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Reduction of biomass in a bioscrubber for waste gas treatment by limited supply of phosphate and potassium ions

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Abstract Elimination of *n*-butanol from the gas phase was examined with a mixed culture in a compact bioscrubber. The extent of the cell concentration was limited by the supply of *n*-butanol, phosphate or potassium, and the growth rate was determined by the dilution rate. With *n*-butanol as the limiting substrate the cellular yield was 0.53 g dry cell weight/g *n*-butanol. Phosphate limitation decreased this yield to 0.34 g and potassium limitation to 0.31 g dry cell weight/g *n*-butanol at a dilution rate of 0.1/h. Under these conditions *n*-butanol was eliminated from the gas phase by 84%–100%. In the same order of limitations the specific degradation rate ranged from 0.19 g to 0.32 g *n*-butanol g dry cell weight^{-1} h^{-1}. The fraction of *n*-butanol required to satisfy the needs for maintenance energy increased significantly depending on the limiting nutrient. Limitation by *n*-butanol, phosphate or potassium caused a maintenance requirement of 0.07, 0.16 and 0.34 g *n*-butanol g dry cell weight^{-1} h^{-1}, thus showing a fivefold increase. This high demand for the carbon source demonstrated the feasibility of operating a bioscrubber under mineral limitation to reduce biomass formation significantly, and to maintain a high degree of substrate elimination from the gas phase.

Introduction

Organic and inorganic compounds in waste gases can be removed by chemical, physical or biological treatment. The chemical treatment requires aggressive additives, causing problems to the environment. Physical processes do not eliminate but transfer the pollutants to a new stream to be treated. They are economically feasible, especially at high organic loads (Ottengraf et al. 1984). Biological waste gas treatment has been predominantly applied to eliminate low concentrations of pollutants. Three modes of treatment are currently used: biofilters, trickling filters and bioscrubbers. The advantage of biological waste gas treatment is in the degradation of the pollutants, the low investment and low operation costs (Bardtke 1990; Eitner 1989). Biological treatment of waste gases with higher organic load is desired to bridge the gap to physical processes (Kok 1992). With increasing organic load, however, biomass formation and its removal are matters of increasing concern (Kirchner et al. 1991).

Biomass formation in nature is limited mainly by the availability of nutrients. Imbalances of nutrient supply may cause an imbalance in composition of biomass. Accumulation of phosphorus as intracellular polyphosphates is observed in the case of excess phosphate supply (Kornberg 1995). Excess of carbon and energy may lead to the intracellular accumulation of polyhydroxy fatty acids (Steinbüchel et al. 1996) or the formation of extracellular polysaccharides and biofilms (Mian et al. 1978).

The organic carbon source is generally used for microbial energy conversion and for biomass formation. Thus, reduction in biomass could be achieved by reducing the efficiency of energy generation which, in turn, increases the specific turnover of the carbon source. Two approaches were considered to reduce biomass formation by maintaining the rate and extent of degradation of the organic load of the waste gases. First, to increase the requirement for maintenance energy by a high mean residence time. Second, to decrease the efficiency of energy generation for dry cell weight (dcw) formation by limitation of a nutrient such as phosphate or potassium.

The principle of maintenance energy, i.e. the requirement for energy to maintain the functions of the cell, has been introduced by Pirt (1965):

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$$\frac{1}{Y} = \frac{m}{\mu} + \frac{1}{Y_{max}} \quad (1)$$

where Y is the yield (g dcw/g substrate), Y_{max} the maximum yield, μ the specific growth rate (h^{-1}), and m the maintenance coefficient (g substrate g $dcw^{-1}h^{-1}$). This principle has been extended by Neijssel and Tempest (1976), who demonstrated in chemostat cultures that the maintenance requirements do not exclusively depend on the dilution rate but are highly dependent on the limiting nutrient.

