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Cultivation of *Escherichia coli* with Mixtures of 3-Phenylpropionic Acid and Glucose: Steady-State Growth Kinetics

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The fate of pollutants in the environment is affected by the presence of easily degradable carbon sources. As a step towards understanding these complex interactions, a model system was explored: the degradation of mixtures of glucose (i.e., an easily degradable substrate) and 3-phenylpropionic acid (3ppa) (a model pollutant) by *Escherichia coli* ML 30 was studied systematically in carbon-limited continuous culture. The two substrates were always consumed simultaneously regardless of the dilution rate applied. Even at dilution rates higher than the maximum specific growth rate for 3ppa ($0.35 \pm 0.05\ h^{-1}$), the two carbon substrates were utilized together. When cells were grown at a constant dilution rate with different mixtures of 3ppa and glucose, in which 3ppa contributed between 5 and 90% of carbon substrate in the feed medium, the steady-state concentrations of 3ppa and glucose were approximately proportional to the ratio of the two substrates in the feed medium. When cells were cultivated at different dilution rates with a 1:1 mixture (based on carbon) of glucose and 3ppa, an overall maximum specific growth rate of $0.90 \pm 0.05\ h^{-1}$ and a Monod substrate saturation constant for 3ppa (K_s) of 600 to 700 $\mu g\ liter^{-1}$, similar to that measured during growth with 3ppa alone, fitted the experimentally determined steady-state 3ppa concentrations. However, due to the highly differing substrate affinity constants for 3ppa and glucose ($K_s \sim 30$ to 70 $\mu g\ liter^{-1}$), the total steady-state carbon concentration in the culture at a constant dilution rate was determined mainly by the steady-state 3ppa carbon concentration, and it increased with increasing proportions of 3ppa in the feed medium.

The development of descriptive growth models in microbiology is based on the assumption that the observed phenomena are perfectly predictable and reproducible. This might be true for growth kinetics such as those proposed by Monod (20), which were derived from laboratory experiments with a pure culture and a single growth-limiting substrate). However, in natural environments, because of the complexity of nutritional and physical conditions (21), microbial growth and substrate utilization are still poorly understood in quantitative terms (e.g., see references 3 and 23). It has been postulated that it is highly likely that under environmental conditions, where growth is mostly carbon limited (e.g., see references 5 and 11), microorganisms utilize a variety of carbon and energy substrates simultaneously (a phenomenon referred to as mixed-substrate growth). Nevertheless, few attempts to elucidate the main kinetic principles of mixed-substrate growth have been made, especially for mixtures of pollutants plus easily degradable carbon substrates.

Over the past two decades, increasing information has become available in the literature on heterotrophic bacteria utilizing mixtures of substrates simultaneously in carbon-limited continuous cultures. Typically, mixed-substrate utilization resulted in steady-state concentrations of individual substrates which were lower than those observed during growth with the single substrates (1, 7, 8, 16, 19). Unfortunately, in most of these publications the steady-state concentration of only one of the carbon compounds used in the mixture has been reported, usually because the analytical methods were not sensitive enough to detect the additional substrates also. Recently, an improved method for the analysis of reducing sugars (24) enabled us to perform more-detailed investigations. The most extensively studied example is the growth of *Escherichia coli* in carbon-limited chemostat cultures with defined mixtures of up to six different sugars (18). The experimental results presented by Lendenmann and coworkers (18) allowed proposal of a tentative model suggesting that during growth at a constant rate, the observed steady-state concentration of a particular sugar was proportional to its contribution to the total sugar concentration in the feed medium. However, the catabolic pathways of all the sugars used in the work described above converge after a few metabolic steps. Therefore, further experiments with mixtures of substrates that differ with respect to their chemical structure, carbon content, degree of carbon reduction, metabolic pathways involved in their degradation, or the physiological function they fulfil are necessary to elucidate whether a more generally applicable principle of mixed-substrate growth kinetics exists.

