Revealing characteristics of mixed consortia for azo dye decolorization: Lotka–Volterra model and game theory

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Abstract

This study provides a novel explanation to put forward, in Lotka–Volterra competition model and game theory, interspecific competition in bioaugmentation using constructed mixed consortia for azo dye decolorization. As mixed cultures are regularly used in industrial dye-laden wastewater treatment, understanding species competition of mixed consortia is apparently of great importance to azo dye decolorization. In aerobic growth conditions, *Escherichia coli* DH5α owned a growth advantage to out-compete *Pseudomonas luteola* due to preferential growth rate of DH5α. However, in static decolorization conditions DH5α surrendered some proportion of its advantage (i.e., a decrease in its competitive power for metabolite stimulation) to enhance color removal of *P. luteola* for total coexistence. In aerobic growth, DH5α had its growth advantage to exclude *P. luteola* for dominance (i.e, conflict strategy) according to competitive exclusion principle. In static decolorization conditions, as the removal of a common dye threat was crucial to both species for survival, both species selected cooperation strategy through metabolite stimulation of DH5α to enhance effective decolorization of *P. luteola* for long-term sustainable management. This analysis of game theory clearly unlocked unsolved mysteries in previous studies.
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Keywords: Lotka–Volterra competition model; Interspecific competition; Game theory; Bacterial decolorization

1. Introduction

Azo dyes are the largest chemical class of dyes frequently used in several industries [1,2]. These industrial effluents often contain residual dye, which deteriorates water quality, and very likely become a threat to public health [3]. Albeit not a direct growth substrate to most of bacteria, azo dye can be transformed or degraded via cometabolism of other energy sources by a variety of aerobic and anaerobic bacteria and fungi [4–6]. As mixed cultures are regularly used in dye-containing wastewater treatment, understanding species competition of mixed consortia is apparently of great importance to azo dye decolorization. To demonstrate some typical combined interactions between bacterial species, *Escherichia coli* (a well-characterized and fast-growing species) and *Pseudomonas luteola* (a most effective and predominant strain isolated from indigenous activated sludges for azo decolorization) were selected in experimental designs [7–9]. As pervious studies [7–9] proposed, species interactions (e.g., competition, mutualism) might be crucial to determine optimal microbial community in bioaugmentation for decolorization. In constructed consortia containing non-decolorizer *E. coli* DH5α and decolorizer *P. luteola*, DH5α owns its growth advantage to exclude *P. luteola* for predominance due to competition exclusion principle. In contrast, in static decolorization DH5α seemed to surrender such an advantage via metabolite enhancement to assist color removal of *P. luteola* [8,9]. However, how species competition in mixed consortia directs this metabolic switch for azo dye decolorization till remained uncertain for discussion. Lack of such information on species interactions in microbial communities apparently makes further applications for bioaugmentation unpredictable and unreliable. Thus, this study tended to adopt the Lotka–Volterra's competitive model in ecology to explain why different competitive outcomes (e.g., mono-species predominance or total coexistence) were resulted for stable or unstable operations in treatment of dye-free and dye-laden environments (refer to Appendix A). In addition, this study is anticipated to uncover why mixed cultures regularly used in industrial wastewater treatment can be

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Nomenclature

C_i	cooperation frequency of player i for mixed strategies (i = A, B or 1, 2)
D_i	defeat (conflict) frequency of player i for mixed strategies (i = A, B or 1, 2)
G_i	payoff gain of player i for mixed strategies (i = A, B or 1, 2)
NC	non-cooperation (or defeat, conflict)
Th_i	threshold ratio of frequency of cooperation strategy divided by conflict strategy (i = A, B or 1, 2)