We used the findings of Pirt (1965) and Neissel and Tempest (1976) to develop a process for waste gas treatment. *n*-Butanol was chosen as a usual component of solvents commonly emitted by lacquer works (Beyritz et al. 1989). We, therefore, used a compact bioscrubber to eliminate *n*-butanol from the gas phase, and significantly reduced the cellular yield with respect to *n*-butanol and increased the specific degradation rate by limitation of phosphate or potassium.

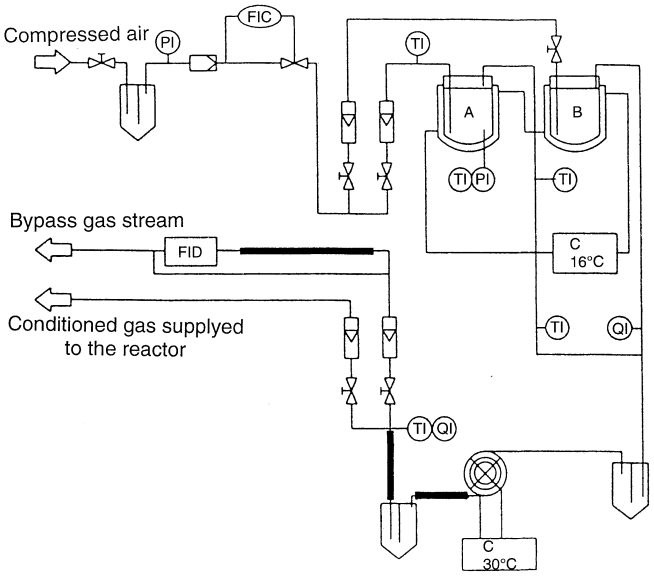

Fig. 1 Gas conditioning facility for supply of the bioscrubber with a defined organic load of the inlet gas. *A* air humidifier; *B* air saturation with *n*-butanol; *C* thermostat; *FIC* flow indication and control; *FID* flame ionization detector heated pipes are shown in bold

Materials and methods

Bacterial mixed culture

Mineral salts medium, as specified below, supplemented with 10 mM *n*-butanol as the carbon source, was inoculated with activated sludge from a local wastewater treatment plant. A stable bacterial mixed culture was obtained after ten transfers. From this mixed culture, isolates were examined for capsules microscopically, for Gram stain, endospore stain, acid-fast stain, oxidase and catalase according to Süssmuth et al. (1987). Examination for pathogens was done according to API Bio-Merieux. An apropriate amount of the mixed culture was used to inoculate batch cultures and the compact bioscrubber.

Media

For batch cultures the mineral medium contained, in 1 l: 1.6 g K_2HPO_4, 0.4 g KH_2PO_4, 3.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.06 g $CaCl_2{\cdot}2H_2O$, 0.05 g ferrous ammonium citrate, and 1.0 ml trace element solution SL6 (Pfennig and Lippert 1966). The pH was adjusted to 7.5 with K_2HPO_4; the concentration of *n*-butanol was 10 mM.

For operation of the *n*-butanol-limited bioscrubber an identical mineral medium was used except that the pH was 7.0 and *n*-butanol was supplied from the gas phase at the concentration indicated. For operation of the potassium-limited bioscrubber the medium contained in 1 l: 2.5 g $Na_2HPO_4{\cdot}12H_2O$, 0.4 g $NaH_2PO_4{\cdot}2H_2O$ and 9.4 mg KCl, equivalent to 5.0 mg potassium/l. For phosphate limitation the medium contained 0.210 g K_2HPO_4/l, equivalent to 115 mg phosphate/l.

