bar{\mu}$	(half) max. growth of bacteria	34	$\text{h}^{-1}$
$K_2$	bact. affinity for digalu	0.14	$M$
Control parameters	Description	value	unit
$\sigma_{in}$	massic external PGA concentration	1	$\text{g/l}$
$\mathcal{M}_{UGA}$	molar mass of dimer UGA	352	$\text{g/mol}$
$s_{in} = \sigma_{in}/(n\mathcal{M}_{UGA})$	external PGA concentration	$1.4 \cdot 10^{-3}$	$M$
$D$	Dilution coefficient	0.1; 0.2; 0.3; 0.4; 0.53	$\text{h}^{-1}$

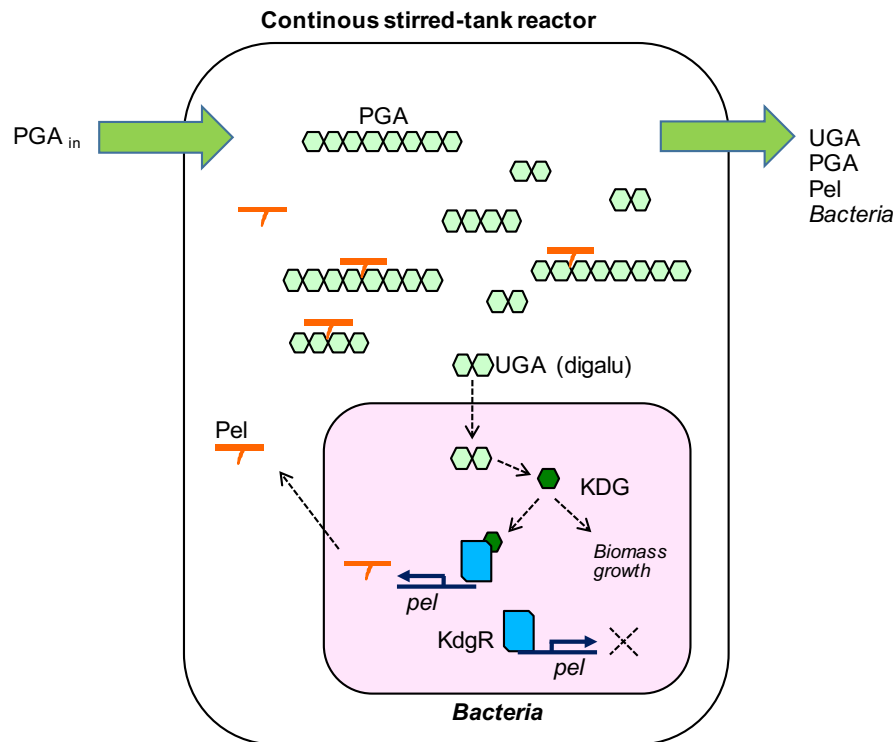
**Table 3:** Properties of pectate lyases reported in (13), supplemented by a comparison of  $\eta_{opt}$  and  $\phi_{opt}$  relative to *D. dadantii*.

Micro-organism (ref)	Mol. mass [kDa]	$K_m$ [g.l <sup>-1</sup> ]	$V_{max}$ [U/mg protein]	$\eta_{opt}$ %	$\phi_{opt}$ [g.l <sup>-1</sup> .h <sup>-1</sup> ]
<i>Dickeya dadantii</i> (Average) (15)	41.2	0.26	2018	76	0.41
<i>Amycolata</i> s.p. (23)	31	0.02	158	74	0.38
<i>Bacillus subtilis</i> (24)	42	0.86	1475	60	0.26
<i>Fusarium solani</i> (25)	26	0.13	202	54	0.21
<i>Pseudomonas fluorescens</i> (26)	41	1.28	419	41	0.12
<i>Azospirillum irakense</i> (27)	44.4	0.08	23	40.5	0.12
<i>Fusarium moniliformae</i> (28)	12.1	1.2	655	33	0.08
<i>Bacillus</i> sp. KSM-P15 (29, 30)	20.3	1.87	300	26	0.05
<i>Bacillus licheniformis</i> (31)	33.4	0.56	35	22	0.03