Greek letters

δ_1	payoff gain of DH5α player under its conflict strategy and cooperation of *P. luteola*
δ_2	payoff loss of DH5α player under its cooperation strategy and conflict of *P. luteola*
δ_3	maximal payoff gain of DH5α player if both players choose cooperation
Δ_1	payoff loss of *P. luteola* player under its cooperation strategy and conflict of DH5α
Δ_2	payoff gain of *P. luteola* player under its conflict strategy and cooperation of DH5α
Δ_3	maximal payoff gain of *P. luteola* player if both players cooperate

stably maintained for long-term operation. As known, there are extreme difficulties to uncover species competition in multiple-species cultures (e.g., practical activated sludge); that was why the binary-species systems were selected for this modeling. Although using only two-species for system analysis is impractical to practices in wastewater treatment, such an approach can specifically separate what the outcomes to be evolved and the possible characteristics on individual species in on-site system are about. In addition, this study also conveyed several typical points for on-site professionals to deal with mixed consortia in wastewater treatment. Unlocking mysteries of mixed cultures is of course with practical values for waste minimization in industry. Although this study only chose an example of azo dye decolorization for demonstration, its novelty is to propose that the evolution of species competition is strongly dependent on the existing toxic and/or inhibitory sources. As different expression systems were switched on or off in specific microorganism in face of diverse environments (e.g., hostile conditions), such metabolic changes of individual species might lead to alterations in combined interactions between species (e.g., competition or mutualism) and in the same species in the whole population.

Competition occurs when organisms of the same species or of different species utilize common resources that are in short supply. Competition may be interspecific (between two or more different species) or intraspecific (between individuals of the same species). Interspecific competition is defined here as a mutually negative (−/−) interaction between two or more species in the culture environment. Negative interactions man-

ifest themselves as reduced population size, decreased growth rate and/or survivorship of species. According to Schoener's classification of species interactions [10], the mechanisms of industrial azo dye decolorization using mixed consortia can be (1) consumptive competition: one species (e.g., DH5α) inhibits another species (e.g., *P. luteola*) by consuming shared nutrient sources; (2) chemical stimulation: metabolites of some species (e.g., DH5α) are expressed as stimulators to enhance color removal and pollutant detoxification for total survival [7–9]. In dye-absent cultures, apparently DH5α owns the advantage of consumptive competition (i.e., higher maximal growth rate) on the culture media to inhibit *P. luteola* (i.e., DH5α tended to be predominated in cultures). In contrast, in dye-laden environments, DH5α assists its partner species—*P. luteola* to implement decolorization more efficiently for two-species coexistence (i.e., both species tended to be equally distributed) as both species are under a common threat of dye pollutant. Here, Lotka–Volterra's model of competition and game theory were used to reveal how the communities with two-species function differently in various environments for evolution in competition. Assume that Lotka–Volterra's two-species competitive model using extensions of the logistic equation is feasible to describe deterministically transient dynamics of two-species as follows [10,11]:

$$\frac{dN_1}{dt} = r_1 N_1 \left(\frac{K_1 - N_1 - \alpha_{12} N_2}{K_1} \right) = f(N_1, N_2), \qquad (1)$$

$$\frac{dN_2}{dt} = r_2 N_2 \left(\frac{K_2 - N_2 - \alpha_{21} N_1}{K_2} \right) = g(N_1, N_2), \qquad (2)$$

where r_i is intrinsic rate of increase (or specific growth rate) of species i, competive coefficient, α_{ij}, denotes a measure of the effect of species j on the growth of species i (α_{ij} = 1 indicated equal effect in depressing the growth of species i), K_i is the carrying capacity of species i. The parameter α_{ij} is also a measure of relative importance per individual of interspecific and intraspecific competition. The cases of $\alpha_{ij} > 1$ and $\alpha_{ij} < 1$ simply imply that the per capita effect of interspecific competition of species j on species i is greater and less than the per capita effect of intraspecific competition of species j, respectively. That is, if $\alpha_{21} < 1$, the intraspecific growth of species 1 is repressed more by the addition of an individual of N_1 than by addition of an individual of species 2. As indicated in equations (1) and (2), there are four equilibrium population densities present in the mixed cultures by setting the differential equations (1) and (2) equal to zero and solving for N (i.e., (0, 0), (K_1, 0), (0, K_2), [($K_2\alpha_{12} - K_1$)/($\alpha_{12}\alpha_{21} - 1$), ($K_1\alpha_{21} - K_2$)/($\alpha_{12}\alpha_{21} - 1$)]) [11]. Note that the equilibrium point (0, 0) is a trivial and unstable point without practicality to be considered for system analysis. To get a grasp of the transient dynamics of competitive interactions and perceive which equilibrium point is stably or unstably achieved, the analysis of phase-plane graph was used [10,11]. In the phase-plane graph (Fig. 1), the x-axis and y-axis represent the abundance of species 1 and 2, respectively. By setting Eq. (1) to be zero, a linear isocline for species 1 can be obtained. The isocline defines the combination of abundances for which species 1 shows zero growth. For points to
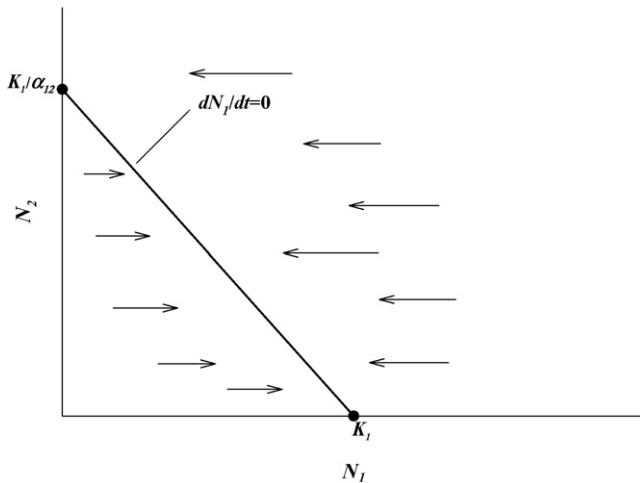
Fig. 1. Linear isocline for species 1 in the Lotka–Volterra competition model. For points to the left of this line, the population of species 1 tends to increase (i.e., right-pointing arrows). For the points to the right of this line, its population decreases (i.e., left-pointing arrows).