Inlet gas conditioning

The facility shown in Fig. 1 allowed precise concentrations of *n*-butanol to be set in the inlet gas for the bioscrubber. Compressed air (500 kPa) was pressure-reduced, and passed through an oil and dust filter and regulated by a model 10 pressure regulator (Fairchild, Winston-Salem, N.C., USA). The air flow rate of 250 l/h was kept constant by a HI-TEC mass flow meter (Mättig, Unna, Germany). This air stream was split and regulated separately by flow meters (Bailey, Fischer & Porter, Göttingen, Germany). One sidestream was saturated with water, the other with *n*-butanol by sparging the air through 2-l gas contactors kept at 16 °C. To avoid subsequent condensation in the Viton tubings, the gas stream was heated to 30 °C, giving a relative humidity of about 60%. The ratio of both gas streams determined the concentration of *n*-butanol in the inlet gas for the bioscrubber. The concentration of *n*-butanol was analysed from a bypass (100 l/h) by a flame ionization detector Compur Multi FID 100 (Hartmann & Braun, Frankfurt, Germany).

Continuous bioscrubber

The *n*-butanol-loaded, moist inlet gas (0.15 Nm^3/h) was passed through a stirred reactor KLF 2000 (Bioengineering, Wald, Switzerland). The reactor (3 l total volume) had a working volume of 2 l and was equipped with a sampling device. It represented a laboratory type of compact bioscrubber according to the concept of Wolff (1989). The medium was kept at 30 °C and at pH 7.0 The flow rate of the mineral medium was maintained at a rate indicated with a MP3 microtube pump (Verder, Düsseldorf, Germany). The culture volume [V (l)] was kept constant by exporting the medium with the exit gas. Subsequently, the gas was separated from the medium by a gas/liquid separator. Steady state was judged from constant cell concentrations in the medium and *n*-butanol concentrations in the exit gas and in the medium. Steady state was usually achieved after three to four volume changes.

Material balances with respect to *n*-butanol were calculated according to Eq. 2:

$$n\text{-Butanol}_{in} - n\text{-butanol}_{out} = n\text{-butanol}_{consumed} \quad (2)$$

Since residual *n*-butanol was present in the exit gas and the outflowing medium, the concentration of *n*-butanol was determined from both. Quantification of *n*-butanol from the gas phase was done by a flame ionization detector, and that from the medium by gas chromatography. The culture fluid was well mixed. Therefore, this system resembled a chemostat except that the carbon source was supplied from the inlet gas while the flow rate [F (l/h)] of the medium determined the specific growth rate (μ) by the dilution rate [D (1/h); Eq. 3]:

$$\mu = D = \frac{F}{V} \quad (3)$$

Analytical methods

The dry cell weight of the bacterial mixed culture growing on *n*-butanol was determined gravimetrically. Culture samples(5–25 ml) were passed through a preweighed nylon membrane filter (pore size 0.2 μm; Nalge, Rochester, USA) and dried overnight at 100 °C. An absorbance at 436 nm (d = 1 cm) correlated to 214 mg dcw/l. Protein from the culture supernatant was determined according to Bradford (1976) with bovine serum albumin as standard.

The concentration of organic carbon from the inlet and the exit gas was determined by flame ionization spectroscopy using a Compur Multi FID 100 (Hartmann & Braun, Frankfurt, Germany) and expressed as g organic carbon/Nm^3. The flame ionization detector was calibrated according to the manufacturer's protocol to convert the concentration from organic carbon to g *n*-butanol/Nm^3. *n*-Butanol from gas samples (100 μl) or from the medium supernatant (0.2 μl) was analysed using a gas chromatograph model 8500 (Perkin Elmer, Düsseldorf, Germany) equipped with a WG-11 column heated to 120 °C and a flame ionization detector. Nitrogen was used as carrier gas.

Total organic carbon was determined from the culture supernatant (100 μl) with a DIMA-TOC 100 total organic carbon analyser (Dimatec, Essen, Germany) at a temperature of 850 °C. Analyses were done in triplicate.

Potassium, either from whole cells or from the culture supernatant, was determined from 10-ml culture samples with an inductive coupled plasma emission spectrometer, (Liberty; Varian, Darmstadt, Germany) at 769 896 nm from 5-ml samples. To determine the potassium content of whole cells, 1.0 ml mixed culture was added to 0.2 ml silicone oil in an Eppendorf cup and subjected to centrifugation for 10 min at 10 000 rpm according to Bakker and Mangerich (1981). Potassium was released from the separated cells by boiling in 1.5 ml 5% (w/v) trichloroacetic acid. Cell debris was separated by centrifugation and 1.0 ml supernatant was diluted with 4.0 ml water and used for inductive coupled plasma analysis. The sensitivity for quantification was 0.1 mg potassium/litre.

