Effects of unsteady state conditions on the biooxidation of methyl ethyl and methyl isobutyl ketone in continuous flow liquid phase cultures

D.P. Geoghegan, G. Hamer, M.A. Deshusses

Abstract The effects of changing operating conditions on the biooxidation of methyl ethyl and/or methyl isobutyl ketone in continuous flow enrichment culture are examined. Particular emphasis is placed on responses to step changes in feed stream concentrations and to substrate pulses injected directly into the culture supernatant. In general, the enrichment culture was better able to handle transients involving methyl isobutyl ketone, the preferred carbon substrate. However, the highly complex response patterns observed clearly indicated major gaps in knowledge concerning the physiology of methyl ketone-oxidizing bacteria. In spite of the two carbon substrates investigated being major environmental pollutants, their removal in waste biotreatment processes is remarkably little understood.

1 Introduction

Bulk organic chemicals are produced either as process feedstocks or as utility products, particularly solvents. The former group of products may become pollutants during their manufacture or their utilization, but the fraction of the total production released in waste streams is relatively small. However, in the case of the latter products, production essentially matches losses in waste streams, and virtually the total production of such compounds finds its way into either aqueous or gaseous waste streams from those industries that utilize such products. Two particular compounds belonging to the latter category are methyl ethyl ketone (MEK) or 2-butanone and methyl isobutyl ketone (MIBK) or 4-methyl-2 pentanone. Production estimates suggest [1] that more than 600,000 tonnes of the former and more than 250,000 tonnes of the latter are produced per annum. The high relative volatilities of both compounds with respect to water suggest that losses in waste gas streams will predominate, although both compounds are also likely to be present in aqueous liquid waste streams. Both MEK and MIBK have been designated

high-priority toxic chemicals [2] in spite of their mode of utilization and as a result were chosen as model pollutants in a study of pollutant elimination from waste gas streams by biofiltration [3–6].

As a group, the ketones have not been subjected to extensive studies as far as biodegradability is concerned, in spite of their high levels of release. Aerobic biodegradability tests [7, 8] have indicated that they are, in general, relatively easily biodegraded, but details of the kinetics of such biodegradation processes are scant, particularly in liquid phase cultures. Accordingly, the work reported here seeks to rectify the lack of kinetic data concerning liquid phase cultures where MEK and MIBK are subject to aerobic biodegradation. The only previous bioreactor study involving the aerobic biodegradation of either MEK or MIBK in liquid phase culture was of batch experiments where mixtures of acetone, MEK and di-ethyl ketone were investigated [9]. In a preliminary study [10], batch experiments in which both MEK and MIBK were subjected to biooxidization were conducted, but simultaneous substrate stripping occurred, even though the bioreactor used was fitted with an outlet gas condenser, thus invalidating the results obtained because of difficulties in differentiating between the removal of the ketone by biooxidation, on the one hand, and by stripping, on the other hand.

In experiments involving carbon limited chemostat cultures, any stripping of either MEK or MIBK would be negligible because of the essentially zero dissolved substrate concentrations involved and in the case of unsteady state continuous flow cultures involving dynamic responses to step changes in feed substrate concentrations and pulse additions of substrates, respectively, would show similar patterns of behaviour irrespective of whether simultaneous substrate stripping was occurring.

2 Materials and methods

2.1 Ketone degrading culture

The samples of microbial biomass used for enrichment were derived from a technical-scale aerobic trickling filter biotreating pharmaceutical industry wastewater at Schering Plough-Avondale Chemicals, Rathdrum, Ireland. Enrichment was carried out at 30 °C over a period of nine months using aerated shake flasks containing a defined buffered mineral medium with MEK and MIBK as carbon energy substrates.