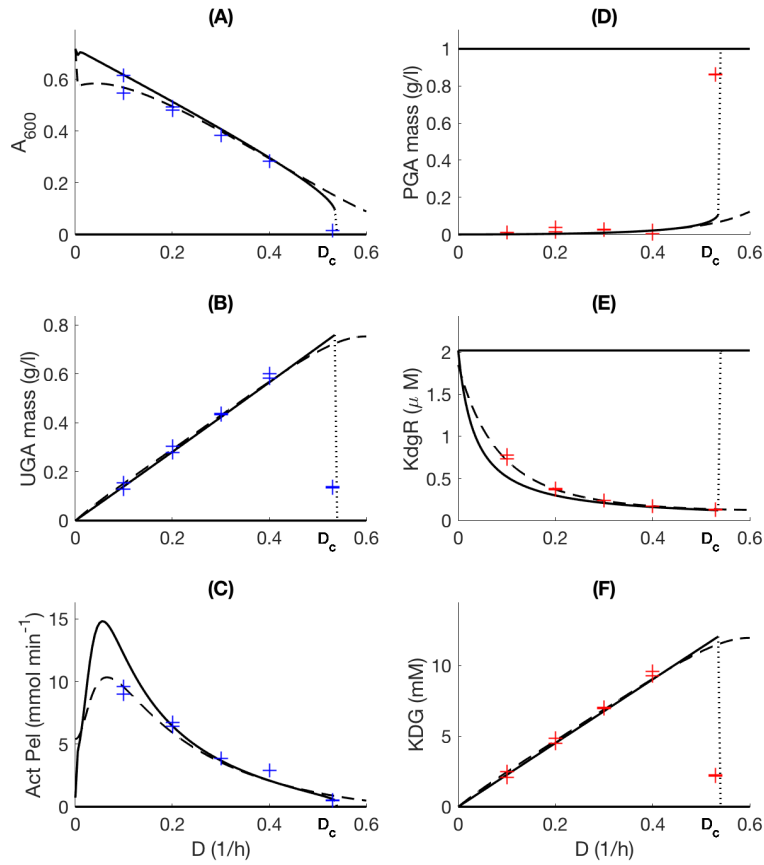
**Table 4:** List of variables.

Extracellular variables	Description	unit
$p(t)$	Pel enzyme	$mM$
$s(t)$	PGA	$M$
$u(t)$	UGA	$M$
$\rho(t)$	fractional bacterial volume	
Intracellular variables	Description	unit
$r(t)$	repressor KdgR	$\mu M$
$i(t)$	inducer KDG	$M$

## FIGURES

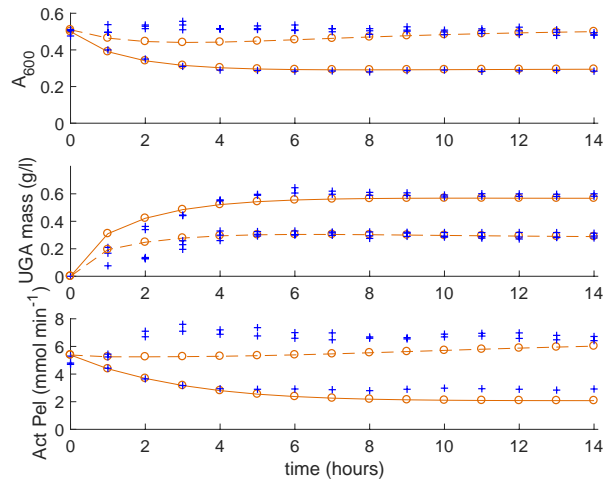


**Figure 1:** A schematic representation of the continuous reactor system. The dynamics can be described qualitatively as follows : The incoming pectin (or  $PGA_{in}$ ) is decomposed into oligogalacturonates (UGA) by the cleavage of Pel enzyme. Extracellular UGA can enter in the bacterium where it is metabolized into KDG allowing the increase in the bacterial biomass. On the other hand KDG acts as an inducer. Indeed in absence of UGA, the genes coding for the enzyme Pel are repressed by KdgR, which is the main regulator mediating directly the catabolism of pectin compounds. Once KDG is produced inside the cell, it can bind to KdgR, and therefore derepresses the expression of the *pel* genes. It entails a synthesis and export of enzymes Pel enzymes in the extracellular medium. Consequently the expression of the *pel* genes is reinforced while the bacteria grow and its population increases. An equilibrium state settles in the continuous reactor when the growth rate of the bacteria is balanced by their outflow from the bioreactor. The objective is to extract from the reactor as much as possible UGA which have a high economical value as compared with the PGA.

