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About biomass overyielding of mixed cultures in batch processes

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Abstract

We study mechanisms that can produce an increase of biomass production in batch processes when considering mixed cultures, compared to pure cultures. We show that growth thresholds or variable yields can produce 'overyielding', while this is not possible in the classical batch model with multiple species. We give sufficient conditions on the characteristics of the species to obtain overyielding, and illustrate these theoretical results with numerical simulations. This work provides new insights on species complementary in models of mixed cultures, without having to consider direct interactions terms between species as, for instance in the well known Generalized Lotka-Volterra model.

Key words: batch culture, complex ecosystems, diversity, overyielding, transient, optimization.

1 Introduction

Microorganisms play an important role in many ecosystems on earth, such as carbon or nitrogen biochemical cycles, or food chains [35]. Today, many industrial bio-processes rely on bacteria or yeasts to transform matter into high-value products, such as in the food industry (winery, bakery, cheese dairy...). Cultivation is operated either in continuous (chemostat) [21], batch [10] or fed-batch modes [26]. Although questions related to biodiversity (exclusion,

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coexistence, persistence of species) have been already well investigated for continuous mode (see e.g. [37]), batch cultures have received less fundamental attention with respect to the study of diversity. One of the main reasons is much probably that most of the industrial batch processes rely on a single species that have been carefully selected for its characteristics (such as yield conversion, growth kinetics, products composition). Another possible explanation is the relative recent availability of genetic tools to discriminate with accuracy the ecosystems composition in these application fields.

In several cultures, it has been reported that species diversity could present better performances than isolated species or less diverse ecosystems (see for instance [6, 23, 31]). When the performance criterion concerns the production of biomass or a product of the bioreaction, we shall say that such situations exhibit an 'overyielding' (we give a precise definition further). Several natural batch ecosystems rely on a certain diversity for their functioning, such as cheese dairy [30] or animal rumen [9]. In such ecosystems, a number of species interact together and are complementary one of each others in terms of functioning. In many cases, a complex ecosystem exhibits better stability and robustness properties with respect to unexpected situations. This is why species interactions have been studied to explain how the main functions of the ecosystems can be ensured by concomitant species. Nevertheless, it appears that few works have investigated the role of ecosystems diversity - from a theoretical view point - in terms of performances of a given function, such as the biomass production. In industrial applications, the paradigm that the best performances are necessarily obtained by the selection of a single species - *the best one* - seems to remain quite strong, in particular in food industry (see for instance [8, 13, 18, 45]). However, some recent investigations show the potential of mixed cultures in these domains [2, 4, 28, 40]. Let us underline that differently to continuous cultures, for which the Competitive Exclusion Principle predicts that under perfectly well-controlled environment the best species excludes the other ones at steady state, the dynamics of batch culture is fundamentally transient. Therefore, alternation of dominance among species is expected to occur during the transients.

The objective of the present work is to study theoretically the possibilities of overyielding in batch cultures with the help of several existing mathematical models [27, 34]. Here we will say that there is biomass overyielding in a batch process if the total production of biomass using an inoculum composed of multiple species is greater than the biomass production obtained when the inoculum is made up of a single species. In other words, we study the role of the diversity of the inoculum with respect to process performances in terms of biomass production. For simplicity, we restrict our attention to single step reactions, which means that we investigate possible mechanisms of overyielding

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in any biological system where the biomass growth is limited by a single limiting substrate. In other terms, we study biomass overyielding in the framework of the competition of n species on a single resource.

The paper is organized as follows. In Section 2, we first introduce assumptions and modeling framework to give a precise mathematical definition of overyielding. In Section 3 we consider the most classical batch model and show, under the simplest hypotheses, that overyielding is not possible (the highest biomass production is obtained when the 'best' species is cultivated alone). For this simplest case however, it is shown that results hold for a very important class of growth functions. Then, we consider more sophisticated models from the literature and highlight key mechanisms for biomass overyielding to occur: constant yield with a threshold in the growth functions of species in Section 4 and variable yields in Section 5. For these two classes of models, we give precise conditions for which overyielding is possible. Summary and comparison of possibilities to obtain overyielding are given in Section 6. Finally, numerical examples which illustrate the possibilities of overyielding for these models are then provided and discussed in Section 7.

2   Model, assumptions and definitions

Under appropriate environmental conditions (temperature, pH,...), we consider a vessel which contents all the biotic resources necessary for microorganisms to grow, except a single limiting resource. At initial time, one or several species and the resource are introduced (in fermentation processes, these are typically one or several yeasts and sugar). We shall denote by $X_i(t)$, $i \in \{1, \cdots, n\}$ the concentrations (or densities) of microorganisms of species i, and by $S(t)$ the concentration of the resource. The general expression of classical consumers-resources model which describes the time evolution of the reactions that take place between X_i and S is given by the following system:

$$
\begin{aligned}
\dot{X}_i &= \mu_i(S)X_i \quad (i = 1 \cdots n) \\
\dot{S} \; &= -\sum_{i=1}^{n} \frac{1}{y_i(S)} \mu_i(S)X_i
\end{aligned}
\tag{1}
$$

where $\mu_i(\cdot)$ is the specific growth rate function and $y_i(\cdot)$ the yield conversion factor of species i. In microbiology, four phases are usually described in batch processes: 1. the lag phase, 2. the growth phase, 3. the stationary phase and 4. the decline phase. As our objective here is to focus on the production of the growth phase, we neglect with this model the lag phase, assuming that at initial time all the species are already in their 'active' phase. We also do not consider the decline phase, and assume that the mortality of micro-organisms

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is negligible during the growth and stationary phase. However, we shall allow $y(\cdot)$ to be possibly resource dependent, as this is commonly met in literature (see e.g. [3, 34]). We make the following assumptions on the functions μ_i and y_i.

Hypothesis 1

(1) The functions $\mu_i(\cdot)$ are Lipschitz continuous from $\mathbb{R}_+$ to $\mathbb{R}_+$ and there exist growth thresholds $\underline{S}_i \geq 0$ $(i = 1 \cdots n)$ such that

$$\mu_i(S) = 0, \ S \in [0, \underline{S}_i], \quad \mu_i(S) > 0, \ S > \underline{S}_i$$

(2) The functions $y_i(\cdot)$ are Lipschitz continuous from $\mathbb{R}_+$ to $\mathbb{R}_+$, with $y_i(S) > 0$ for any $S \in \mathbb{R}_+$ $(i = 1 \cdots n)$).