To investigate the validity of the simple model of mixed-substrate growth proposed by Lendenmann and coworkers (18), we set up an experimental system that fulfilled some of the requirements mentioned above. This system consisted of *E. coli* ML 30 growing with glucose and 3-phenylpropionic acid (3ppa) as the only sources of carbon and energy. In continuous culture, the concentrations of both substrates could be conveniently measured down to a few micrograms per liter. In parallel studies, both the dynamics of growth with mixtures of 3ppa and glucose (14) and the inducibility of the 3ppa-degrading system have been investigated in more detail (13). (Table 1 summarizes the nomenclature used throughout.)

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MATERIALS AND METHODS

Medium and culture conditions. *E. coli* ML 30 (DSM 1329) was grown at 37°C in mineral medium (24) supplemented with glucose and/or 3ppa as the sole sources of carbon and energy. In the bioreactors (MBR, Wetzikon, Switzerland

TABLE 1. Nomenclature

Term	Definition	Unit
D	Dilution rate (specific growth rate in chemostat)	h^{-1}
DW	Dry weight	mg liter^{-1}
K_s	Substrate saturation constant	μg liter^{-1}
R	Ratio as defined by equations 5 and 5a	
s, s_i	Steady-state substrate concentration, steady-state concentration of substrate i	μg liter^{-1}
s_0	Substrate concentration in feed medium	mg liter^{-1}
$s_{0,i}$	Substrate concentration in feed medium with respect to substrate i	mg liter^{-1}
$s_{100\%,i}$	Steady-state substrate concentration during single-substrate growth with substrate i	μg liter^{-1}
$q_{s,i}$	Specific substrate consumption rate with respect to substrate i	mg (mg [DW] h)$^{-1}$
q_{tot}	Total specific consumption rate	mg (mg [DW] h)$^{-1}$
q_{max}	Maximum specific consumption rate	mg (mg [DW] h)$^{-1}$
Y	Yield coefficient	mg DW (mg of C)$^{-1}$
Y_i	Yield coefficient with respect to particular substrate i	mg DW (mg of C)$^{-1}$
μ	Specific growth rate	h^{-1}
μ_{max}	Maximum specific growth rate	h^{-1}
μ_{3ppa}, μ_{glc}	Specific growth rate contribution of 3ppa or glucose, respectively	h^{-1}

[working volume, 1.5 liter], and Bioengineering, Wald (ZH), Switzerland [working volume, 1 liter]), the pH was maintained at 7 ± 0.05 and oxygen saturation was always >90% air saturation. The cultivation conditions have been described in detail elsewhere (24).

Glucose analysis. Glucose concentrations were analyzed by high-pressure liquid chromatography as described by Senn et al. (24).

3ppa analysis. The high-pressure liquid chromatography method for the analysis of 3ppa was described elsewhere (14).

Biomass determination. Biomass was measured as DW and/or optical density at 560 nm. Details are given by Kovárová et al. (14).

Specific substrate consumption rates. The excess glucose or 3ppa consumption capacity (given in milligrams of substrate per milligram [DW] per hour) of the cells grown in continuous culture was measured immediately after the cells were withdrawn from the bioreactor by a modified method (17) originally reported by Neijssel and coworkers (22). An excess noninhibiting concentration of substrates (5 mg liter^{-1}) was used in these experiments. The precision of such measurements was in the range of ±0.15 mg of substrate (mg [DW] h)$^{-1}$. Additionally, the actual specific consumption rate was also calculated by using equation 1. The directly measured and calculated data were compared (e.g., see Fig. 4).

$$q_{s,i} = \frac{(s_i - s_0)}{\mathrm{DW}} \cdot D \tag{1}$$

Data analysis. (i) Monod growth kinetics. The relationship between substrate concentration and the specific growth rate was described by the conventional growth kinetic model proposed by Monod (20) (equation 2):

$$\mu = \mu_{max} \cdot \frac{s}{K_s + s} \tag{2}$$

The models were fitted to the experimental data by nonlinear parameter estimation (NPE) (for details, see reference 15).