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2.2
Media

For enrichment cultures the medium employed comprized: $Na_2HPO_4 \cdot 2H_2O$ – 1.4 kg, KH_2PO_4 – 0.7 kg, NaCl – 0.1 kg, $MgSO_4 \cdot 7H_2O$ – 0.6 kg, $CaCl_2 \cdot 2H_2O$ – 0.1 kg, $(NH_4)_2SO_4$ – 2.0 kg, EDTA Na_2 – 0.1 kg and trace element solution[11] – 1.0 dm^3 per m^3 deionized water. The pH of this medium was 6.7. For continuous flow cultures, the medium employed comprized: H_3PO_4 (85% solution) – 0.2 dm^3, K_2SO_4 – 0.18 kg, NaCl – 0.05 kg, $MgSO_4 \cdot 7H_2O$ – 0.12 kg, $CaCl_2 \cdot 2H_2O$ – 0.05 kg, $(NH_4)_2SO_4$ – 1 kg, EDTA Na_2 – 0.05 kg and trace element solution [11] – 1.0 dm^3 per m^3 tap water. The pH of this medium was 2.5, in order to suppress any growth of potentially contaminating biota in the medium reservoir. Carbon energy substrates, MEK and MIBK, were added as required. During experiments, the pH of the medium was adjusted to 6.7 by the addition of an equimolar 0.5 M NaOH/KOH aqueous solution. All chemicals used were either Analar or GPR grade.

2.3
Bioreactor

The bioreactor employed was a 14 l baffled cylindrical impeller agitated stainless steel vessel. It was fitted with impeller speed, temperature, and pH control and a medium inlet pump and culture overflow weir to allow a constant liquid operating volume of 6.9 l under aerated conditions. The culture pH was maintained by automatic addition of aqueous alkali solution. The temperature was maintained constant at 30 °C, while an impeller speed of 900 rpm and an air flow of 4 l min^{-1} were used throughout the experimental programme. An outlet gas stream condenser was provided.

The growth medium was stored in a magnetically stirred reservoir.

2.4
Analytical procedures

Optical density The optical density of samples of culture from the bioreactor was measured at 546 nm in 10 mm cuvettes with a model CE 303 spectrophotometer (Cecil Instruments Ltd., Cambridge, England) after dilution with chilled 0.9% NaCl solution, where appropriate. The values obtained were converted to dry weights using a linear calibration.

Dry weight Culture dry weights were obtained by filtering 20 ml aliquots of culture through a tared 0.45 μm pore size 25 mm diameter type WCA cellulose acetate membrane filter (Whatman Ltd., Maidstone, England). After washing with 10 ml 0.9% NaCl solution, each filter was dried at 90 °C to constant weight (24 h), and cooled in a desiccator prior to reweighing.

Dissolved ketone analyses The concentrations of MEK and MIBK in aqueous samples were determined by gas chromatography using a Model 280 gas chromatograph (Gow-Mac, Shannon, Ireland) fitted with a 2 m nickel column packed with 5% carbowax 20 M on carbopack B, and a flame ionisation detector. Injections, which varied in size from 0.2 μl to 0.8 μl, were introduced manually. The injection port was maintained at 150 °C, the column at 100 °C, and the detector at 170 °C. Helium was used as the carrier gas. Diethyl ketone was used as an internal standard. Integration of the chromatographs was performed using a model 3395 integrator (Hewlett Packard, Dublin, Ireland) equipped with a baseline program.

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Results

Chemostat cultures were conducted with substrate mixtures containing 200 mg l^{-1} each of MEK and MIBK at a dilution rate of 0.15 h^{-1}. Under such operating conditions carbon substrate limitation prevailed, with residual concentrations of MEK and of MIBK essentially zero. Transients, involving changes in individual substrate feed stream concentrations were investigated by abruptly doubling either the feed stream MIBK or the feed stream MEK concentration from 200 to 400 mg l^{-1} while maintaining the concentration of the other carbon substrate in the feed constant at 200 mg l^{-1}.

In the case of the step change in MIBK concentration (Fig. 1), a slight accumulation of MIBK, which was eliminated within 5 h, occurred in the culture supernatant, while the MEK concentration in the culture supernatent remained essentially zero. The biomass concentration increased markedly and exceeded, for some 15 h, the ultimate steady state concentration that was established. In contrast, for the step change in MEK concentration (Fig. 2), MEK accumulation occurred in the culture supernatent and persisted for some 10 h, while the MIBK concentration remained essentially zero throughout. Furthermore, a small, but discernible reduction in the culture biomass concentration occurred.