The threshold $\underline{S}_i$ represents the minimal value of the substrate concentration under which a growth cannot occur [42]. This value is often imposed to be equal to 0, as in the classical Monod model. However, in practice it is rare to have observations for low values of the substrate concentration to identify such threshold. Nevertheless, it has been observed that for some strains, this threshold is not negligible [38, 41].

Under this hypothesis, one can easily check that solutions of the system of differential equations (1) are well defined and bounded at any positive time, whatever the initial condition is in $\mathbb{R}_+^{n+1}$.

Recall that we shall speak of overyielding when the production of biomass of an inoculum composed of multiple species is greater than the production obtained when the inoculum is made up of a single species. Let us formalize this definition from a mathematical viewpoint: let us denote by $\mathcal{S}$ the simplex in the positive orthant of $\mathbb{R}^n$

$$\mathcal{S} := \left\{ p \in \mathbb{R}_+^n; \ \sum_i p_i = 1 \right\}$$

and its vertices σ^i $(i = 1 \cdots n)$ as vectors of the canonical basis:

$$\sigma_i^i = 1, \quad \sigma_j^i = 0, \ j \neq i$$

We shall also denote by X the vector in $\mathbb{R}_+^n$ of components X_i.

Definition 2 Let B_0, S_0 be two positive numbers. For any $p_0 \in \mathcal{S}$, consider the solution of (1) for the initial condition

$$X(0) = p_0 B_0, \quad S(0) = S_0$$

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and define the biomass production as the number

$$R(p_0) := B_\infty - B_0 \quad where \quad B_\infty := \lim_{t \to +\infty} B(t) \quad with \quad B(t) = \sum_i^n X_i(t)$$

System (1) *is said to present* overyielding *for the pair* (B_0, S_0) *when the inequality*

$$\max_{p_0 \in \mathcal{S}} R(p_0) > \max_{i \in \{1 \cdots n\}} R(\sigma^i) \tag{2}$$

is fulfilled.

In other words, overyielding in this precise framework means that there exists a composition of the initial biomass with different species that gives a greater production of biomass than the one we obtain when the initial biomass is made up of a single species.

In the definition of the function R, we consider asymptotic values B_∞ of the biomass, whereas we have previously mentioned that the model do not take into consideration the decline phase of the biomass which occurs for large times. Therefore, those asymptotic values may not be reached in practice. However, if one considers that the stationary phase corresponds to biomass levels close to these asymptotic values, one may reasonably assume that the comparison order between the biomass productions determined with the asymptotic values of the model is also verified at the stationary phase.

Remark 3 *In some works [14, 36], the weaker condition $R(p_0) > \sum_{i=1}^n p_{0,i} R(\sigma^i)$ is considered as the definition for overyielding, and condition (2) refers then as* transgressive *overyielding. We believe that this difference makes sense when the criterion is static or deals with steady-states. Here the proportions of species in the consortium are changing with time, which makes us choose a stricter definition of overyielding, as we shall see later on.*

To study the possibilities of biomass overyielding, it is useful to first characterize the residual concentration of substrate, with the following lemma.

Lemma 4 *For any initial condition in $\mathbb{R}^n_+$, the solution of* (1) *verifies*

$$S_\infty := \lim_{t \to +\infty} S(t) = \min \left(S(0), \min_{i=1 \cdots n} \{\underline{S}_i \ s.t. \ X_i(0) > 0\} \right)$$

PROOF. Posit $l = \min \left(S(0), \min_{i=1 \cdots n} \{\underline{S}_i \ \text{s.t.} \ X_i(0) > 0\} \right)$.

Notice first, from equations (1), that $S(\cdot)$ is a decreasing function bounded from below by 0. Therefore, it admits a limit $S_\infty \leq S(0)$. Moreover, the

functions $X_i(\cdot)$ are non decreasing. One can then write

$$\sum_{i=1}^{n} \frac{\mu_i(S(t))}{y_i(S(t))} X_i(t) \geq \sum_{i=1}^{n} \min_{S \in [S_\infty, S(0)]} \left\{ \frac{\mu_i(S)}{y_i(S)} \right\} X_i(0), \quad t \geq 0$$

If $S_\infty > l$, there exists $j \in \{1, \cdots, n\}$ such that $X_j(0) > 0$ with $l = \underline{S}_j < S(0)$. Then, let

$$\eta = \min_{S \in [S_\infty, S(0)]} \frac{\mu_j(S)}{y_j(S)} X_j(0) > 0$$

and one has $\dot{S}(t) \leq -\eta$ for any $t > 0$, which contradicts the positivity of the variable S for any time.

If $S_\infty < l$, there exists $\tilde{S} \in (0, l)$ and $T > 0$ such that $S(T) = \tilde{S}$. Then, $(X(T), \tilde{S})$ is a steady-state of (1), which contradicts the uniqueness of solutions of (1).

We conclude that the equality $S_\infty = l$ is satisfied.

Remark 5 *When there is no threshold i.e. $\underline{S}_i = 0$, we find the well-known fact that there is no residual substrate at the end of the reaction.*

3   Overyielding in the classical 'batch competition model'

We consider here the model (1) with constant yields $y_i(\cdot) = Y_i$ and growth functions with no threshold i.e. $\underline{S}_i = 0$. We denote by $R_c(\cdot)$ the corresponding production function $R(\cdot)$.

Proposition 6 *Let B_0, S_0 be two positive numbers.*

1. One has

$$R_c(\sigma^i) = Y_i S_0, \quad i = 1 \cdots n$$

2. The system (1) does not present any overyielding, and best species $i^\star$ are the ones with the highest $Y_{i^\star}$.

PROOF. From Lemma 4, one has $S_\infty = 0$ whatever is the initial composition p_0 of the biomass. From (1), one can write

$$\frac{d}{dt} \left(\sum_{i=1}^{n} \frac{X_i(t)}{Y_i} + S(t) \right) = 0$$

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and then, integrating between $t = 0$ and $t = +\infty$, one obtains

$$\sum_{i=1}^{n} \frac{X_i(\infty) - X_i(0)}{Y_i} = S_0$$

Posit

$$q_i = \frac{X_i(\infty) - X_i(0)}{Y_i S_0}, \quad i = 1 \cdots n$$

Then, for any initial proportion $p_0 \in \mathcal{S}$ of the species, one has

$$R_c(p_0) = \sum_{i=1}^{n} X_i(\infty) - X_i(0) = \sum_{i=1}^{n} q_i Y_i S_0$$

For the proportions σ^i $(i = 1 \cdots n)$, one has

$$R_c(\sigma^i) = Y_i S_0, \quad i = 1 \cdots n$$

which proves the point 1. One can then write

$$R_c(p_0) = \sum_{i=1}^{n} q_i R_c(\sigma^i)$$

As the numbers q_i belongs to $[0, 1]$ and their sum is equal to 1, we conclude that any $R_c(p_0)$ cannot be above the largest $R_c(\sigma^i)$ with $i \in \{1, \cdots, n\}$, which proves point 2.