(ii) Specific growth rate during mixed-substrate utilization. The consumption of carbon and energy substrates (equations 1 and 3) supports a particular specific growth rate:

$$\mu = f(q_i, Y_i) \tag{3}$$

This μ can be achieved by utilizing either one substrate at a high rate ($q_i = q_{tot}$) or several substrates simultaneously ($q_{tot} = q_1 + q_2 \ldots + q_i$) at reduced rates with respect to the individual substrates (here, glucose and 3ppa). Assuming additive behavior, this can be described in the simplest way by equation 4:

$$\mu = \mu_{glc} + \mu_{3ppa} \cong q_{glc} \cdot Y_{glc} + q_{3ppa} \cdot Y_{3ppa} \tag{4}$$

(iii) Steady-state concentration as a function of the mixture composition. An empirical model (equation 5; for its theoretical foundation, see references 8 and 18) was used for the description of the relationship between the steady-state substrate concentrations and the corresponding proportions of these substrates in the inflowing medium. In this model, the steady-state substrate concentration is a function of the specific rates of consumption of these substrates and, implicitly, of their proportions in the feed medium.

$$s_i = s_{100\%} \cdot \frac{q_i}{\Sigma q_i} \cong s_{100\%} \cdot \frac{s_{0,i}}{\Sigma s_{0,i}} \tag{5}$$

These proportions can be expressed in different ways and not only by the weight contributions of individual substrates (see Fig. 2a). Therefore, equation 5 can be rewritten in a more general form (equation 5a), namely, showing that when mixtures of substrates are utilized, the steady-state concentration of the particular substrate can be predicted from the medium composition (via the ratio R) and the steady-state substrate concentration during single-substrate growth.

$$s_i = s_{100\%} \cdot R \tag{5a}$$

RESULTS

Relationship between steady-state substrate concentration and specific growth rate. The growth kinetics of *E. coli* during growth with either 3ppa alone or a mixture of 3ppa and glucose (1:1, based on carbon concentration) was studied in carbon-limited continuous culture at different growth rates. With 3ppa as the sole carbon and energy source, *E. coli* was able to grow up to a dilution rate (specific growth rate) of 0.35 ± 0.05 h^{-1}. The substrate affinity constant (K_s) fitted by NPE was 600 to 700 μg liter^{-1} (Fig. 1a; Table 2). No statistically significant difference was obtained between fits of K_s within this range. (Note that previously the growth kinetics of *E. coli* on glucose as the sole energy and carbon substrate has been systematically studied by Senn et al. [24] and Kovárová et al. [15].)

In contrast to the case for batch cultivation (14), the two substrates were consumed simultaneously in continuous culture, independent of the dilution rate applied (range tested, 0.15 $h^{-1} \leq D \leq 0.6$ h^{-1}). The steady-state concentrations of both 3ppa and glucose were reduced in comparison to the concentrations measured during single-substrate growth (Fig. 1a and 2). The formation of the biomass from the two substrates occurred in an additive manner, which suggests that the growth yields did not change during the growth with mixtures in comparison to growth with single substrates (14) (Table 2). When cells were grown with a 1:1 mixture of glucose and 3ppa (based on carbon), a growth rate of 0.90 ± 0.05 h^{-1} was fitted by NPE to the experimentally determined steady-state 3ppa concentrations. The fitted Monod substrate saturation constant for 3ppa was 600 to 700 μg liter^{-1} and hence similar to that measured during growth with 3ppa alone (Fig. 1a). The *s*-versus-*D* relationships for growth with 3ppa, glucose, and a 1:1 (C:C) mixture of glucose and 3ppa are compared in Fig. 1a, and the kinetic parameters are collected in Table 2.

The specific consumption rates for 3ppa and glucose during growth at different dilution rates are given in Fig. 1b. In this case, a particular specific rate of carbon substrate consumption (q_{tot}) supports a corresponding specific growth rate (μ = D).