Phosphate from the culture supernatant was determined by ion chromatography using model DX 300/IC2-0 equipped with a conductivity detector (Dionex, Idstein, Germany). The precolumn Dionex Anion Micro Membrane Suppressor AMMS-1 was used for suppression of the eluent and anion-exchange column Dionex Ion Pac AS9, and 3 mM sodium carbonate was used as eluent for anion separation. Prior to ion chromatography analysis, the culture supernatant (10 ml) was membrane-filtered (pore size 0.2 μm) and subjected to Chromabond C18 filtration (Macherey & Nagel, Düren, Germany) to eliminate traces of solvents. When required, samples were diluted up to tenfold and 1.0 ml was subjected to ion chromatography analysis. Phosphate from whole cells was determined spectrophotometrically according to the ammonium molybdate procedure after hydrolysis of organic phosphates with boiling perchloric acid as detailed by Daniels et al. (1994).

Results

Growth characteristics of batch cultures

The feasibility of a nutrient-limited continuous bioscrubber for waste gas treatment depends on how efficiently it absorbs pollutants and on its potential for reduced biomass formation as compared to nutrient-sufficient batch culture. Since it appeared not to be practical to maintain pure cultures for waste gas treatment, a mixed culture was used to compare the various cellular yields as well as the rates of *n*-butanol degradation. The mixed culture grew in batch culture in mineral medium at pH 7 and 30 °C with *n*-butanol as carbon source with a mean generation time of 0.43/h and a cellular yield of 0.90 g dcw/g *n*-butanol. To estimate suitable concentrations of biomass for phosphate- and potassium-limited cultivation the cellular yields of the respective nutrients were also determined from batch cultivation. For phosphate the cellular yield was in the range 16.4–34.5 g dcw/g phosphate, equivalent to 2.9%–6.1% (data not shown). For potassium the yield ranged from 286 g dcw/g to 333 g dcw/g potassium, equivalent to a potassium content 0.3%–0.35%. The range in yield on phosphate or potassium depended on the degree of formation of storage material.

Yield studies in the continuous bioscrubber

The conditions for formation of low biomass were determined from the dependence of (i) the cell concentration, (ii) the cellular yield and (iii) the specific degradation rate on the dilution rate of cells grown under *n*-butanol, phosphate or potassium limitation. The bioscrubber was continuously operated with the mixed culture grown in mineral media. *n*-Butanol was supplied from the gas phase (Fig. 1). Carbon was limited to a concentration of 8.74 g *n*-butanol/Nm^3 and a gas flow rate of 0.15 Nm^3/h at a dilution rate of 0.1–0.3/h, and 98%–100% of the *n*-butanol was degraded. The cell concentration X (g dcw/l) in the medium decreased with increasing dilution rate (Fig. 2A). At a dilution rate of 0.1/h the cellular yield was 0.53 g dcw/g of *n*-butanol (Table 1). This yield increased with dilution rate (Fig. 2B). Also, the productivity, P (g dcw l^{-1}h^{-1}), of biomass formation (Eq. 4) increased from 0.34 g dcw l^{-1}h^{-1} to 0.46 g dcw l^{-1}h^{-1} (data not shown):

Table 1 Characteristics of the *n*-butanol-degrading mixed culture in the continuous bioscrubber.