The second pair of transients examined sought to allow determination of the ability of cultures to accommodate to removal from and subsequent addition to the bioreactor feed of each individual substrate. Steady state conditions, as described previously, were established with a feed stream containing 200 mg l^{-1} each of MEK and MIBK,
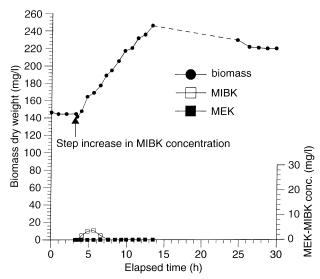

Fig. 1. Effect of a step increase in feed MIBK concentration from 200 to 400 mg l^{-1}, while maintaining the feed MEK concentration constant at 200 mg l^{-1}, at a dilution rate of 0.15 h^{-1}. Substrate stripping was ignored
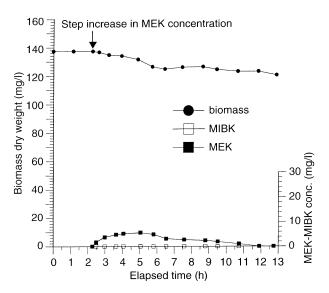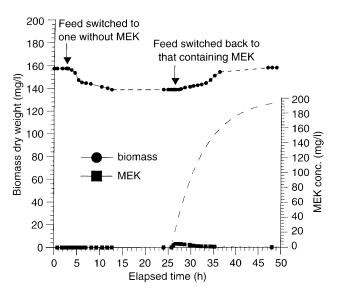
Fig. 2. Effect of a step increase in feed MEK concentration from 200 to 400 mg l^{-1}, while maintaining the feed MIBK concentration constant at 200 mg l^{-1} at a dilution rate of 0.15 h^{-1}. Substrate stripping was ignored

When the feed stream of a steady state continuous flow culture, operating under previously described conditions, was abruptly changed to one containing 200 mg l^{-1} of MEK and no MIBK, and then, after a new steady state had been established, the feed stream containing 200 mg l^{-1} each of MEK and MIBK was restored, it can be seen from Fig. 4 that neither step change resulted in accumulation of either MIBK or MEK. However, the first change did result in a dramatic reduction in biomass concentration, although not to the extent that might have been expected from the previous experiment. Restoration of MIBK to the feed stream resulted in a major increase in the suspended biomass concentration, but because of wall growth development late in the experiment, the original steady state suspended biomass concentration was not re-established.

Experiments in which pulses of either MIBK or MEK were added directly to chemostat cultures operating at a dilution rate of 0.15 h^{-1} with carbon substrate feed concentrations comprizing 400 mg l^{-1} of either MIBK or MEK or 200 mg l^{-1} each of MIBK and MEK under carbon substrate limitation, to give a dissolved concentration in the culture of 200–250 mg l^{-1}, were conducted in order to examine the response of the enrichment culture to operating changes of a type most likely to be encountered as a result of either a restricted accidental spillage in wastewater biotreatment or under typical unsteady state operating conditions in waste gas biotreatment. In all cases some stripping of the pulsed ketone must have occurred, but experiments conducted to measure elimination of MIBK and MEK from aqueous solutions by sparged air stripping [10] indicate elimination rates markedly inferior to the elimination rates observed in the series of experiments under discussion, even after ketone wash-out is taken into account.
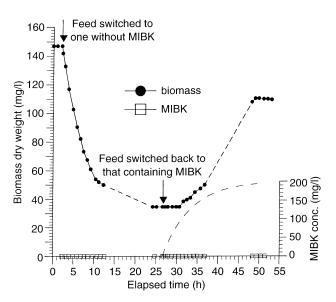
prior to switching to a feed containing no MEK. Subsequently, the MEK was restored in the feed. The response of the culture is shown in Fig. 3. The removal of MEK from the feed stream resulted in continued complete removal of MIBK accompanied by a minor reduction in the biomass concentration of ca. 11 percent. Upon restoration of MEK in the feed stream, very minor accumulation of MEK, but no accumulation of MIBK, occurred. Restoration was also accompanied by re-establishment of the original suspended biomass concentration.