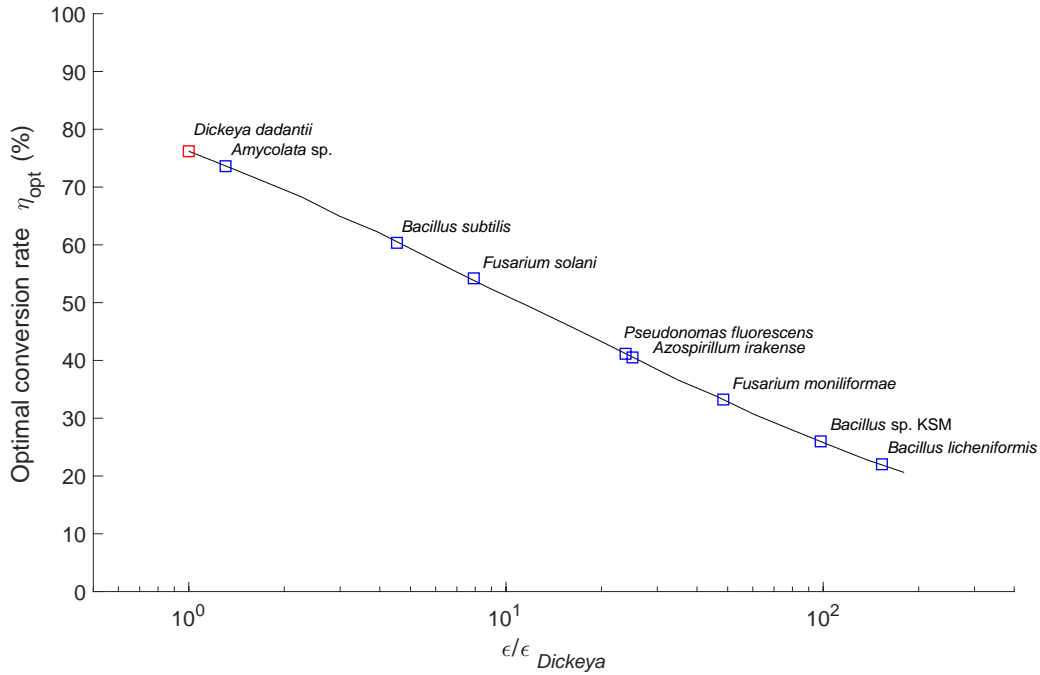


**Figure 2:** Stationary states of the system variables in function of the dilution coefficient  $D$ . The figure shows curves computed by numerical simulations, as well as experimental data for five values of  $D$  (0.1, 0.2, 0.3, 0.4, 0.53). Dashed curves correspond to the pre-equilibrium seen for  $t = 14h$ . The continuous curves are the theoretical steady states. The dilution coefficient  $D_c$  is a bifurcation point beyond which the non-zero stationary states cease to exist and the washout phenomenon occurs. (A) Optical absorbance at 600 nm is proportional to the bacterial biomass. (B) UGA mass concentration is extracted from the measurements of the absorbance at 230 nm. (C) The enzymatic activity is proportional to the concentration of the pectate lyases. (D) Observer of the mass concentration of PGA inside the culture vessel is deduced from the conservation of the mass-equivalent UGA (eq.(13)). (E) Observer of the concentration of the repressor KdgR. (F) Observer of the KDG concentration. Experimental data points are plotted with symbol '+'.