the left of this line (i.e., $K_1 - N_1 - \alpha_{12}N_2 > 0$; Fig. 1), the population of species 1 increases, indicated by the right-pointing horizontal arrows. In contrast, for points to the right of this line (i.e., $K_1 - N_1 - \alpha_{12}N_2 < 0$), the population of species 1 decreases (i.e., the right-pointing arrows). Similarly, the isocline for species 2 can be obtained by setting $dN_2/dt = 0$ (i.e., $K_2 - N_2 - \alpha_{21}N_1 = 0$ in Eq. (2); Fig. 2). The downward-pointing and upward-pointing vertical arrows for species 2 denoted the population of species 2 decreases (at points above isocline) and increases (at points below isocline), respectively. By combined use of two isoclines in the phase-plane graph, four patterns of possible outcomes of competition in the Lotka–Volterra equation can be found (Fig. 3(a)–(d)). By solving $dN_1/dt = 0 = dN_2/dt$ in equations (1) and (2) at equilibrium, the arrows in these four possible geometric configurations could be determined by vector addition (Fig. 3(a)–(d)). Several crucial points for the competi-

tive principle could be obtained from these configurations. First, there is an equilibrium of two-species if the diagonal curves cross each other. As indicated in Fig. 3(a) and (b), there is no equilibrium as one species tends to increase its population in which the second species must decrease (i.e., complete competitive exclusion for one-species predominance). Second, if diagonal lines cross, the equilibrium point may be either stable (i.e., all vectors are directed toward the equilibrium point; Fig. 3(c) or unstable (i.e., all vectors are directed away from the equilibrium point; Fig. 3(d)). Using these specific characteristics of the equilibrium points, we could explain unsolved results shown in previous studies [7–9].

From static game theorist perspectives, metabolite expression of DH5α and effective decolorization activity of *P. luteola* was found as a cooperative strategy in the evolution of mixed consortia during decolorization. This approach in game theory is useful in finding plausible answers to mysteries of species evolution not completely uncovered in previous studies [7,8]. Game theory was first applied in economics to determine what rational "individuals" (viz. groups or coalitions) should do in playing a game to attain different outcomes (or payoffs) in the presence of interactions (e.g., competition (conflict) or mutualism (cooperation)). Axelrod [12] mentioned from the perspectives of microbiology and biochemistry that "bacteria have a basic capacity to play games". For example, Chen [13] mentioned that due to host-range mutation between bacteriophage λ and *E. coli*, interactions of competitive exclusion tended to be in neutralism (i.e., mutation from virulent λ (conflict) to avirulent λ (cooperation)). Chen [14] indicated that virulence reduction of bacterial virus λ to *E. coli* is crucial to coexistence for sake of total survival. Although prior studies [7,8] showed that DH5α behaved metabolically dormant to dye decolorization, its metabolites apparently enhanced color removal performance of *P. luteola* [8,15]. In the dye-free environment, DH5α tended to be dominated according to competition exclusion principle. In contrast, in the presence of dye threat DH5α surrendered its growth advantage and expressed metabolites to synergistically enhance dye decolorization performance of *P. luteola*. Thus, mixed consortia tended to keep cultures free of competition and/or threat for a "win-win situation" in survival.