Remark 7 *Notice that Proposition 6 remains valid whatever the expressions of $\mu_i(\cdot)$ are. The only condition is that these functions are positive when S is positive, so that one has $\underline{S}_i = 0$. Indeed, the only condition for this result to hold is the mass conservation, under the property that $S_\infty = 0$. This implies that the terms $\mu_i(\cdot)$ appearing in the dynamics of X_i be the same than the ones appearing negatively in the dynamics of S_i, whatever are the growth rates (provided to be positive for non-null S): they can involve any state of the system or any external 'inputs' or environmental variables like the pH or the oxygen concentration, or even include direct interaction terms. From a chemical engineering viewpoint, this result is easily understandable: since our performance index - the biomass production - is essentially related to the yield of the biomass, the final state does not depend on the transitory but only on the initial resource available.*

In the next sections, we study two different mechanisms for biomass overyielding to appear.

4 Overyielding with constant yields and growth thresholds

In this section, we denote by capital letters functions $y_i(\cdot)$ when constant, that is $y_i(S) = Y_i$, and by $R_t(\cdot)$ the corresponding production function $R(\cdot)$.

Proposition 8 *Fix B_0, S_0 two positive numbers.*

1. *One has*
$$R_t(\sigma^i) = Y_i \max(0, S_0 - \underline{S}_i), \quad i = 1 \cdots n \tag{3}$$

2. *Let $j \in \{1, \cdots, n\}$ be such that $R_t(\sigma^j) = \max_{i \in \{1 \cdots n\}} R_t(\sigma^i) > 0$. If there exists $k \in \{1, \cdots, n\} \setminus \{j\}$ such that $Y_k > Y_j$, then for $S_0 > \underline{S}_k$, the model (1) presents an overyielding.*

PROOF.

1. For pure cultures i.e. proportions p_0 equal to one of the σ^i, $i \in \{1, \cdots, n\}$, one has from equations (1)

$$\frac{d}{dt}(X_i(t) + Y_i S(t)) = 0$$

Integrating this equality between $t = 0$ and $t = +\infty$ gives

$$X_i(\infty) - X_i(0) = Y_i(S_0 - S_\infty)$$

where $S_\infty = \min(S_0, \underline{S}_i)$ by Lemma 4. Therefore, one obtains the equality $R_t(\sigma^i) = Y_i \max(0, S_0 - \underline{S}_i)$.

2. Consider an initial proportion p_0 with the presence of the species j and k only. Note that $Y_k > Y_j$ implies necessarily to have $\underline{S}_k > \underline{S}_j$ (as $R_t(\sigma^j) > R_t(\sigma^k)$). When $S_0 > \underline{S}_k$, the two species grow and one has $S_\infty = \underline{S}_j$ (by Lemma 4). From equations (1), we write

$$\frac{d}{dt}\left(\frac{X_j(t)}{Y_j} + \frac{X_k(t)}{Y_k} + S(t)\right) = 0$$

and by integration, one has

$$\frac{X_j(\infty) - X_j(0)}{Y_j} + \frac{X_k(\infty) - X_k(0)}{Y_k} = S_0 - \underline{S}_j$$

From $Y_k > Y_j$, one gets

$$R_t(p_0) = X_j(\infty) + X_k(\infty) - X_j(0) - X_k(0) > Y_j(S_0 - \underline{S}_j) = R_t(\sigma^j)$$

which shows that a mixed culture of species j and k gives a better production than the best species in pure culture.

This result shows that having different growth thresholds could be a way to obtain overyielding, playing with a *complementary effect* among species. Typically, this happens when a species j has the best biomass production among single species, and another species k has an even better conversion factor Y_k but suffers from a relatively large growth threshold $\underline{S}_k$ (so that it can no longer grow when the resource level S is below $\underline{S}_k$). Then, the presence of species k in addition to species j boosts the performances of the ecosystem when S is above $\underline{S}_k$ due to the better conversion factor of species k. Later on, species j carries on the conversion of the resource alone, ensuring a better total production at the end of the batch when compared to situations where k or j are cultivated alone.

Remark 9 *When the numbers $\underline{S}_i$, $i \in \{1, \cdots, n\}$ are all identical, one can straightforwardly extend the result of Proposition 6 to show that no overyielding is possible.*

The mechanism we have identified for overyielding is based on growth thresholds, assuming that the species do not consume any substrate when there is no growth. We investigate now another mechanism that could produce overyielding.

5   Overyielding with variable yields

In this section, we explore another mechanism based on variable yields, that can produce overyielding. To properly separate this mechanism from the one induced by growth thresholds that has been studied in Section 4, we come back to the hypothesis used in Section 3 and consider growth thresholds $\underline{S}_i$ all equal to 0.

Evidence of variable yield cultures could be deduced from observed oscillations in both continuous [11, 15] or batch cultures [24, 43], based on the fact that models without variable yields cannot exhibit oscillations [1, 25]. Moreover, models with several species and variable yields have been also investigated in the literature but for continuous cultures [20, 32] and not - at the best of author's knowledge - to characterizing overyielding in batch, as we do in the present work.