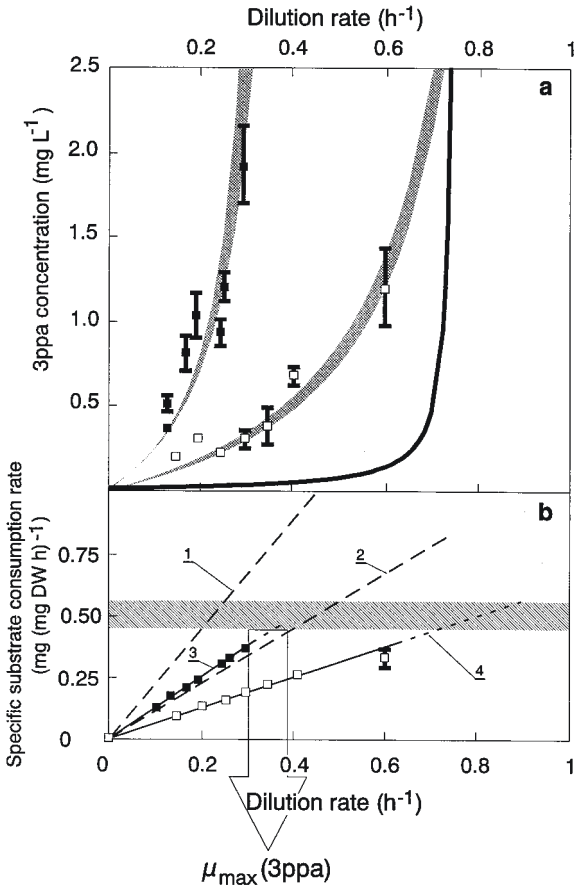

FIG. 1. (a) Steady-state concentrations of 3ppa during growth of *E. coli* in carbon-limited continuous culture with 3ppa only (■) and with a 1:1 (C:C) mixture of 3ppa (□) and glucose, as a function of dilution rate. Each datum point is the average of 4 to 10 single steady-state measurements. Predicted steady-state substrate concentrations based on kinetic data are given in Table 2 (shaded areas) and, for comparison, the steady-state concentration for growth of *E. coli* with glucose alone (heavy curve, from reference 14) are also shown. (b) Specific substrate consumption rates for 3ppa when cells were cultivated with 3ppa only or fed with a mixture of glucose and 3ppa (1:1; C:C) (lines 3 and 4, ■ and □, respectively) and for glucose when cells were cultivated with glucose only or fed with a mixture of glucose and 3ppa (1:1; C:C) (lines 1 and 2, respectively). The range of the maximum specific consumption rate for 3ppa is indicated (shaded area).

This can be achieved either by consuming one substrate at a high rate or by consuming several substrates at lower rates (for formulas, see Materials and Methods). For growth with glucose only, 3ppa only, or a 1:1 mixture of the two, the specific consumption rates increased linearly with increasing growth rates; however, the slope was steeper when *E. coli* was grown with single substrates (Fig. 1b). Hence, when the bacterium was supplied with a 3ppa-glucose mixture 3ppa was utilized well above the maximum specific growth rate with 3ppa alone, i.e., 0.35 ± 0.05 h^{-1}. This was probably because the cells growing with the mixture achieved the maximum possible specific consumption rate for 3ppa, 0.51 ± 0.05 mg of 3ppa (mg [DW] h)$^{-1}$, only at a dilution rate of approximately 0.8 ± 0.1 h^{-1}.

Growth with mixtures at a constant dilution rate. (i) Steady-state substrate concentrations as a function of the mixture composition. In the experiments described above, it was observed that both the 3ppa and the glucose steady-state concentrations were always reduced during growth with a 1:1 (C:C) mixture of the two substrates, compared to those during growth at the same dilution rate with 3ppa or glucose alone. To study this phenomenon in more detail, cells were cultivated at a constant dilution rate and were fed with different defined mixtures of 3ppa and glucose. The substrates were added to the feed medium in such a way that the total biomass concentration in the culture remained constant at 45 ± 5 mg liter^{-1}. (The composition of the mixtures supplied was calculated under the assumption that the particular substrates contributed to the total biomass proportionally to the yield coefficients determined during growth with individual substrates [14].) This experiment was performed at a dilution rate of 0.6 h^{-1} because during growth at lower dilution rates, the steady-state concentrations of the two substrates were expected to be close to the detection limit.

Over the whole range of mixtures containing 5 to 90% 3ppa (based on carbon), the two substrates were utilized simultaneously. The resulting steady-state concentrations were approximately proportional to the ratio of these substrates in the inflowing medium (Fig. 2), and, as tested for 3:7 and 1:1 mixtures of 3ppa-glucose (C:C), the steady-state concentrations were independent from the total carbon concentration in the feed medium (tested were media containing 20, 40, and 80 mg of total C liter^{-1}) and, hence, independent from the biomass concentration.