2. Results and discussion

2.1. Analysis of competitive exclusion

To grasp competitive interactions of bacterial species, the analysis of Lotka–Volterra's equations was used [11]. Considering algebraic solutions to the Lotka–Volterra equations, we may obtain the stability criteria for the persistence of individual species. Obviously, if species 1 does not go extinct at the worst situation, it can always persist. Under this critical circumstance, if species 1 almost dies out (i.e., $N_1 \cong 0$) and the abundance of its competitor N_2 is near carrying capacity (i.e., $N_2 \cong K_2$ or a maximal competitive pressure to species 1), N_1 still has a positive specific growth (i.e., $(dN_1/dt)(1/N_1) > 0$). Apparently, species 1 will then persist for all conditions. That is, Eq.
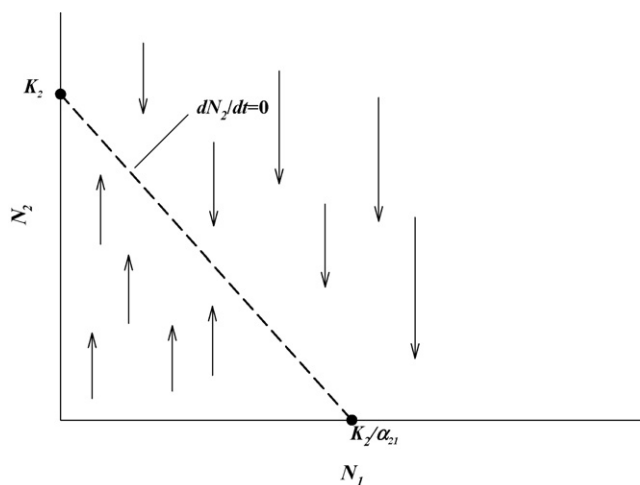