Fig. 3. Effects of a step decrease in feed MEK concentration from 200 to 0 mg l^{-1} and of a subsequent step increase in feed MEK concentration from 0 to 200 mg l^{-1}, while maintaining the feed MIBK concentration constant at 200 mg l^{-1} at a dilution rate of 0.15 h^{-1}. The supernatant MIBK concentration was below the detection limit throughout and the dashed curve represents possible maximum MEK build-up. Substrate stripping was ignored

Fig. 4. Effects of a step decrease in feed MIBK concentration from 200 to 0 mg l^{-1} and of a subsequent step increase in feed MIBK concentration from 0 to 200 mg l^{-1}, while maintaining the feed MEK concentration constant at 200 mg l^{-1} at a dilution rate of 0.15 h^{-1}. The supernatant MEK concentration was below the detection limit throughout and the dashed curve represents possible maximum MIBK build-up. Substrate stripping was ignored
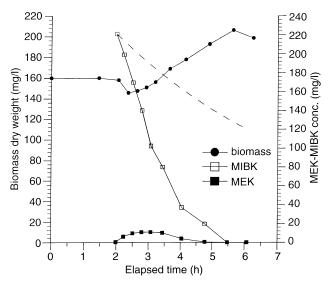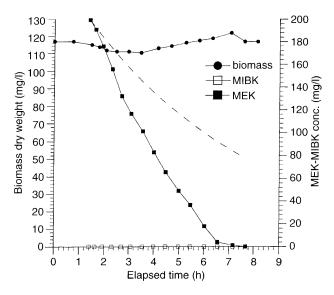
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Fig. 5. Response of MEK/MIBK-limited chemostat culture operating with a feed concentration of 200 mg l^{-1} each of MEK and MIBK at a dilution rate of 0.15 h^{-1} to a 200 mg l^{-1} pulse addition of MIBK. The dashed curve represents the theoretical wash-out of MIBK based on a total absence of elimination mechanisms. Substrate stripping was ignored

Fig. 6. Response of a MEK/MIBK-limited chemostat culture operating with a feed concentration of 200 mg l^{-1} each of MEK and MIBK at a dilution rate of 0.15 h^{-1} to a 200 mg l^{-1} pulse addition of MEK. The dashed curve represents the theoretical wash-out of MEK based on a total absence of elimination mechanisms. Substrate stripping was ignored

In the case of a pulse of MIBK to a chemostat operating under dual MIBK/MEK limitation (Fig. 5), the response involved an immediate but low accumulation of MEK for 1 h after the pulse followed by a return to complete MEK elimination some 2.5 h after the pulse, marked biooxidation of the pulsed MIBK until complete elimination was achieved some 3.5 h after the pulse, and an initial decline followed by an increase to a peak in the biomass concentration some 3.5 h after the pulse. From an initial steady state biomass concentration of 160 mg l^{-1}, the peak level achieved was 208 mg l^{-1}, giving an apparent MIBK based biomass yield coefficient of 0.22. Comparing this with the corresponding yield coefficient that resulted from a step increase in the feed MIBK concentration, i.e., 0.405, suggests that some 46 percent of the pulsed MIBK was eliminated by the combined actions of stripping and wash-out. This is, of course, an over estimate as biomass was also subject to wash-out, but confirms biooxidation as the major elimination mechanism for MIBK.

Turning to the corresponding pulse addition of MEK to a dual carbon substrate limited chemostat (Fig. 6), the response involved MEK elimination over 5.5 h until complete elimination occurred, no accumulation of MIBK, and a barely discernible decline and subsequent increase in the biomass concentration to a peak some 5.5 h after the pulse addition of MEK. These results again indicated an extremely low MEK based biomass yield coefficient as was expected from the results for step changes of MEK to chemostat feeds, but the peak in biomass concentration confirmed oxidation as a removal mechanism, but again this was very largely uncoupled from growth.