(Note that the well-known chemostat theory [12], stating that the steady-state substrate concentration is not affected by the concentration of substrate in feed medium, was formulated for growth with single limiting substrates. For the simultaneous utilization of mixtures, not only the total substrate (carbon) concentration but additionally the composition of the mixture in the feed is important. Also here, steady-state concentrations are independent from the total carbon concentration in the feed medium, but this statement is now restricted to a particular mixture of constant composition.)

Because the steady-state concentrations of 3ppa were 1 to 2 orders of magnitude higher than those of glucose (Fig. 2b), the total steady-state carbon concentration in the culture was determined mainly by the 3ppa concentration. As a consequence, the steady-state total carbon concentration followed closely the steady-state concentration of 3ppa carbon and increased with increasing proportions of 3ppa in the feed medium. This is in contrast to the experiments performed by Lendenmann et al. (18) with mixtures of sugars, in which the total steady-state carbon concentration was approximately constant and did not

TABLE 2. Growth parameters of *E. coli* ML 30

Growth substrate	μ_{max} (h^{-1})	Yield (mg [DW] [mg of C]$^{-1}$)	K_s (μg liter^{-1})
Glucose	0.92 ± 0.05[a]	1.13 ± 0.07	51.5–177.0[d]
	0.76 ± 0.10[b,f]		32.8[f]
3ppa	0.42 ± 0.05[a]	0.75 ± 0.06	600–700[e]
	0.35 ± 0.05[b]		
3ppa-glucose mixture (1:1, C:C)	0.90 ± 0.10[b,c]	—[g]	600–700[e]

[a] Determined from batch culture data (14).
[b] Determined by NPE from continuous-culture data.
[c] The overall growth rate on the mixture determined from 3ppa concentrations.
[d] From reference 24.
[e] No statistically significant difference between the fits of K_s could be determined within this range. The 3ppa value is given for the mixture.
[f] From reference 15, using an extended form of the Monod model.
[g] —, additive yield.