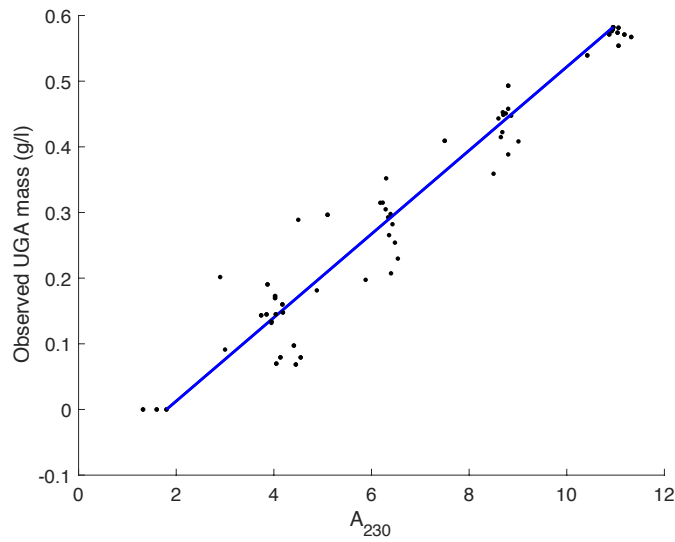




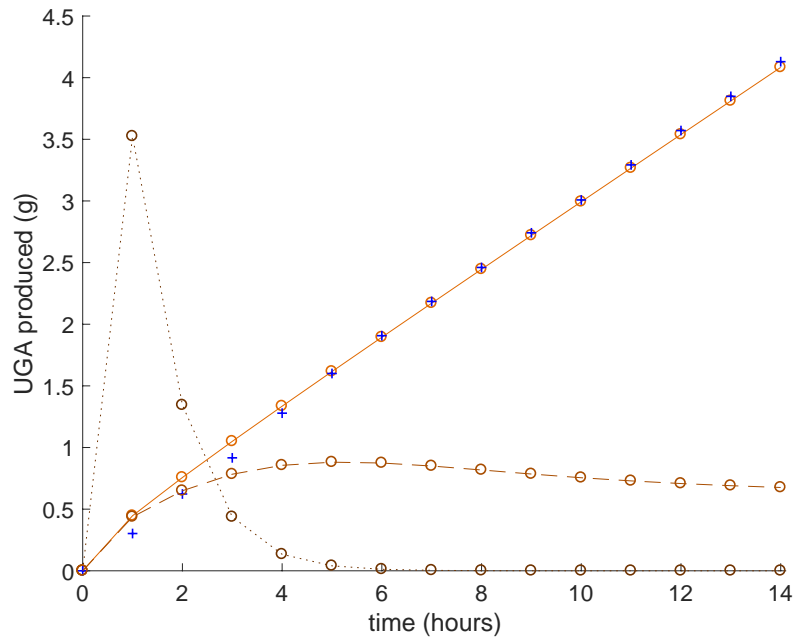
**Figure 3:** Time-evolutions of 3 variables of interest in the chemostat, for two different values of the dilution coefficient ( $D = 0.2 \text{ h}^{-1}$  in dashed lines,  $D = 0.4 \text{ h}^{-1}$  in continuous lines) comparing the experimental measures and the numerical simulations of the theoretical model described by Eqs.(6) with parameters given in Table 2. Experimental data points are plotted with symbol '+'.



**Figure 4:** The optimal conversion efficiency  $\eta_{opt}$  of PGA into UGA, that is the upper limit of produced mass of UGA divided by the incoming mass of PGA, is plotted as a function of the dimensionless parameter  $\epsilon$  (in lin-log scale) for the nine microorganisms reported on Table 3. Recall that  $\epsilon$  is a dimensionless parameter defined in eq. (3) depending on the system parameters and such that the smaller it is, the larger is  $\eta_{opt}$ . The abscissa is scaled in function of the value of  $\epsilon$  corresponding to *Dickeya dadantii*. The present graph, comparing the efficiency of nine microorganisms, is performed on basis of their biochemical parameters listed on Table 3 ( $K_m$ ,  $V_{max}$  and molar mass of Pel). The other biochemical parameters entering in  $\epsilon$  being unknown, they are assumed to be equal to the ones of *Dickeya dadantii*. In this approximation, *Dickeya dadantii* appears to be the bacteria possessing the best conversion efficiency of PGA into UGA.



**Figure 5:** The blue line shows an affine model (mass concentration of UGA (in g/l) =  $64 \cdot 10^{-3} A_{230} - 0.11$ ) for the relationship between the absorbance at 230 nm and the mass concentration of the free UGA in the continuous reactor. Each point corresponds to a measure performed at a given time (each hour between 0 and 14h), and a given dilution coefficient  $D$  among (0.1, 0.2, 0.3, 0.4). This affine transformation is used in Figs. (2)–(3) to plot the mass concentration of UGA measured via the absorbance  $A_{230}$ . The abscissa of each point is a measurement of  $A_{230}$  which is matched with the “expected” UGA that is observed via the UGA-equivalent variable  $w$  (see text). A salient feature of this relation is that when the amount of UGA vanishes, the absorbance  $A_{230}$  tends to a non-zero value that can be interpreted as a residual absorption of the bulk at 230 nm.



**Figure 6:** Assuming the same consumption of PGA, comparison of the mass production of UGA predicted by the numerical simulation of our model (6)-(12) adapted to 3 types of biochemical reactors. (i) The continuous line : continuous reactor with a constant dilution coefficient  $D = 0.4 \text{ h}^{-1}$  and a reactor volume  $V_0 = 1.2 \text{ l}$  crossed by a constant volume debit  $DV_0 = 0.48 \text{ lh}^{-1}$ . After  $t_f = 14 \text{ h}$ , the total consumed volume is  $V_f = DV_0 t_f = 6.72 \text{ l}$ . Experimental data of UGA measured from the optical absorbance  $A_{230}$  are superposed to the theoretical curve. (ii) the dashed-line describes the production of UGA in a fed-batch reactor, fed with a constant debit  $F_{in} = 0.4 \text{ lh}^{-1}$  filling a vessel of initial volume  $V_0$  and reaching a final volume  $V_f = 6.72 \text{ l}$  after  $t_f = 14 \text{ h}$ . (iii) The dotted-line shows the production of UGA in a batch reactor of volume  $V_f = 6.72 \text{ l}$  containing from the beginning the same mass of PGA consumed in the previous cases (i) and (ii).