In the case of pulses of MIBK and of MEK to MIBK-limited chemostats shown in Figs. 7 and 8, respectively, the response to the MIBK pulse involved elimination of the pulsed MIBK within 3 h and a decline and subsequent peak in biomass concentration also 3 h after the pulse. For

Fig. 7. Response of a MIBK-limited chemostat culture operating with a feed MIBK concentration of 400 mg l^{-1} at a dilution rate of 0.15 h^{-1} to a 200 mg l^{-1} pulse addition of MIBK. The dashed curve represents the theoretical wash-out of MIBK based on a total absence of elimination mechanisms. Substrate stripping was ignored
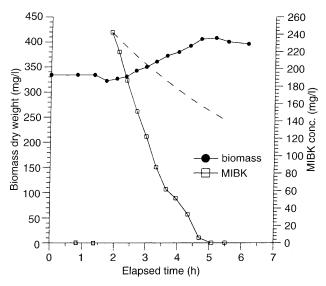
the MEK pulse, a trivial increase in biomass concentration accompanied MEK elimination over 8 h, but no concomitant accumulation of MIBK occurred. In the case of the latter pulse, the contributions of wash-out and stripping to MEK elimination were probably more significant. It should be noted that the MIBK based yield coefficients for the chemostats prior to pulsing were 0.83 and 0.80, respectively.

The final pulse experiment (Fig. 9) involved pulsing an MEK-limited chemostat with MIBK. In this case the response was dramatic, with rapid elimination of the pulsed

Fig. 8. Response of a MIBK-limited chemostat culture operating with a feed MIBK concentration of 400 mg l^{-1} at a dilution rate of 0.15 h^{-1} to a 200 mg l^{-1} pulse addition of MEK. The dashed curve represents the theoretical wash-out of MEK based on a total absence of elimination mechanisms. Substrate stripping was ignored
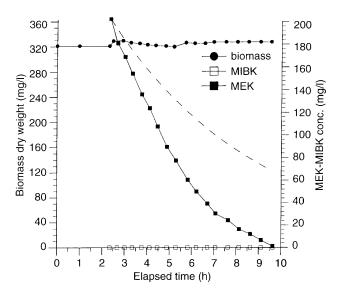

Fig. 9. Response of a MEK-limited chemostat culture operating with a feed MEK concentration of 400 mg l^{-1} at a dilution rate of 0.15 h^{-1} to a 200 mg l^{-1} pulse addition of MIBK. The dashed curve represents the theoretical wash-out of MIBK based on a total absence of elimination mechanisms. Substrate stripping was ignored
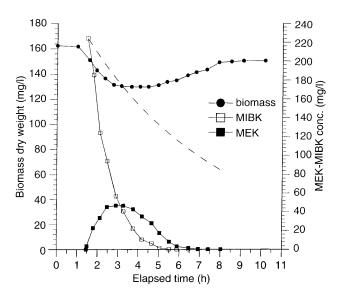
MIBK within 3.5 h, an accumulation to a peak value of 46 mg l^{-1} and subsequent elimination of MEK over a period of 5 h and a decline, accompanying MIBK elimination, and subsequent recovery in the biomass concentration over a period of 6.5 h with a slight reduction in the final MEK based yield coefficient of some 5 percent. The initial MEK based yield coefficient was 0.41.

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Discussion

In experiments where the enrichment culture was grown in the batch mode [10], it was found to exhibit maximum specific growth rates of 0.19 h^{-1} and 0.20 h^{-1} with MEK and MIBK as single carbon substrates, respectively. These rates were markedly higher than those reported earlier by Deshusses [12]. When the enrichment culture was grown on mixtures of MEK and MIBK, simultaneous utilization of the two substrates occurred and a maximum specific growth rate of 0.21 h^{-1} was observed. Because of significant stripping, it was not possible to determine carbon substrate based biomass yield coefficients in batch culture. However, for chemostat cultures growing under carbon substrate limitation on each individual substrate, biomass yield coefficients of 0.41 for MEK and of 0.815 for MIBK, respectively, were determined (Figs. 7–9). These yield coefficient values are real values because stripping under conditions of strict carbon substrate limitation is negligible. Deshusses [12] reported yield coefficients for MIBK and for MEK, obtained in enclosed suspension cultures, between 0.45 and 0.81 and between 0.26 and 0.39, respectively, depending on supernatant substrate concentration.