Growth-limiting substrate	Substrate supply rate (mg l^{-1} h^{-1})	Yield[a] (g dcw/ g *n*-butanol)	Specific *n*-butanol degradation rate[a] g *n*-butanol g dcw^{-1} h^{-1}	Maintenance coefficient g *n*-butanol g dcw^{-1} h^{-1}
n-Butanol	655.5	0.53	0.19	0.07
Phosphate	11.5	0.34	0.27	0.16
Potassium	0.5	0.31	0.32	0.34

[a] D 0.1/h

$$X \cdot D = P \quad (4)$$

The specific rate of *n*-butanol degradation was 0.19 g *n*-butanol g $dcw^{-1}h^{-1}$ at D = 0.1/h, increasing with dilution rate (Fig. 2C).

Phosphate-limited continuous growth was obtained when cells grew with *n*-butanol as carbon source and a phosphate concentration in the medium reduced to 115 mg phosphate/l. Under these conditions the cell concentration was significantly reduced to 2.0 g dcw/l at a dilution rate of 0.1/h as compared to 3.4 g dcw/l for *n*-butanol limitation (Fig. 2A). Under phosphate limitation the degree of *n*-butanol degradation was 84–94% (data not shown). The cellular yield with respect to *n*-butanol was 0.34 g dcw/g *n*-butanol at D = 0.1/h (Table 1). The yield decreased by 16% to 36%, depending on the dilution rate (Fig. 2B) as compared to limitation of *n*-butanol. In accordance with these data the specific degradation rate was generally higher as compared to *n*-butanol limitation, increasing with growth rate (Fig. 2C). This was especially evident when the bioscrubber was operated at a dilution rate of 0.1/h under different nutrient limitations.

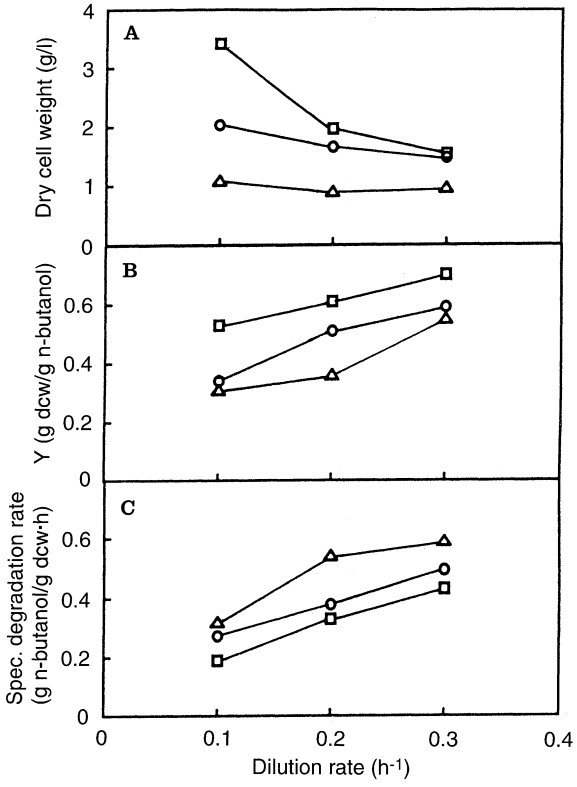

Fig. 2A–C Effect of nutrient limitation on culture characteristics in the continuous bioscrubber. The bioscrubber was operated as described in Materials and methods. Dependence of cell concentration (**A**), cellular yield (**B**) and specific *n*-butanol degradation rate (**C**) on the dilution rate. *n*-Butanol was supplied at a concentration of 8.74 g/Nm^3 and a rate of 0.15 Nm^3/h (□). Phosphate limitation was set to match the supply of *n*-butanol at 210 mg K_2HPO_4/litre (O). Potassium limitation was set to match the supply of *n*-butanol at 9.42 mg KCl per litre (△)

In waste gas treatment the crucial criteria are the degree and the rate of elimination of the pollutant. To evaluate the feasibility of mineral nutrient- limitation for waste gas treatment, phosphate was given as the substrate limiting the extent of growth, while the specific growth rate was kept constant at a dilution rate of 0.1/h. As expected, the rate of *n*-butanol degradation revealed a linear dependence on cell concentration (data not shown), resulting in a specific degradation rate of 0.27 g *n*-butanol/g dcw (Table 1, Fig. 2).