Fig. 2. Linear isocline for species 2 in the Lotka–Volterra competition model. For points below this line, the population of species 2 tends to increase (i.e., upward-pointing arrows). For the points above this line, its population decreases (i.e., downward-pointing arrows).
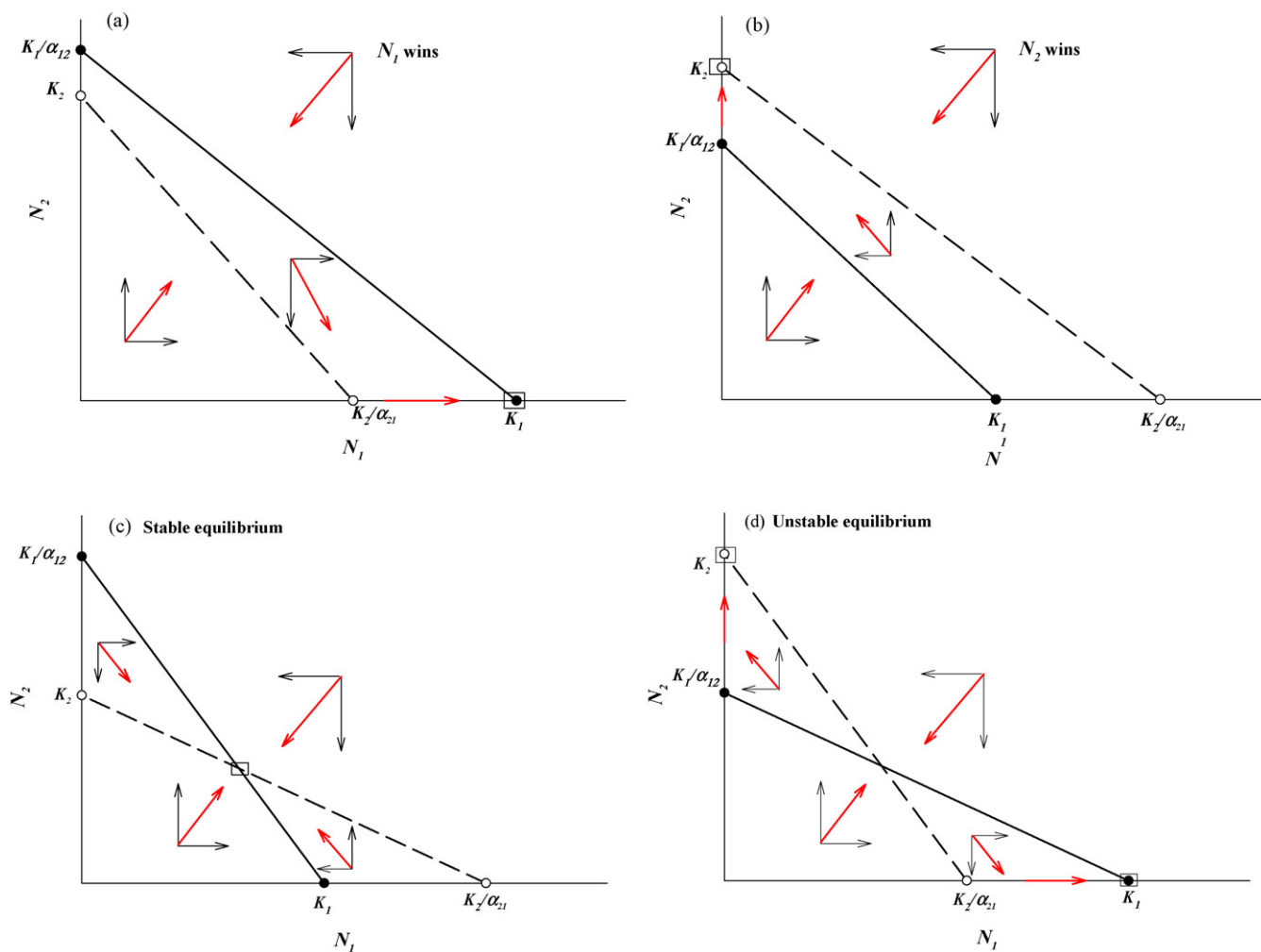
Fig. 3. Case (a): competitive exclusion of species 2 by species 1. Thin arrows denote the trajectories of each population. Red arrows indicate the trajectories of joint vector of both populations. The outcome of this competition is an equilibrium for species 1 at carrying capacity K_1. Case (b): competitive exclusion of species 1 by species 2. Thin arrows denote the trajectories of each population. Red arrows indicate the trajectories of joint vector of populations. The outcome of this competition is an equilibrium for species 2 at carrying capacity K_2. Case (c): coexistence in a stable equilibrium. These two isoclines cross, and all the joint vectors tend to stabilize toward the equilibrium point. Case (d): competitive exclusion in an unstable equilibrium. Two isoclines also cross and form an equilibrium point. However, all the joint vectors point away from this unstable equilibrium, leading to stabilize at an equilibrium at carrying capacity for species 1 or 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article or [11].)

(1) can be rewritten as

$$\left(\frac{dN_1}{dt}\right)\left(\frac{1}{N_1}\right) \cong r_1\left(\frac{K_1 - 0 - \alpha_{12}K_2}{K_1}\right). \qquad (1')$$

Therefore, the inequality $((K_1 - \alpha_{12}K_2)/K_1) > 0$ (i.e., $K_1/K_2 > \alpha_{12}$) must hold to augment for the persistence of N_1, as r_1 is always positive. It implies that if species 1 can successfully "invade" in the community, the ratio of the carrying capacities must exceed the competitive effect of species 2 on species 1. Similarly, using Eq. (2), we may also find the inequality for the persistence of species 2: $K_2/K_1 > \alpha_{21}$. Thus, with the expressions for whether N_1 will persist (i.e., $K_1/K_2 > \alpha_{12}$) or not persist (i.e., $K_1/K_2 < \alpha_{12}$) and whether N_2 will persist (i.e., $K_1/K_2 < 1/\alpha_{21}$) or not persist (i.e., $K_1/K_2 > 1/\alpha_{21}$), we can evaluate on species competition for azo dye decolorization. In aerobic growth, DH5α (species 1; N_1) wins for its growth