Several biological mechanisms could justify a variable yield, such as extracellular material or transporters [12, 17]. Typically, the production of other

material by different metabolic ways, concomitantly to the bacterial growth, could impact the conversion yield of substrate into pure biomass. However, the most frequently encountered justification is related to maintenance terms [22, 33, 34] Typically, in continuous culture, a maintenance term mX (where m is a positive constant) is subtracted from the substrate kinetics, as follows:

$$\dot{X} = \mu(S)X - DX$$
$$\dot{S} = -\frac{1}{Y}\mu(S)X + D(S_{in} - S) - mX \tag{4}$$

where D represents the dilution rate. Nevertheless, although this formalism has been successfully validated in chemostats [22], this writing is not satisfactory in batch mode (i.e. when $D = 0$) because the solutions of the differential equations (4) can take negative values of S. This issue has been addressed by several authors that have proposed more complex models, incorporating microbial death [5, 39, 44]. However, non-constant maintenance terms have been observed experimentally (see e.g. [7, 19]). This is why we consider here the maintenance term m as a function of S with $m(0) = 0$ (which gives no substrate consumption at $S = 0$) instead of a constant coefficient. One can take, for instance, an expression of the form $m(S) = \min(kS, m_c)$ (where k and m_c are constant parameters) to recover the constant case $m = m_c$ when $S > m_c/k$. Therefore, the model (1) with constant yield Y_i becomes

$$\dot{X}_i = \mu_i(S_i)X_i \quad (i = 1 \cdots n)$$
$$\dot{S} = -\sum_{i=1}^{n} \frac{1}{Y_i}\mu_i(S)X_i - m_i(S)X_i \tag{5}$$

We shall consider the following assumption

Assumption 10 *The function $m_i(\cdot)$ are Lipschitz continuous from $\mathbb{R}_+$ to $\mathbb{R}_+$ with $m_i(0) = 0$ and $m_i(S) > 0$ for $S > 0$. Moreover, for each $i \in \{1, \cdots, n\}$, one has*

$$\exists \lim_{S>0, S \to 0} \frac{m_i(S)}{\mu_i(S)} < +\infty$$

Under this assumption, one can define for each $i \in \{1 \cdots n\}$, the function

$$\alpha_i(S) := \frac{m_i(S)}{\mu_i(S)}, \quad S \geq 0$$

and consider functions $y_i(\cdot)$ (denoted in lowercase letters) defined as follows

$$y_i(S) := \frac{Y_i}{1 + \alpha_i(S)Y_i}, \quad S \geq 0$$

Then, formally, one can check that system (5) is equivalent to (1). In this section we denote by $R_v(\cdot)$ the corresponding production function $R(\cdot)$.

Proposition 11 *Fix B_0, S_0 two positive numbers.*

1. *One has*
$$R_v(\sigma^i) = \int_0^{S_0} y_i(s)ds, \quad i = 1 \cdots n \ . \tag{6}$$

2. *Assume there exist j, k in $\{1, \cdots, n\}$ such that*

   (i) $R_v(\sigma^k) = \max_{i \in \{1 \ldots n\}} R_v(\sigma^i)$,

   (ii) there exist numbers $\tilde{S}$ and $S^\star$ with $0 < \tilde{S} < S^\star < S_0$ such that

   (a) $S \neq S^\star \Rightarrow (y_j(S) - y_k(S))(S^\star - S) > 0$,

   (b) $S \in (0, \tilde{S}) \Rightarrow \mu_k(S) < \alpha\mu_j(S)S^{1+\beta}$ with $\alpha, \beta > 0$,

   then, the model (5) presents an overyielding when the initial proportion of species j is sufficiently small.

PROOF.

1. For pure cultures, equations (1) give

$$\frac{d}{dt}X_i(t) + y_i(S(t))\frac{d}{dt}S(t) = 0$$

Integrating this equality between $t = 0$ and $t = +\infty$ gives

$$X_i(\infty) - X_i(0) = -\int_0^{+\infty} y_i(S(t))\dot{S}(t))dt.$$

By Lemma 4 one has $S_\infty = 0$ and from (1) $S(\cdot)$ is a decreasing function from $[0, \infty)$ to $(0, S_0]$. Therefore, we obtain

$$R_v(\sigma^i) = \int_0^{S_0} y_i(s)ds.$$

2. We consider ecosystems with species j and k only and show that overyielding occurs when the initial proportion $p_{0,j}$ of species j is small enough. As $S(\cdot)$ is decreasing with time, there exist finite times $0 < t^\star < \tilde{t}$ such that $S(t^\star) = S^\star$ and $S(\tilde{t}) = \tilde{S}$. We proceed in three steps.

Step 1: $t \leq t^\star$.

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From equations (1), let us write $\dot{S}$ as follows

$$\dot{S} = -\frac{\dot{X}_j}{y_j(S)} - \frac{\dot{X}_k}{y_k(S)} = -\frac{\dot{X}_j + \dot{X}_k}{y_k(S)} + \left(\frac{1}{y_k(S)} - \frac{1}{y_j(S)}\right)\dot{X}_j$$

and define the number

$$\eta := \max_{s \in [S^\star, S_0]} \left(\frac{y_k(s)}{y_j(s)} - 1\right).$$

From hypothesis (ii)-(a), η is a positive number. As $S(t) \in [S^\star, S^0]$ and $\dot{X}_j(t) > 0$ for $t \in [0, t^\star]$, one can then write

$$\dot{B}(t) = \dot{X}_j(t) + \dot{X}_k(t) \geq -y_k(S(t))\dot{S}(t) - \eta\dot{X}_j(t), \quad t \leq t^\star.$$

Integrating this inequality between $t = 0$ and $t = t^\star$ gives

$$B(t^\star) - B_0 \geq \int_{S^\star}^{S_0} y_k(s)ds - \eta(X_j(t^\star) - X_j(0)). \qquad (7)$$

On another hand, consider the proportion variable $p_j(t) = X_j(t)/B(t)$. One has straightforwardly from equations (1)

$$\dot{p}_j = (\mu_j(S(t)) - \mu_k(S(t)))p_j(1 - p_j), \quad p_j(0) = p_{0,j}.$$

Then, one has the inequality

$$\dot{p}_j(t) \leq m\,p_j(t), \quad t \leq t^\star \qquad (8)$$

with

$$m := \max\left(0, \max_{s \in [S^\star, S_0]} \mu_j(s) - \mu_k(s)\right) \geq 0.$$

From equations (1), one can also write

$$\dot{S}(t) \leq -rB(t) \leq -rB_0, \quad t \leq t^\star$$

with

$$r := \min_{s \in [S^\star, S_0]} \left(\frac{\mu_j(s)}{y_j(s)}, \frac{\mu_k(s)}{y_k(s)}\right) > 0$$

which provides a bound on $t^\star$:

$$t^\star \leq \frac{S_0 - S^\star}{rB_0}. \qquad (9)$$

Then, inequalities (8) and (9) give

$$p_j(t^\star) \leq \lambda\,p_{0,j}.$$

with

$$\lambda := e^{m\frac{S_0 - S^\star}{rB_0}} \geq 1.$$

In this way, one obtains

$$X_j(t^\star) - X_j(0) \leq \lambda p_{0,j}(B(t^\star) - B_0) \tag{10}$$

and combining inequalities (7), (10) raises

$$B(t^\star) - B_0 > \frac{1}{1 + \eta\lambda p_{0,j}} \int_{S^\star}^{S_0} y_k(s)ds$$

from which we deduce the inequality

$$B(t^\star) - B_0 > \int_{S^\star}^{S_0} y_k(s)ds - A\, p_{0,j} \tag{11}$$

where we posit the number

$$A := \eta\lambda \int_{S^\star}^{S_0} y_k(s)ds.$$

Let us underline that this number does not depend on $p_{0,j}$ (it depends only on S_0 and B_0).