When mixed enrichment cultures growing on binary carbon energy substrate mixtures in carbon limited chemostat culture are subjected to step increased in the individual carbon substrate concentration in their feeds, it is the acceleration of the *in situ* respiration rate of either a component strain within or the whole culture, that governs the response that occurs. In the present study, where the mixed culture was growing at an imposed specific growth rate corresponding to 71.5 percent of the maximum rate observed for growth of the mixed culture on the binary carbon substrate mixture, the potential capacity, with respect to respiration rate acceleration, would be restricted, and the minor accumulation of MIBK and MEK after step increased shown in Figs 1 and 2, respectively, would be expected. However, it was the contrasting effects on biomass concentration that were caused by the step increases in feed MIBK and MEK concentrations that were anomalous.

Steady state biomass concentrations for the mixed enrichment culture growing on a feed containing 200 mg l^{-1} each of MIBK and MEK were 143, 137, 156, and 147 mg l^{-1} on a dry basis (Figs. 1–4). On the basis of the maximum individual substrate based biomass yield coefficients, 0.815 for MIBK and 0.41 for MEK, the expected steady state dry bacterial biomass concentration would have been 245 mg l^{-1}. As shown in Fig. 1, the steady state biomass concentration when a new steady state had been established after the step increase in MIBK feed concentration to 400 mg l^{-1} was only 224 mg l^{-1}, although a peak value of 248 mg l^{-1} was achieved prior to this. The new steady state value indicates that 200 mg l^{-1} MIBK produced 81 mg l^{-1} dry biomass giving a biomass yield coefficient for biomass production from MIBK of 0.405. If this yield coefficient applied to the conversion of all the MIBK supplied, 162 mg l^{-1} of biomass would have been derived from MIBK and 62 mg l^{-1} of biomass would have been derived from MEK, indicating a corresponding yield coefficient of 0.31. The results suggest inter-substrate interactions in the case of simultaneous MIBK/MEK biooxidation.

In the case of the chemostat culture which was subjected to a step increase in its MEK feed concentration (Fig. 2),

initial conditions showed a steady state biomass concentration of 137 mg l^{-1}, which, after the step change, reduced to 124 mg l^{-1}, when MEK elimination was again complete. This particular result suggests that MEK was eliminated with virtually no corresponding biomass production either by cooxidation [13] or by the uncoupling of respiration from growth [14], possibly as a result of magnification of the inhibitory nature of MEK when present as a component in a carbon substrate mixture [12].

In a subsequent experiment (Fig. 3), where the feed was switched from one containing 200 mg l^{-1} each of MIBK and MEK to one containing only 200 mg l^{-1} of MIBK, the biomass concentration was reduced from 157 mg l^{-1} to 140 mg l^{-1}, suggesting a biomass from MIBK yield coefficient of 0.70 and a biomass from MEK yield coefficient of 0.085. After switching to the original feed concentrations, the biomass concentration was re-established at 160 mg l^{-1}, indicating an MEK based biomass yield coefficient of 0.10. However, in a similar experiment (Fig. 4), where MIBK was removed from the feed stream and MEK retained at a concentration of 200 mg l^{-1}, the biomass concentration dropped to 35 mg l^{-1}, indicating an MEK based biomass yield coefficient of 0.175, i.e., an intermediate value, markedly below the maximum that had been observed, but distinctly higher than the minimum values observed in the previous experiment.

Interactions between carbon substrates in either mono- or mixed cultures have received little study particularly in continuous flow cultures when simultaneous substrate utilization was occurring. In the case of the methylotrophic yeast, *Hansenula polymorpha* growing on methanol/glucose mixtures, Egli *et al.* [15] showed, under steady state conditions, simultaneous and complete substrate utilization even at dilution rates exceeding the equivalent maximum specific growth rate for growth on methanol alone. Although they did not report directly on biomass yield coefficients, their results indicated a range of glucose based yield coefficients between 0.54, for growth on glucose alone, and 0.41, for growth on glucose in a methanol/glucose mixture containing 22.6 weight percent glucose and a range of methanol based yield coefficients between 0.39, for growth on methanol alone, and 0.52, for growth on methanol in a methanol/glucose mixture containing 19.3 weight percent methanol, suggesting marked substrate interaction.