advantage, but *P. luteola* (species 2; N_2) cannot persist [7–9]. This implies that the condition of $(1/\alpha_{21}) < K_1/K_2 > \alpha_{12}$ must hold. That is, "complete competitors could not coexist" (i.e., competition exclusion) as both species exploited the same limiting nutrients in the culture. But, in static decolorization, both species are able to stably persist for total coexistence (i.e., $(1/\alpha_{21}) > K_1/K_2 > \alpha_{12}$). This analysis clearly concluded that for total survival in a hostile environment containing hazardous azo dye, species 1 surrendered its growth advantage (i.e., a decrease in the competitive power). Due to this marked decrease in the competitive effect of species 1 on species 2, α_{21}, DH5α bypassed portion of energy to express metabolic stimulators for a significant enhancement of color removal of *P. luteola* (i.e., DH5α yielded its growth advantage to its competitor). In addition, it was suspected that *P. luteola* might also release "signal factors" to provoke DH5α for metabolite

stimulation. Thus, the Lotka–Volterra's competitive equations (1) and (2) could be modified as the following [10,11]. In static decolorization, the growth rate of species 2 is significantly increased by $\delta N_1 N_2$. Introduce this combined interaction to N_2 in which the rate at which two-species N_1 and N_2 interact is proportional to the product $N_1 N_2$. That is, for clarification the following equation should be amended to explain why metabolite stimulation of DH5α could protect species 2 from extinction.

$$\frac{dN_2}{dt} = r_2 N_2 \left(\frac{K_2 - N_2 - \alpha_{21} N_1}{K_2} \right) + \delta N_1 N_2. \tag{2'}$$

For the sake of survival of both species in such a hostile environment, metabolic changes of both species for efficient decolorization and toxicity reduction are inevitable. In summary, DH5α owned the competitive predominance in dye-free cultures, but apparently yielded its growth advantage to *P. luteola* in the dye-bearing cultures, in particular for static decolorization conditions [7,8].

2.2. Assessment of game theory

Evidently, characteristics of competitive interactions between species can also be explained by game theory. Previous studies [7–9] indicated that in dye-absent cultures, DH5α owned its growth advantages (e.g., higher growth rate) over *P. luteola*, showing its competitive dominance for the persistence. That is, DH5α tended to select a conflict strategy in face of arms race of two players (i.e., DH5α and *P. luteola*) for its predominance. In contrast, in toxic azo dye-laden environments, either player has to seek for the maximal benefits of compromise (i.e., total survival) as a cooperative strategy since *P. luteola* is the sole dye decolorizer to remove dye threat effectively for survival. Note that in aerobic conditions, dissolved oxygen can repress activities of azo reductase and/or non-specific enzymes for color removal in *P. luteola*. Thus, azo dye decolorization could only carry out at dissolved oxygen-depleted environments (e.g., cultures of static decolorization). Evidently, if both players chose cooperation strategy in conditions of static decolorization, there was a marked rise in color removal for total survival due to metabolite enhancement of DH5α to decolorization of *P. luteola*. Thus, this cooperation situation was stably maintained in mixed cultures for dye decolorization [7,8]. In fact, according to Axelord and Hamilton [16] if two bacteria have the continuous contact (e.g., mixed consortia in closed systems like batch reactors or shake-flask cultures) and each organism can benefit from mutual cooperation, then each one can also do even better by exploiting the cooperative efforts of others. In contrast, if "two individuals" destined never to meet again (e.g., mixed cultures in continuous flow systems like CSTR and rivers), the strategy of defeat or total conflict is apparently the only solution to the game. That is, for a short-term relationship each one will choose a "non-cooperative move", as there is no way for them to threaten each other. But, all the individual species in mixed cultures of a closed system must "meet more than once" [12]. Therefore, for a long-term sustainable management, both players will choose to reach a symbiotic relationship instead of competitive one for coexistence; in particular, in face of dye pollutant as a common threat for survival.