Step 2: $t \in (t^\star, \tilde{t}]$.

For any $t > t^\star$, one has $S(t) < S^\star$ and by hypothesis (ii)-(a) the inequality $y_j(S(t)) > y_k(S(t))$ is verified, which allows to write

$$\dot{S}(t) = -\frac{\dot{X}_j(t)}{y_j(S(t))} - \frac{\dot{X}_k(t)}{y_k(S(t))} \geq -\frac{\dot{B}(t)}{y_k(S(t))}, \quad t \in (t^\star, \tilde{t}]$$

Integrating between $t^\star$ and $\tilde{t}$ gives

$$B(\tilde{t}) - B(t^\star) > \int_{\tilde{S}}^{S^\star} y_k(s)ds \tag{12}$$

Step 3: $t \in (\tilde{t}, +\infty)$.

In a similar way to step 1, we write

$$\dot{B} = -y_k(S)\dot{S} + \left(1 - \frac{y_k(S)}{y_j(S)}\right)\dot{X}_j$$

where $\dot{X}_j > 0$, and define the number

$$\gamma = \min_{s \in [0,\tilde{S}]} \left(1 - \frac{y_k(s)}{y_j(s)}\right)$$

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which is positive by hypothesis (ii)-(a). One has then

$$B_\infty - B(\tilde{t}) \geq \int_0^{\tilde{S}} y_k(s)ds + \gamma(X_j(+\infty) - X_j(\tilde{t})). \qquad (13)$$

Consider first the species k. One has

$$\dot{S} < -\frac{\dot{X}_k}{y_k(S)} \Rightarrow \dot{X}_k < -y_k(S)\dot{S}$$

and integrating with respect to time between 0 and $t > 0$ gives an upper bound on X_k:

$$X_k(t) < X_k(0) + \int_{S(t)}^{S_0} y_k(s)ds < \overline{X}_k := B_0 + \int_0^{S_0} y_k(s)ds, \quad t > 0.$$

Concerning the species j, let us write

$$\dot{X}_j = \frac{\mu_j(S)X_j}{\dfrac{\mu_j(S)X_j}{y_j(S)} + \dfrac{\mu_k(S)X_k}{y_k(S)}}(-\dot{S}),$$

where $\dot{S}$ is always negative. For $t > \tilde{t}$, one has $S(t) < \tilde{S} < S^\star$ and with Hypothesis (ii)-(a) one can write

$$\dot{X}_j(t) > y_k(S(t))\frac{X_j(t)}{X_j(t) + \phi(S(t))\overline{X}_k}(-\dot{S}(t)), \quad t > \tilde{t}$$

where the function ϕ is defined as:

$$\phi(s) = \frac{\mu_k(s)}{\mu_j(s)}, \quad s > 0.$$

As X_j is an increasing function of the time, we can also write

$$\dot{X}_j(t) > y_k(S(t))\frac{X_j(0)}{X_j(0) + \phi(S(t))\overline{X}_k}(-\dot{S}(t)), \quad t > \tilde{t}. \qquad (14)$$

Consider then the number

$$S^\dagger := \left(\frac{X_j(0)}{\alpha\overline{X}_k}\right)^{\frac{1}{1+\beta}} \qquad (15)$$

Note that for $X_j(0)$ small enough, one has $S^\dagger < \tilde{S}$. With Hypothesis (ii)-(b), one has the property

$$\phi(s)\overline{X}_k \leq \alpha s^{1+\beta}\overline{X}_k < \alpha(S^\dagger)^{1+\beta}\overline{X}_k = X_j(0), \quad 0 < s < S^\dagger. \qquad (16)$$

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Let $t^\dagger > \tilde{t}$ be such that $S(t^\dagger) = S^\dagger$. Inequalities (14), (16) gives

$$\dot{X}_j(t) > \frac{1}{2}y_k(S(t))(-\dot{S}(t)) \geq \frac{1}{2}\underline{y}_k(-\dot{S}(t)), \quad t > t^\dagger$$

where

$$\underline{y}_k = \min_{s\in[0,\tilde{S}]} y_k(s) > 0.$$

Then, as X_j is an increasing function of time and $S(t) \to 0$ when $t \to +\infty$, one can write

$$X_j(+\infty) - X_j(\tilde{t}) > X_j(+\infty) - X_j(t^\dagger) = \int_{t^\dagger}^{+\infty} \dot{X}_j(t)dt > \frac{1}{2}\underline{y}_k S^\dagger.$$

Finally, from the expression (15) of $S^\dagger$ and (13), one obtains the inequality

$$B_\infty - B(\tilde{t}) > \int_0^{\tilde{S}} y_k(s)ds + \Gamma\, p_{0,j}^{\frac{1}{1+\beta}} \tag{17}$$

where

$$\Gamma := \frac{1}{2}\gamma\underline{y}_k\left(\frac{B_0}{\alpha\overline{X}_k}\right)^{\frac{1}{1+\beta}}.$$

Let us underline that this number does not depends on $p_{0,j}$ (it depends only on S_0 and B_0).

Gathering inequalities (11), (12) and (17) raises

$$B_\infty - B_0 > R_v(\sigma^k) - A\, p_{0,j} + \Gamma\, p_{0,j}^{\frac{1}{1+\beta}}$$

As the numbers A and Γ are independent of $p_{0,j}$, we conclude that an over-yielding occurs when $p_{0,j}$ is smaller than $\left(\frac{\Gamma}{A}\right)^{\frac{1+\beta}{\beta}}$.