In a subsequent evaluation of an earlier culture study [16], Hamer [17] drew attention to unexpected results concerning yield coefficients based on acetone and isopropanol. In the original study [16], it was claimed that isopropanol was cooxidized by an acetone utilizing consortium in the mixed culture, but that when the ratio of acetone: isopropanol in the feed was less than 1.18 : 1 on a weight basis, isopropanol accumulated in the culture supernatant. Based on strict definition, carbon substrates that are the subject of cooxidation should not contribute to biomass production. However, data taken from the original study showed that for growth on acetone alone, the acetone based biomass yield coefficient was 0.60, but that for a 2 : 1 mixture of acetone: isopropanol on a weight basis, the biomass yield coefficient increased to 0.75 on the basis of the combined weight of acetone and isopropanol

biooxidized [17], suggesting that energy derived from isopropanol oxidation was utilized for the conversion of acetone carbon into biomass carbon.

Linton and Stephenson [18] examined the relationship between maximum biomass yield coefficients for a wide range of carbon substrates and their respective heats of combustion. Their study clearly indicated a linear relationship between the maximum yield coefficient and the heat of combustion, when both were expressed on a unit of substrate carbon basis, provided the heat of combustion of the substrate was less than that for dry bacterial biomass on the same basis. For substrates with heats of combustion that exceed the value for biomass, their greater availability of energy did not contribute to energy efficient conversion of substrate carbon into biomass carbon, but rather to wasteful heat dissipation during growth. The main development from this approach was the concept of mixed carbon substrate fermentations [19–22] where substrates were classified as either energy deficient or energy excess and, for chemostat cultures, could be combined in order to maximize biomass yield coefficients and minimize heat dissipation.

Heats of combustion data for MIBK and MEK indicate that the former is an energy deficient and the latter an energy excess substrate. Hence, as dual substrates in chemostat culture, simultaneous utilization of these two ketones would be expected to result in some degree of biomass yield coefficient maximization. On the basis of the correlation proposed by Linton and Stephenson [18], the theoretical maximum substrate biomass yield coefficients, on a mass of anhydrous biomass per unit mass of carbon substrate utilized basis, for MIBK and MEK are 0.94 and 0.68, respectively, values that are in sensible correspondence with experimentally determined biomass yield coefficients for the two ketones, i.e., 0.815 and 0.41, respectively.

Most studies of the auxiliary substrate effect have been based on binary substrate mixtures that were utilized by means of markedly different metabolic pathways, whereas with MIBK and MEK, one might expect, in the absence of firm evidence, the involvement of a common pathway for their metabolism, thereby eroding any benefits that might have accrued from the effect, as is the case when simultaneous utilization of members of a homologous series of compounds, such as *n*-alkanes, occurs [23].

In early studies concerning the oxidation of *n*-alkanes, Leadbetter and Foster [24] showed that when the methanotrophic bacterium, then described as *Pseudomonas methanica*, was growing on methane, it simultaneously cooxidized propane to acetone and *n*-butane to MEK, respectively, while Lukins and Foster [25] showed that when propane grown cells of *Mycobacterium smegmatis* 422 were supplied with mixtures of air and *n*-butane, *n*-pentane, and *n*-hexane, the products formed were MEK, methyl butyl ketone and methyl pentyl ketone, respectively. Such results implied that, not only were methyl ketones intermediates in oxidation sequences for *n*-alkanes, but that they could also be expected to be utilized by *n*-alkane-oxidizing bacteria, a fact that was confirmed for *M. smegmatis* 422, *M rhodochrous* 382 and *M. fortuitum* 389, when these *n*-alkane-oxidizing strains were shown to grow

on acetone, MEK, methyl butyl ketone, methyl undecyl ketone and methyl heptadecyl ketone by Lukins and Foster [25], the *n*-alkane-oxidizing *Mycobacterium* sp. strain OFS, which was shown to grow on methyl tetradecyl ketone by Dunlap and Perry [26] and for the *n*-pentane-oxidizing *Brevibacterium* sp. strain JOB5, whose growth on acetone was reported by Vestal and Perry [27]. In addition, Lowery *et al.* [28] showed that five of eight *n*-alkane-oxidizing yeast strains were capable of growth on several of a wide variety of ketones tested, particularly on methyl pentyl ketone and on methyl hexyl ketone.