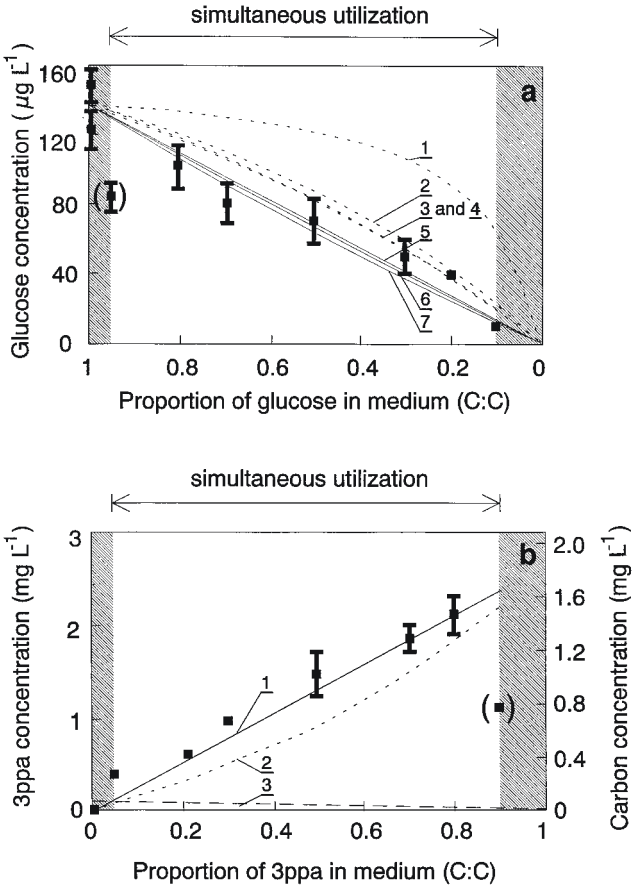

FIG. 2. Steady-state concentrations of glucose and 3ppa during growth of *E. coli* in carbon-limited chemostat cultures with mixtures of the two substrates at a constant dilution rate of 0.6 h^{-1}. The total biomass concentration was always 45 mg (DW) $liter^{-1}$. (a) Glucose concentrations (■) as function of the mixture composition; the numbered curves give the model predictions when the original equation proposed by Lendenmann and coworkers (18) was modified so that different ways to express the mixture composition and the contribution (*R*) of an individual substrate were used in the calculation (equation 5a). *R* was expressed in the following ways: curve 1, Gibbs free energy of formation from elements (in kilojoules per liter of feed medium); curve 2, substrate concentration (milligrams per liter) in feed medium; curve 3, substrate concentration (millimoles per liter) in feed medium; curve 4, substrate concentration (milligrams per liter) in feed medium multiplied by the particular yield coefficients; curve 5, substrate concentration (milligrams of carbon per liter) in feed medium;); curve 6, Gibbs free energy for full oxidation reaction (in kilojoules per liter of feed medium, calculated according to reference 4); and curve 7, moles of O_2 per liter of feed medium required for full oxidation of the substrates. (b) 3ppa concentrations (■) as a function of the mixture composition. Line 1, model predictions (equation 5a) when considering the contribution of carbon of the two substrates; line 2, 3ppa substrate concentrations predicted by equation 5 when weight proportions of the substrates are used; line 3, steady-state concentrations of glucose (given in milligrams of carbon per liter).

significantly change when mixtures of different compositions were supplied.

(ii) Contributions of the individual substrates to the overall specific growth rate. Although the dilution rate of 0.6 h^{-1} applied in this experiment was higher than the maximum specific growth rate achieved by *E. coli* with 3ppa as the only source of carbon and energy (0.42 ± 0.05 h^{-1} in batch culture [14] or 0.35 ± 0.05 h^{-1} in continuous culture; this study), the two substrates were utilized simultaneously and contributed to the formation of biomass. This implies that each of the substrates contributed to the overall growth rate (equation 4). Assuming that the mixed-substrate kinetics can be derived from the relationship between specific growth rate and steady-state substrate concentration during single-substrate growth, the contributions of the individual substrates to the overall growth rate were determined from the measured steady-state concentrations and the single-substrate Monod growth kinetics (using equations 1 and 4; for kinetic parameters, see Table 2). On the basis of this assumption, the calculated contribution of 3ppa was never higher than the specific growth rate with 3ppa as the sole substrate (Fig. 3). The resulting overall specific growth rate with glucose and 3ppa was always 0.60 ± 0.05 h^{-1}, indicating that the assumption made with respect to constant yield coefficients for the two substrates was correct.

Because of wall growth problems, it was impossible to cultivate the bacterium with media containing 3ppa at a proportion higher than 90 to 95% (percent carbon; compare also the specific consumption rates of individual substrates in Fig. 1b). When cultures were fed mixtures containing less than 5 mg of 3ppa $liter^{-1}$, the 3ppa-degradative pathway could not be induced when the cells had been previously grown with glucose only (for details, see reference 13).

(iii) Evaluation of the mixed-substrate models. By using the experimental data, the mixed-substrate model proposed by Egli et al. (8) and Lendenmann et al. (18) was evaluated for its ability to predict the steady-state substrate concentrations from the composition of the feed medium. The experimental system used, *E. coli* utilizing mixtures of up to six sugars, is so far the best-studied example of mixed-substrate utilization, which allowed proposal of an empirical equation (equation 5) for the description of the relationship between steady-state substrate concentrations and mixture composition (based on weight). Unfortunately, all of these sugars have the same carbon content, a degree of carbon reduction of 0, and the same energy content (with respect to both the Gibbs free energy of formation from elements [25] and the reaction for full oxidation). Therefore, when steady-state substrate concentrations are being related to substrate proportions in feed medium, it is impossible to judge how to express the substrate ratio (equation 5a) in order to best describe the mixed-substrate kinetics exhibited.