Potassium-limited continuous growth was obtained when the mixed culture grew with *n*-butanol as carbon source and a potassium concentration in the inlet medium reduced to 5.0 mg potassium/l. Under these conditions reduction in biomass was most pronounced. At D = 0.1/h the cell concentration was reduced by 65% to 1.2 g dcw/l and the yield reduced by 42% to 0.31 g dcw/g *n*-butanol (Table 1) as compared to that obtained with *n*-butanol limitation. In accordance to these data the specific degradation rate increased 1.7-fold. Both the yields and the specific degradation rates increased with increasing dilution rate (Fig. 2B, C).

The cellular yields decreased in the following order of limitation: *n*-butanol, phosphate and potassium, while the specific degradation rates increased. This observation was supported by the significant increase in maintenance requirements as deduced from the data shown in Fig. 2B. The maintenance requirement under *n*-butanol limitation was 0.07 g *n*-butanol g $dcw^{-1}h^{-1}$, increasing to 0.16 g and 0.34 g *n*-butanol g $dcw^{-1}h^{-1}$ under phosphate and potassium limitation respectively (Table 1). *n*-Butanol carbon may be converted to dry-cell-weight carbon, carbon dioxide or to a water-soluble, non-volatile organic product according to Eq. 5:

$$C_{butanol} = C_{dcw} + C_{CO_2} + C_{product} \quad (5)$$

Therefore, the medium was analysed for organic carbon. Media supernantants of cultures grown under *n*-butanol, phosphate, or potassium limitation were analysed for total organic carbon. Negligible amounts of 31–71 mg total organic C/l were observed, affecting the cellular yield by 2% at most. Also, no proteins were detected in the culture supernants (data not shown).

Effect of *n*-butanol concentration

n-Butanol is a constituent of solvents, being fairly water-soluble. Solvents are generally deleterious to cells especially to their cytoplasmic membrane. The consequences of a high *n*-butanol load in the inlet gas for the *n*-butanol degradation rate were examined under potassium-limited growth. Potassium limitation was chosen since it is well established that membrane-active compounds exert potassium efflux (Heipieper et al. 1991). Therefore, concentrations of 6.8–11.0 g *n*-butanol/Nm^3 were applied at a dilution rate of 0.1/h. Under these conditions, significant concentrations of 9.6–18.9 mM *n*-butanol accumulated in the medium at steady state (Fig. 3A).