Condition (i) of point 2. of Proposition 11 means that species k has the highest biomass production when cultivated alone. Condition (ii) assumes that species k has a larger yield compared to another species j, but only for high levels of substrate concentration. On the opposite, for small concentrations of substrate, species j has a better yield but should have also a larger kinetics that behaves much differently than the k one close to 0 (cf condition (ii)-(b)). Graphical illustrations of these conditions are depicted on Fig. 1. In short, species k and j are complementary, k being "specialist" of large concentrations while j is more efficient for small ones. Species j being faster with a better yield at the end of the batch process, can boost the biomass production already achieved by species k, and produce then overyielding even if it is initially present in a small proportion. Such kind of ecological succession is illustrated in Section 7. Notice that differently to the constant yield case (Proposition 8), conditions on the growth functions are required here.
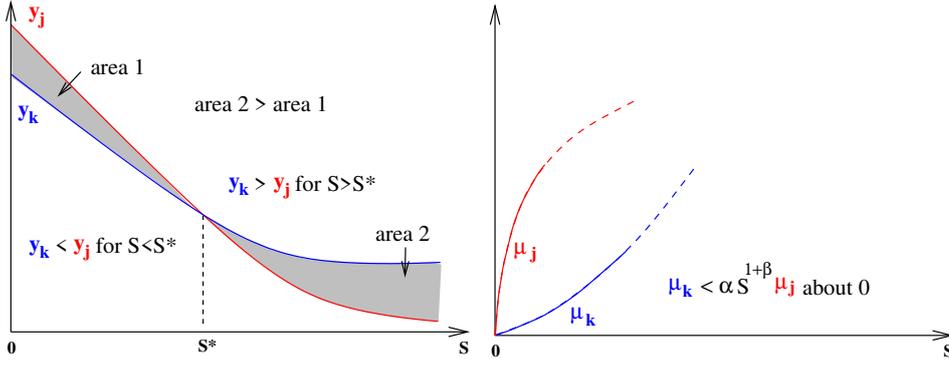
Fig. 1. Graphical illustration of sufficient conditions for overyielding.

6 Summary and comparison

We summarize on Fig. 2 the results obtained in Sections 3, 4 and 5.

	classical model	*threshold model*	*variable yield model*
growth	any without threshold on S	threshold on S	function of S without threshold
yield	constant	constant	variable
	no overyielding	overyielding when the best species does not have the highest yield	overyielding when the best species is overtaken by another one for small S

Fig. 2. Conclusions about possibilities of overyielding for the three different models

One can see, as already underlined in Remark 7, that under constant yields, it is not possible to have overyielding whatever are the growth functions and their dependency, but on the single condition that growth functions are non null when $S > 0$. Conversely, threshold is not sufficient to have overyielding: a complementary between species with both high yield and high threshold versus lower yield and lower threshold is required. Notice that here also no particular condition on the kinetics, apart the existence of thresholds, is required. For variable yields, we give more sophisticated sufficient conditions for overyielding, in terms of complementarity between species involving both yield and kinetics functions. Although different thresholds or different variable yields both reflect complementary between species, the underlying mechanisms in the transient are quite different.

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7 Numerical simulations and discussions

In this section, we illustrate the results of Propositions 8 and 11 on numerical simulations with growth functions of the literature. The parameter values do not correspond to precise known species but their magnitude orders are inspired from the literature (with units corresponding to hour for time and mg/l for concentrations). For simplicity and ease of results interpretation, we show examples of overyielding with two species only. Of course, more sophisticated examples with more species can be provided.

7.1 Case of constant yields

According to Proposition 8, we consider non-null growth thresholds. Take for instance two species with growth function of Moser type [29] with threshold:

$$\mu_i(S) = \begin{cases} 0, & S < \underline{S}_i \\ \dfrac{\mu_{max,i} S^{\alpha_i}}{K^{\alpha_i} + S^{\alpha_i}}, & S \geq \underline{S}_i \quad (\alpha_i > 1) \end{cases} \tag{18}$$

Other choices of growth functions are possible. The Moser expression possesses the advantage to be smooth at $S = \underline{S}_i$. Parameter values of the two species are given in Table 1, while the graphs of their growth functions are plotted in Fig. 3.

	Y_i	$\underline{S}_i$	$\mu_{max,i}$	K_i	α_i
$i = 1$	1	0.5	0.3	0.7	2
$i = 2$	1.4	1	0.6	0.5	2

Table 1
Parameter values of the model (18) for each species.

Consider now initial conditions with $B_0 = 0.01$ and $S_0 = 2$. One can check that $R_c(\sigma^1) = Y_1 \max(0, S_0 - \underline{S}_1) = 1.5 > R_c(\sigma^2) = Y_2 \max(0, S_0 - \underline{S}_2) = 1.4$. Therefore, species 1 is the best in pure culture. However, one has $Y_2 > Y_1$ and according to Proposition 8, any mixture of both species brings a better final biomass production. This is verified numerically, as illustrated in Table 2 and Fig. 4, with for instance the initial proportion $p_0 = 0.4$.

In Table 2, one can observe that one has $S(\infty) = \underline{S}_i$, $B(\infty) = R_c(\sigma^i)$ $(i = 1, 2)$ as expected from Lemma 4 and formula (3). For the mixed culture, one has $S(\infty) = \min(\underline{S}_1, \underline{S}_2)$, consistently with Lemma 4. As predicted by Proposition 8, the mixed culture presents a systematic overyielding with an increase of the production about 21.7% for $p_{0,1} = 0.4$, compared to the best species in pure
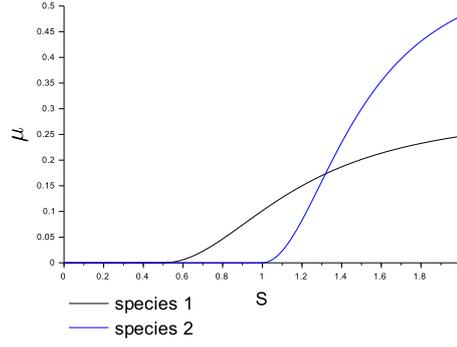
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Fig. 3. Graphs of the growth rate functions given by expression (18) and parameters in Table 1.