Here, we tested whether the concept of Lendenmann and

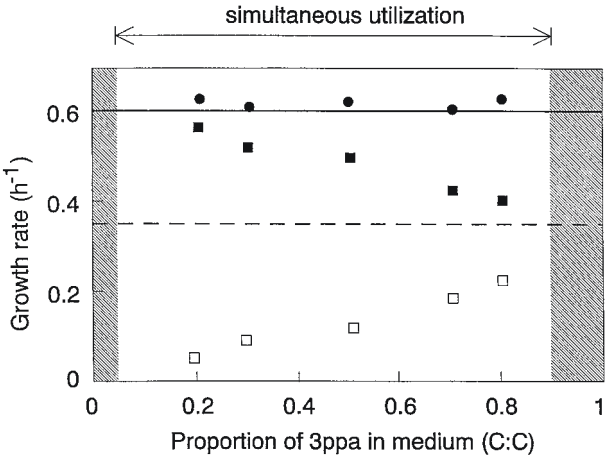

FIG. 3. Predicted contributions of individual substrates to the overall specific growth rate when *E. coli* is cultivated in a chemostat at a *D* of 0.6 h^{-1} with different mixtures of 3ppa and glucose. The contributions of 3ppa (□) and glucose (■) were calculated via equation 4; the maximum specific growth rate for *E. coli* growing with 3ppa as the sole carbon substrate in the chemostat (dashed line), the dilution rate of 0.6 h^{-1} (solid line), and the sum of the growth rates for glucose and 3ppa (●) are shown.

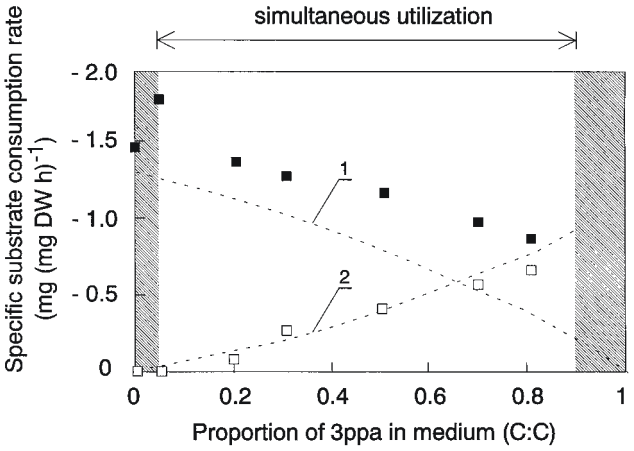

FIG. 4. Specific rates of consumption of excess substrate for glucose (■) and 3ppa (□) during growth of *E. coli* in a carbon-limited chemostat culture with mixtures of the two substrates at a constant dilution rate of 0.6 h^{-1}. The actual specific substrate consumption rates for glucose (line 1) and 3ppa (line 2) in the chemostat as calculated from equation 1 are shown.

coworkers (18) is also applicable to a mixture of two substrates that are metabolized via two completely different metabolic pathways, i.e., 3ppa and glucose (Fig. 4). Additionally, the regulation of the enzymes of the two pathways is distinctly different. Whereas the 3ppa-degrading system is inducible, that for glucose is constitutive (14). The curves in Fig. 2a visualize the model predictions of steady-state glucose concentrations by the simple empirical equation using different ways of expressing the contribution of a particular substrate to the mixture composition. The predicted values were in good agreement when the calculation was based on the contribution of carbon of a substrate. Similarly, good predictions were obtained when the relative energy content of the substrates (here expressed as the Gibbs free energy of the reaction for the full oxidation of the two substrates) or the molar ratio of oxygen needed for their complete oxidation to CO_2 and H_2O was used. Both of these characteristics are indirect expressions of the degree of carbon reduction in the compound. It should be pointed out that this is all based on the (reasonable) assumption that the substrates are perfectly substitutable (i.e., the two substrates fulfil the same physiological functions as sources of carbon and energy).

In Fig. 2b, either weight or carbon proportions of the substrates in the feed were used in the calculation of steady-state 3ppa concentrations. Although both calculation methods resulted in reduced steady-state concentrations during utilization of mixtures of carbon substrates, the predictions made by the originally proposed model (8) using weight proportions systematically deviated from our experimental data. However, simply replacing the substrate weight proportions in equation 5 by the proportions of carbon led to good agreement of the predicted steady-state concentrations of glucose and 3ppa with the experimental data.