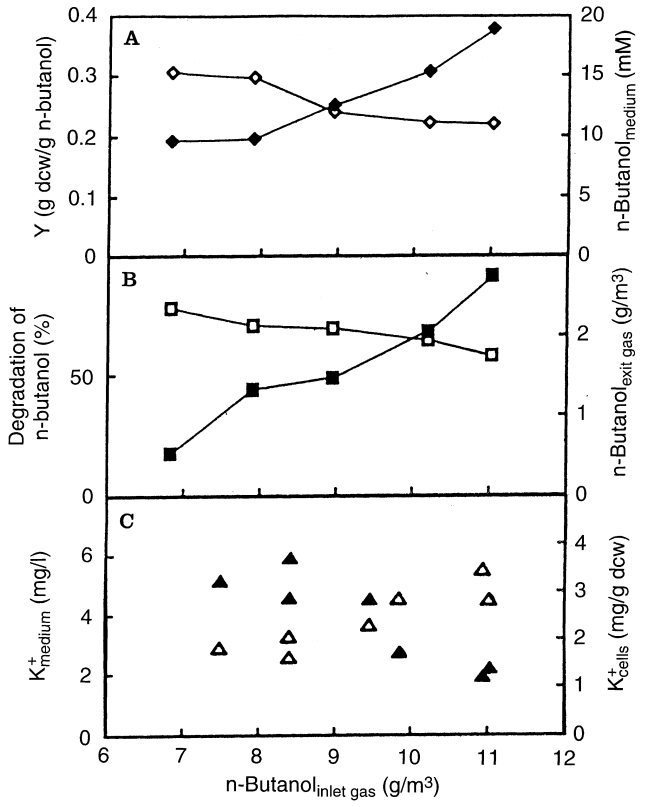

Fig. 3A–C Effect of *n*-butanol surplus on culture characteristics in the continuous bioscrubber. The bioscrubber was operated at $D = 0.1$/h with 9.42 mg KCl/l. The dependence of cellular yield (**A**), efficiency of *n*-butanol degradation (**B**) and potassium uptake (**C**) on the *n*-butanol concentration in the inlet gas. ◇ Cellular yield, ◆ concentration of *n*-butanol in the medium, □ degradation of *n*-butanol, ■ concentration of *n*-butanol in the exit gas, △ concentration of potassium in the medium, ▲ concentration of potassium in the cells

Because of the equilibrium of the gas/liquid phase, the concentration of *n*-butanol increased accordingly in the exit gas from 0.53 g to 2.75 g *n*-butanol/Nm^3. Under these growth conditions the yield of *n*-butanol decreased from 0.31 g dcw/l to 0.22 g dcw/l (Fig. 3A) and the total rate of degradation decreased from 78% to 65% (Fig. 3B). A significant fraction of potassium ions of 2.2–3.5 mg/l was observed in the medium at a concentration of 7.4–11.0 g *n*-butanol/Nm^3. The concentration of potassium in the medium increased with increasing *n*-butanol concentration. A complementary amount was associated with the cells (Fig. 3C). The potassium balance of cells grown under potassium limitation was generally close to 95% (data not shown).

Discussion

n-Butanol is frequently used as model substrate for waste gas purification and can be completely eliminated from such gases (Bardtke and Fischer 1983; Beyritz et al. 1989). Previous communications have predominantly dealt with the most important parameter, the degree of elimination. However, data on cellular yields and specific degradation rates have not been communicated. An organic substrate may be converted to organic products other than dry cell weight and carbon dioxide, according to Eq. 5. Conversion of glucose to gluconic acid, 2-oxogluconic acid, 2-oxoglutaric acid, exopolysaccharides or proteins was observed for *Klebsiella aerogenes* growing under limitation of ammonia or of potassium (Neijssel and Tempest 1975). We have used a mixed culture, although its composition with respect to bacterial species may have changed at different steady states, at different growth rates or under different nutrient limitations. Irrespective of possible changes in composition, the degree of elimination at steady state was constant with time. With a mixed culture for *n*-butanol degradation, evidence for its conversion to other products than dry cell weight and carbon dioxide has not been obtained, since only negligible amounts of non-volatile total organic carbon were observed in culture supernantants.

Therefore, a reduction in biomass could only be achieved by decreasing the efficiency in energy conversion and shifting the amount of carbon source required for the formation of biomass towards energy generation. Phosphate is essential for energy-rich anhydride formation in ATP synthesis, for example. Aerobic respiration is usually highly productive in ATP synthesis and very phosphate-demanding. Thus, phosphate limitation may have caused a decreased P/O quotient, i.e. decreasing the efficiency of phosphoanhydride formation. Phosphate limitation has been applied to *K. aerogenes* growing aerobically with glucose as carbon source. This resulted in an increased requirement for glucose and oxygen for respiration to satisfy the needs of cellular syntheses (Neijssel and Tempest 1976). The energetic requirement for potassium uptake and its consequences for growth under potassium limitation have been described by Mulder et al. (1986). Thus, the increased energy demand resulting from limitation of growth by potassium ions led to a decreased cellular yield. In fact, a further decrease in yield by *n*-butanol toxicity was achieved under potassium-limited growth of the *n*-butanol-degrading mixed culture. This was possibly because of the chemical nature of *n*-butanol as solvent. Very likely this property caused disorientation of the cytoplasmic membrane and led to potassium efflux at higher concentrations of *n*-butanol in the medium.

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