n-Alkane oxidation is an important property of a wide range of aerobic microorganisms and the linking of methyl ketone oxidation and *n*-alkane oxidation indicates that a substantial natural resource of microbiota is available for methyl ketone biodegradation. However, with the exception of acetone biodegradation, only the work of Irvine *et al.* [9] and the present study have sought to examine patterns of ketone elimination from liquid phase cultures. In the former study, aerobic batch biooxidation by a mixed culture resulting from combination of heterogeneous cultures enriched separately on acetone, MEK and diethyl ketone, of a substrate mixture comprizing 400 mg l^{-1} acetone, 400 mg l^{-1} MEK and 850 mg l^{-1} diethyl ketone was reported. The bacterial biomass, measured as mixed liquor suspended solids increased from an initial concentration of 250 mg l^{-1} to a maximum of 400 mg l^{-1} after 6.5 h with the characteristics of typical batch growth. During growth, acetone decreased slowly for 5 h and then abruptly after that. MEK decreased linearly over six hours, suggesting a combination of biodegradation and stripping as the elimination mechanisms, while the removal of diethyl ketone showed two distinct phases, but with complete removal occurring after six hours. Two unidentified intermediates accumulated and were subsequently removed, in sequence, during the elimination of the three substrates investigated.

The pulse experiments that were conducted in the present study emphasized both the fact that MIBK was the preferred substrate for the mixed enrichment culture, unlike the enrichment culture that biooxidized these same carbon substrates in the vapour phase [3] where the reverse was true, and the fact that the oxidative capacity, particularly with respect to MEK, appeared to become largely saturated. In some respects, the pattern observed resembled the behaviour of chemostat cultures of *Escherichia coli* growing at imposed specific growth rates (dilution rates) approaching their maximum when challenged with an increased availability of glucose [29]. Obviously, the responses of the ketone oxidizing enrichment culture to transients in carbon substrate availability at low imposed specific growth rates need investigation.

In many respects, dynamic responses resulting from step changes imposed on biofilters that are oxidizing volatile organic carbon compounds, resemble dynamic responses resulting from pulse additions of carbon substrates to chemostat cultures with the ability to oxidize the substrate in question. In the case of the former, Deshusses *et al.* [6] showed that a marked reduction in the inlet concentration of MEK resulting from a doubling of the air flow rate caused a short lived break-through of MEK in the biofilter outlet stream, while an increase in MEK concentration at a constant flow rate of air resulted in a prolonged breakthrough of MEK in the outlet stream of a biofilter which had the potential to completely eliminate MEK. In addition, a pulse of MEK to a biofilter eliminating a MEK/MIBK pollutant mixture resulted in markedly less effective MIBK removal. Clearly, organic vapour elimination in waste air biofilters involves a number of complex phenomena such as gas-liquid partition, substrate sorption/desorption, biooxidation under restricted nutrient availability and local process culture acclimation/deactivation that are absent in chemostat cultures. Therefore, beside similarities in the reactor responses, some of the transient responses observed in biofilters [5, 6] must be partially attributed to effects other than those discussed in the present study.

Results from the present study, together with studies concerning the dynamic response of biofilters to changes in carbon substrate availability, emphasize a need for fundamental studies of the physiology of ketone degrading microorganisms, particularly those capable of biooxidizing such major pollutants as MEK and MIBK.

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Conclusions

The present study clearly demonstrates a major lack of knowledge concerning the mechanisms involved in the biooxidation of binary mixtures of methyl ketones, particularly when unsteady state operating conditions apply. In both waste treatment processes and polluted natural environments, unsteady state conditions are the rule rather than the exception. The development of a reliable predictive basis for effective and efficient industrial pollutant degradation in biotreatment processes is an essential requirement if acceptable levels of environmental protection are to be achieved. However, this will not be achieved until the performance of process cultures in process situations becomes a matter of design rather than one of chance.

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