However, how can both species of seemingly conflicting interests be reconciled? To understand why a cooperative relationship (e.g., total coexistence in an almost competition-free mixed consortia; [7,8]) was resulted, this strategic game could be quantitatively evaluated by introduction of average payoff to the player 1 (DH5α) and player 2 (*P. luteola*) as G_1 and G_2, respectively [17]. Let C_1 and C_2 stand for mixed strategies in which the strategies so assigned are selected with frequencies C_1 and C_2 ($0 \le C_1 \le 1; 0 \le C_2 \le 1$) for cooperation. In addition, the payoffs of players are defined as (a) Δ_i and δ_i ($i = 1, 2, 3$) > 0, where $\delta_3 > \delta_1$ and $\Delta_3 > \Delta_2$ (player 1 = DH5α, player 2 = *P. luteola*); (b) *E. coli* DH5α loses a payoff δ_2, but *P. luteola* gains a payoff Δ_2 due to metabolite enhancement. In contrast, if *E. coli* DH5α gains a payoff δ_1, *P. luteola* loses a payoff Δ_1 (refer to [18] for payoff gain and loss). The conflict-and-cooperation game for *E. coli/P. luteola* impasse may be regarded as a "non-zero-sum two-player game" in the normal-form strategic game matrix as follows [18]:

E. coli DH5α side (1)	*P. luteola* side (2)	
	Cooperation(C2) (effective decolorization)	Conflict(NC2) (basal decolorization)
Cooperation(C1) (metabolite enhancement)	(δ_3, Δ_3)	$(-\delta_2, \Delta_2)$
Conflict(NC1) (no metabolite enhancement)	$(\delta_1, -\Delta_1)$	$(0, 0)$

Then the defeat frequency $D_i = 1 - C_i$ ($i = 1, 2$) and the payoffs G_i ($i = 1, 2$)

$$G_1 = C_1 C_2 \delta_3 + C_1 (1 - C_2)(-\delta_2) + (1 - C_1) C_2 \delta_1, \tag{3}$$

$$G_2 = C_1 C_2 \Delta_3 + C_1 (1 - C_2) \Delta_2 + (1 - C_1) C_2 (-\Delta_1). \tag{4}$$

One may obtain partial differential terms for equations (3) and (4) as follows:

$$\frac{\partial G_1}{\partial C_1} = C_2 \delta_3 - (1 - C_2)\delta_2 - C_2 \delta_1$$
$$= C_2(\delta_3 - \delta_1) - (1 - C_2)\delta_2, \tag{5}$$

$$\frac{\partial G_1}{\partial C_2} = C_1 \delta_3 - C_1(-\delta_2) + (1 - C_1)\delta_1$$
$$= C_1(\delta_2 + \delta_3 - \delta_1) + \delta_1, \tag{6}$$

$$\frac{\partial G_2}{\partial C_1} = C_2 \Delta_3 + (1 - C_2)\Delta_2 + C_2 \Delta_1$$
$$= C_2(\Delta_3 - \Delta_2 + \Delta_1) + \Delta_2, \tag{7}$$

$$\frac{\partial G_2}{\partial C_2} = C_1 \Delta_3 - C_1 \Delta_2 - (1 - C_1)\Delta_1$$
$$= C_1(\Delta_3 - \Delta_2) - (1 - C_1)\Delta_1. \tag{8}$$