DISCUSSION

How general are the concepts of mixed-substrate kinetics? Up to now, the biodegradation of pollutants has been investigated either in complex systems consisting of undefined mixtures of cultures and substrates (e.g., directly in natural or technical environments and in laboratory microcosms) or in pure culture with a single pollutant supplied as the only carbon source. However, neither of these experimental strategies is sufficient to deduce principles of pollutant biodegradation under the complex nutritional and physical conditions that pertain in natural and engineered environments. On the one hand, the many reported die-away studies of pollutants using water or soil samples are essentially black-box systems (e.g., see reference 10) and therefore difficult to interpret, although sometimes the observed degradation for some compounds seems to follow a rather simple pattern. On the other hand, the traditional single-substrate laboratory approach totally ignores the reality of the broad spectrum of substrates available for microorganisms in complex systems.

The use of a well-defined laboratory system, namely, a carbon-limited chemostat with pure cultures and defined mixtures of substrates, allowed us to make some general predictions on the behavior of pollutant-degrading organisms and, additionally, to describe them in quantitative terms (compiled by Egli [5]). These studies offer a conceptual framework within which a number of observations concerning the fate of chemicals in real environmental and technical systems must be discussed. A first emerging pattern is the lowering of steady-state concentrations during simultaneous utilization of mixed carbon sources by heterotrophic microorganisms at a given specific growth rate, as was observed in several laboratory systems (8, 18, 26). This is certainly a general phenomenon that can be extended to growth with complex mixtures. (However, the general applicability of, for example, equation 5a should be further supported with experimental data for growth with other substrates, especially substrates that are more oxidized than sugars or 3ppa.) Also, it should be pointed out that the resulting patterns of steady-state (extracellular) substrate concentrations are closely linked to the concentration of enzymes in a metabolic pathway. In this respect, the almost linear behavior of the steady-state concentration patterns obtained for growth of *E. coli* with either a mixture of up to six sugars (18) or glucose and 3ppa (this study), suggesting that all enzyme systems involved were more or less fully induced and operating far from saturation, might perhaps be untypical. Certainly, the data obtained for the simultaneous utilization of methanol and glucose by a methylotrophic yeast demonstrate that not only linear, but also complex patterns can be expected (6, 7) and that such patterns might be linked to the patterns of expression of the enzymes involved in the degradation of these compounds.

These observations considerably change our perception of microbial growth under environmental conditions. First, the experimental data indicate that for microbial competition at low environmental concentrations, in addition to K_s, the catabolic potential to utilize different (homologous) substrates simultaneously has to be taken into account. All the examples given in the literature (reviewed by Egli [5]) indicate that under carbon- and energy-limited conditions, the simultaneous derepression of many different catabolic enzymes is not wasteful but allows an organism to respond quickly to changes in substrate availability. Hence, this will allow a generalist to grow at reduced concentrations of substrates despite its seemingly poor K_s and to compete with more catabolically specialized strains. That this strategy can be successful has been demonstrated for facultatively autotrophic thiobacilli that under mixed-substrate conditions were able to outcompete specialist strains (9).

Second, the experimental data now available for *E. coli* with mixtures of substrates in continuous culture (this paper and reference 18), and also those for mixed-substrate growth of other microorganisms (6, 7, 16), give an answer to the question how microorganisms are able to reduce concentrations of carbon substrates in ecosystems to the low levels observed and to

still grow reasonably fast under such conditions. It should be added here that in comparison to growth with the individual carbon sources, in many cases a stimulation in the maximum specific growth rate was observed also in laboratory batch cultures when mixtures of substrates were used simultaneously (e.g., reference 2).

Third, the results presented in this paper indicate that further improvement of the existing biodegradation-biotransformation processes will be possible due to better understanding of the behavior of microbial cultures with respect to mixed substrates. When at a constant growth rate several substrates are consumed at the same time, the flux through the catabolic pathways of individual substrates will be reduced (Fig. 1b; more examples of this kind have been discussed in, e.g., reference 5). Implicitly, this might enable higher rates of biodegradation of substrates (pollutants) when they are degraded in the presence of additional substrates (2) (equation 4). In this context, the example studied here of *E. coli* utilizing glucose and 3ppa clearly demonstrates that not only the concentration of an individual compound but also the total carbon concentration in, for instance, a wastewater effluent stream might be reduced by proper system design.

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