$p_{0,1}$	$X_1(+\infty)$	$X_2(+\infty)$	S_∞	$B\infty$
1	1.5	0	0.5	1.5
0	0	1.4	1.5	1.4
0.2	$\simeq 0.596$	$\simeq 1.262$	0.5	$\simeq 1.858$
0.4	$\simeq 0.664$	$\simeq 1.169$	0.5	$\simeq 1.832$
0.6	$\simeq 0.745$	$\simeq 1.058$	0.5	$\simeq 1.803$

Table 2
Final values of concentrations of individual biomass X_1, X_2, substrate S and total biomass B, depending on the initial proportion $p_{0,1}$.

culture. This example shows that even with species of similar performances in pure culture, the mixed culture could bring a significantly better biomass production. In Fig. 4, one can see that for the particular choice of the growth rate functions μ_i, the second species grows the fastest in mixed culture, as long as the level of substrate is above its growth threshold $\underline{S}_2$, in a similar way of what it does in pure culture. On the contrary, the first species is somewhat penalized in the competition and increases less quickly than if it were in pure culture. This is due to the fact hat the growth rate of the second species is the largest for large values of substrate (see Fig. 3). But, differently to the second species, the first species keeps growing when S goes below $\underline{S}_2$, and as a consequence, its stationary phase is reached later. As noted in the last remark of section 3, it is worth to be underlined that the final biomass in pure culture does not depend on the growth rate function (the growth rate impacts the kinetics but not the yield). In mixed culture, although overyielding is systematic, the interplay between the growth rates impacts the final gain. Its mathematical analysis appears to be quite complex, because of richness of the nonlinear nature of the growth functions.
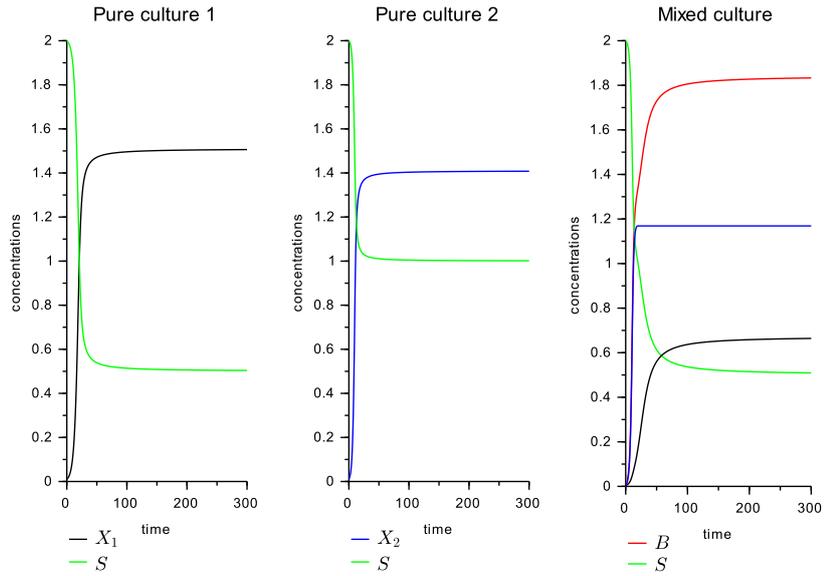
Fig. 4. From left to right: pure culture of species 1 (X_1), pure culture of species 2 (X_2) and mixed culture ($B = X_1 + X_2$) with initial proportion $X_1(0) = 0.4B(0)$. In green the concentration of substrate (S).

7.2 Case of variable yields

We have considered here a first species with a Monod law and a decreasing yield against a second species with a Moser law and a constant yield. Decreasing variable yields are typically met in alcohol fermentation [16, 19]. The expressions of the growth and yield functions are given in Table 3 and their graphs are depicted in Fig. 5.

	$\mu_i(S)$	$y_i(S)$
$i = 1$	$\dfrac{0.5\,S}{0.1 + S}$	$3.0\,e^{-0.2\,S}$
$i = 2$	$\dfrac{0.8\,S^4}{625 + S^4}$	0.8

Table 3
Expressions of the growth and yield functions considered for the simulations.

Their biomass production when cultivated separately can be easily computed, as

$$R_v(\sigma^1) = \int_0^{S_0} y_1(s)ds = 15(1 - e^{-0.2S_0})\,, \ \ R_v(\sigma^2) = \int_0^{S_0} y_2(s)ds = 0.8S_0\,.$$

Therefore, species 1 cannot have a production $R_v(\sigma^1)$ larger than 15, while
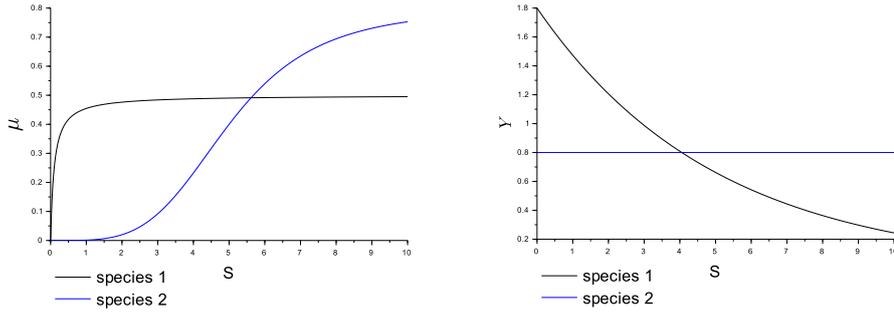
Fig. 5. Graphs of the growth rate functions (on the left) and yield functions (right) given in Table 3.

species 2 necessarily satisfies $R_v(\sigma^2) > R_v(\sigma^1)$ when S_0 is above $15/0.8 = 18.75$. For the simulations, we have considered initial conditions with $B_0 = 0.1$ and $S_0 = 10$, so that species 2 has the highest production in pure culture. Let us now check that conditions (ii) of Proposition 11 are fulfilled with $j = 1$ and $k = 2$. The graphs of y_1 and y_2 cross at $S^\star = (\log(1.8) - \log(0.8))/0.2 \simeq 6.61$, which is lower than S_0. Condition (ii)-(a) is then fulfilled. For $S > 0$, we write the ratio

$$\frac{\mu_2(S)}{\mu_1(S)} = 1.6S^3 \frac{0.1 + S}{625 + S^4} \implies \frac{\mu_2(S)}{\mu_1(S)} < 1.6S^3, \ S \in (0, 1).$$

Condition (ii)-(b) is thus satisfied taking for instance $\tilde{S} = 1 < S^\star$, $\alpha = 1.6$ and $\beta = 2$.