As shown in equations (6) and (7) for all C_1 and $C_2 \in [0, 1]$, the relationships $\partial G_2/\partial C_1 > 0$ and $\partial G_1/\partial C_2 > 0$ hold, indicating that the payoff of one player will increase if another player tends to be more cooperative. Eq. (5) also states that if relative ratio of frequency of cooperation to conflict strategy for player 2 (*P. luteola*) is greater than the payoff loss δ_2 at the player 2's non-cooperative strategy divided by the payoff gain $(\delta_3 - \delta_1)$ at player 2's cooperation (i.e., $C_2/D_2 = C_2/(1 - C_2) > \delta_2/(\delta_3 - \delta_1) = \mathrm{Th}_2$), player 1 DH5α will gain payoff (i.e., $\partial G_1/\partial C_1 > 0$) by the cooperation of its own. In addition, Eq. (8) indicates that if relative ratio of frequency of cooperation to conflict strategy for player 1 (DH5α) is greater than the payoff gain Δ_1 at player 1's conflict strategy divided by the payoff gain $(\Delta_3 - \Delta_2)$ at player 1's cooperative strategy (i.e., $C_1/D_1 = C_1/(1 - C_1) > \Delta_1/(\Delta_3 - \Delta_2) = \mathrm{Th}_1$), player 2 *P. luteola* will gain payoff (i.e., $\partial G_2/\partial C_2 > 0$) by the cooperation of his own. In batch cultures, both players of course met each other more than once, thus inherited generations of DH5α and *P. luteola* progressively perceived marked rises in payoffs (e.g., phenotypic selection for significant enzyme expression in dye resistance) for their survival if both populations were inclined to be more cooperative. Thus, the frequencies of cooperation C_1 and C_2 for both players would gradually increase as time went by. According to game theory, once the ratio C_i/D_i ($i = 1, 2$) exceeded the threshold value Th_i, the agreement of total cooperation strategy for both sides would be achieved since no one tended to deviate this "best" situation for propagation. In biochemical terms, significant increases in the ratio C_i/D_i might simply explain that metabolite stimulation of DH5α (i.e., phenotypic expression) assisted *P. luteola* for effective decolorization as indicated previously [7,8]. The existence of the threshold level Th_i might simply suggest the presence of a critical dye concentration to switch species interactions from total conflict toward total cooperation. At dye concentration above this threshold level, DH5α has to give up its competitive exclusion strategy to accept a strategy of metabolite stimulation for survival. Thus, this game-theoretic model explained that in dye-absent cultures both sides would selected total conflict strategy (i.e., growth predominance via competition) for their own survival. In contrast, in the dye-laden cultures both players would choose a cooperation strategy of their own for long-term sustainable management. Therefore, this game-theoretic model clearly unlocks mysteries unsolved in previous works [7–9].

3. Conclusion

In aerobic conditions, *P. luteola* would be restricted for propagation due to its relatively lower growth rate than DH5α. In contrast, in static decolorization conditions DH5α reduced its competitive power (i.e., decrease in α_{21}) to assist *P. luteola* for color removal, total coexistence of both organisms was experimentally resulted [7–9]. Game theory indicated that even competing species in mixed consortia might cooperate to be altruistic because of "reciprocity" for survival in the life-threatening environments [19,20]. This study used an unstructured modeling on species competition in biosystems for azo dye decolorization; however, there are some mysteries still needed to be unlocked in the follow up studies. For example, azo dye decolorization is not growth associated; that is, *P. luteola* should not be young in age to conduct color removal (only growing cells are young-aged). Thus, part of metabolic changes (e.g., metabolite stimulation or repression) during species competition might be age-dependent and growth phase-related and whether such events are directly correlated to species competition is remained to be discussed.

Acknowledgments

Appendix A. Stability criteria for the Lotka–Volterra competition model [21]

Let $\underline{X}_1^{\mathrm{T}} = (N_1^*, N_2^*)^{\mathrm{T}}$ be an equilibrium point of the plane autonomous system $\underline{X}' = \underline{G}(\underline{X}) = (f(N_1, N_2), g(N_1, N_2))^{\mathrm{T}}$, where $f(N_1, N_2)$ and $g(N_1, N_2)$ have continuous first partials in a neighborhood of $\underline{X}_1$. The original system $\underline{X}' = \underline{G}(\underline{X})^{\mathrm{T}}$ may be approximated in a neighborhood of the equilibrium point $\underline{X}_1$ by the linear system $\underline{X}' = \underline{A}(\underline{X} - \underline{X}_1)$, where

$$\underline{A} = \begin{pmatrix} \dfrac{\partial f}{\partial N_1}\bigg|_{(N_1^*, N_2^*)} & \dfrac{\partial f}{\partial N_2}\bigg|_{(N_1^*, N_2^*)} \\ \dfrac{\partial g}{\partial N_1}\bigg|_{(N_1^*, N_2^*)} & \dfrac{\partial g}{\partial N_2}\bigg|_{(N_1^*, N_2^*)} \end{pmatrix}.$$

Therefore, the significance of Fig. 3 can be termed as follows:

(a) If the eigenvalues of $\underline{A} = \underline{G}'(\underline{X}_1)$ have negative real part, then $\underline{X}_1$ is an asymptotically stable equilibrium point (i.e., stabilized toward $\underline{X}_1$).
(b) If $\underline{A} = \underline{G}'(\underline{X}_1)$ has an eigenvalue with positive real part, then $\underline{X}_1$ is an unstable equilibrium point (i.e., instabilized outward $\underline{X}_1$).

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