Table 4 gives the final values for pure and mixed cultures. One can see that here

$p_{0,1}$	$X_1(+\infty)$	$X_2(+\infty)$	S_∞	B_∞
1	$\simeq 15.725$	0	0	$\simeq 15.725$
0	0	$\simeq 8.599$	0	$= 16.100$
0.7	$\simeq 9.166$	$\simeq 6.662$	0	$\simeq 15.828$
0.3	$\simeq 6.181$	$\simeq 11.174$	0	$\simeq 17.355$
10^{-4}	$\simeq 2.626$	$\simeq 15.327$	0	$\simeq 17.953$

Table 4

Final values of concentrations of individual biomass X_1, X_2, substrate S and total biomass B, depending on the initial proportion $p_{0,1}$.

overyielding is not systematic, differently to the case of constant yields: the initial proportion of species 1 has to be small enough. The gain for $p_{0,1} = 0.3$ is approximately equal to 7.8% at the end of the batch (11.6% at time 50), while it reaches 11.5% (15.4% at time 50) for $p_{0,1} = 10^{-4}$. Fig. 6 shows how species 1 contributes to end rapidly the batch in mixed culture, while species 2 in pure culture takes a longer time to convert all the substrate. Fig. 7 shows

even more clearly this succession. Although species 1 is initially present in very small quantity, and increases very slowly as long as species 2 has a faster growth, it overtakes species 2 in consuming the substrate in a second stage and contribute efficiently to improve the total production of biomass in a short time horizon.
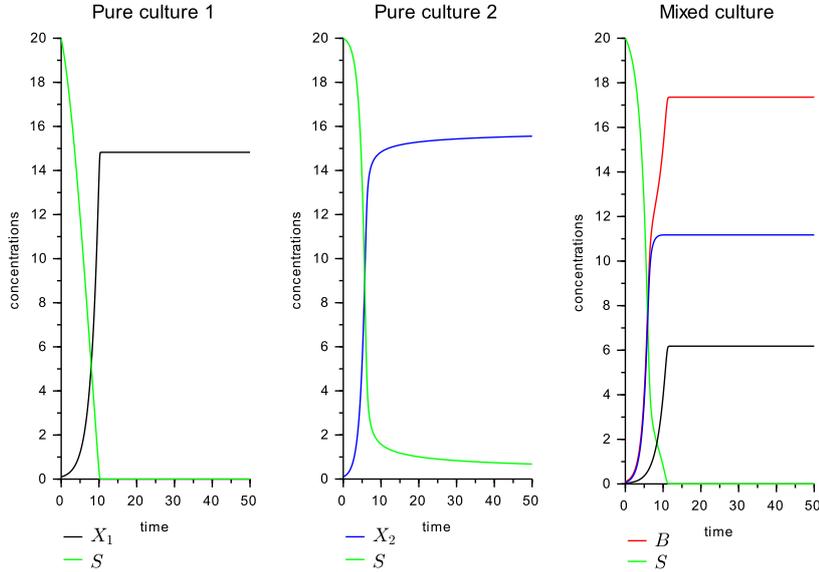

Fig. 6. From left to right: pure culture of species 1 (X_1), pure culture of species 2 (X_2) and mixed culture ($B = X_1 + X_2$) with initial proportion $X_1(0) = 0.3B_0$. In green the concentration of substrate (S).

The simulations presented in this section show that significant overyielding can occur with mixed culture of two species only. Practically, this means that the addition of a well chosen strain could boost the production and moreover help the batch to end earlier (as this is has been reported for instance in alcoholic fermentation [2, 28]), which presents potential interests in industry. Even better production could be expected with more than two species, and this could lead to several optimization problems, such as 1. choosing the best sub-set among a set of available species (whose growth characteristics are known) and 2. choosing the best times to add the different species (and not necessarily all of them present at the initial time).

8 Conclusion

In this work, we have investigated mechanisms that could explain a gain in biomass production with mixed cultures of micro-organisms, compared to pure cultures, that we defined as "overyielding". Our main message is that the na-
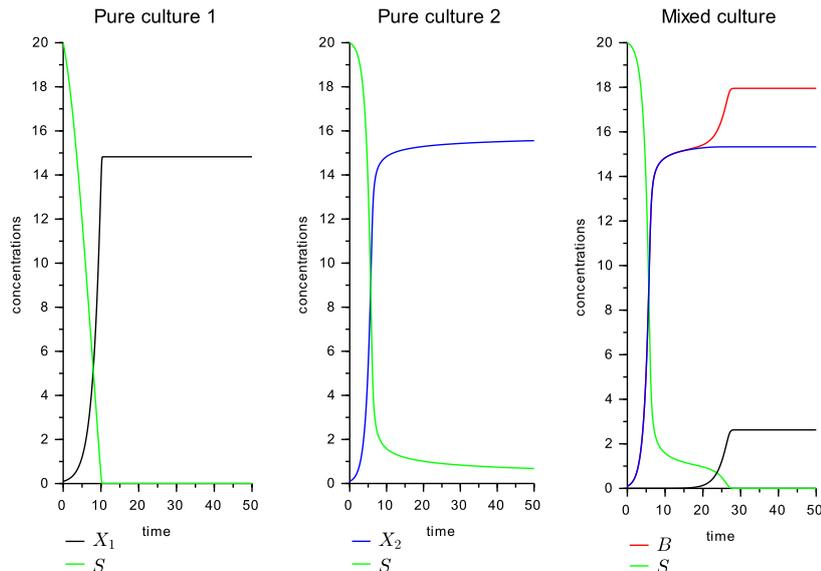
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Fig. 7. From left to right: pure culture of species 1 (X_1), pure culture of species 2 (X_2) and mixed culture ($B = X_1 + X_2$) with initial proportion $X_1(0) = 10^{-4}B_0$. In green the concentration of substrate (S).

ture of the non-linearities of the growth characteristics (specific growth rate functions and yields) is enough to produce overyielding. In particular, there is no need to have direct interaction between species to obtain overyielding. It was first shown that overyielding is not possible with the classical batch models, such as the Monod model with multi-species. To play with complementarity between species, we have to face some additional features. We have exhibited two kinds of situations, with features reported in the literature :

(1) different growth thresholds and (constant) yields (see Proposition 8),
(2) variable yields coupled with conditions on the growth functions (see Proposition 11),

that have been illustrated with numerical simulations. The underlying mechanism is based on the utilization of the resources during the transients, with a succession of stages where each species contributes to its best. Of course, those mechanisms can be added to each other or other ones to obtain overyielding, may be even better. The analysis we performed here allowed to check a potential overyielding from the single knowledge of growth characteristics in pure culture, which can then guide the choices of experiments among the combinatorics of possible mixtures. In this study, we have considered the biomass production criterion because it is often of primer interest in batch cultures. However, for applications for which production speed is also important, the productivity criterion could be considered instead. For other applications such as waste treatments, the quantity of remaining substrate could be a more rel-

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evant criterion. Investigations of overyielding for such other criteria could be the matter of a future work.

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