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EFFECT OF NICKEL(II) ON THE BIOMASS YIELD OF THE ACTIVATED SLUDGE

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ABSTRACT

Biomass yield of microorganisms is important in applied microbiology since it is the ultimate factor determining the amount of product produced regardless of whether product is growth-linked or not. In the case of environmental microbiology the opposite is true and minimizing the biomass produced, or the sludge in the relevant jargon, often is the prime goal. In this paper, a unique means of manipulating the microbial biomass yield of a heterogeneous culture to fulfil either of the two goals is presented. 5.0 mgl^{-1} Ni(II) in the feed composition to a completely mixed, once- through, activated sludge was found to induce the observed biomass yield of the microbial culture developed from sewage. As compared with the base-line study without Ni(II), where the reactor received synthetic wastewater only, true biomass yield was found to have increased along with the increased decay constant with the net effect of lowering observed biomass yield drastically at lower dilution rates and increasing it over that observed in the base-line study at higher dilution rates. At 10.0 mgl^{-1} influent Ni(II) concentration the culture conditions almost reverted back to the base- line study and at 25 mgl^{-1} Ni(II) concentration a truly steady-state condition could not be attained. Copyright © 1996 IAWQ. Published by Elsevier Science Ltd.

KEYWORDS

Activated sludge; biomass yield; Ni(II); maintenance; stimulation.

INTRODUCTION

Although trace amounts of certain metals are required by microbes for optimum growth, heavy metals such as Ni, Cu, Zn, Cd and Pb, which may be fairly soluble in aqueous environments under certain circumstances, are usually regarded as toxic to most microorganisms even at very low concentrations (Hartz *et al.*, 1985). However, given the proper acclimatization period, microorganisms can often successfully adapt to these metals.

Research to this day has basically concentrated on unacclimatized microorganisms and has often neglected the fact that the tolerance of microorganisms to toxic metals may be greatly be enhanced by proper acclimatization. During acclimatization either resistant organisms are selected and/or microorganisms are adapted to the metal environment metabolically. According to the current hypothesis the metal-ion-damaged enzymes are replaced by new enzyme synthesis, or new shunt pathways are created to replace the inoperative ones (Chang *et al.*, 1986).

Toxicity of heavy metals to biological systems depends on several factors including metal species and concentration, pH, influent type and strength, amount of biomass present in the system and the extent of

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system acclimatization. One common finding in metal toxicity studies is that increased biological solids retention time decreases metal toxicity to the activated sludge (Neufeld and Hermann, 1976; Weber and Sherrard, 1980; Hartz *et al.*, 1985; Sujarittanonta and Sherrard, 1981; Trahern *et al.*, 1980). In a summary report, Barth *et al.* (1965) indicated that the aerobic biological treatment processes which they have tested could tolerate up to 10 mg l^{-1} of single or combination doses of Cr, Cu, Ni or Zn without significant loss in treatment efficiency. Sujarittanonta and Sherrard (1981) showed that the maximum biomass yield, Y_m, and the maintenance coefficient, b, values for the reactors receiving 1.0 and 5.0 mgl^{-1} Ni(II) were greater than those without Ni(II).

There is an indication that anaerobic bacteria are also stimulated by Ni(II). Speece *et al.* (1983) showed that when a culture of methanogenic bacteria was supplied with 10 mg l^{-1} Ni(II), the observed biomass yield increased almost fourfold as compared to the base-line condition without Ni(II). The specific substrate utilization rate of methanogenic culture was also found to have increased by about 3 to 6-fold upon addition of yeast extract and Ni(II) into the feed solution. Clearly, the increase in the uptake rate was largely due to the Ni(II) alone.

There is now accumulating evidence that a variety of biological systems, notably blue- green bacteria (Henrikson and DaSilva, 1978), a culture of Chlorella (Bertrand and Dewolf, 1967) and activities of certain enzymes (Hutchinson, 1973) are stimulated by Ni. Conversely, Poon and Bhayani (1971) applied inhibited enzyme kinetics to simulate oxygen uptake by bacteria in the presence of metal ions, i.e. Cu, Cr, Ni, Ag and Zn. As a result of respirometric studies they have concluded that all these metals noncompetetively inhibit bacterial metabolism. Moreover, Lewandowski *et al.* (1985) evaluated the value for noncompetetive inhibition coefficient, KI, for Cr(VI) as 87 mg l^{-1}, by using an oxygen electrode. They, too, adapted Michaelis-Menten type enzyme kinetics to describe the effects of Cr(VI) on sewage microorganisms.

BIOMASS YIELD AS AN INDEX OF METABOLIC COUPLING

The presence of microbial growth was initially the sole interest of microbiologists and often a slight turbidity in the test tubes was taken as sufficient indication of growth and the phenomenon was investigated no further. However, upon realization of the biotechnological potential inherent in microorganisms, attention has since shifted to the biomass yield of microorganisms, simply for the sake of higher product gains. In the case of environmental microbiology, the purpose is different and the interest in biomass yield is often towards minimising the excess sludge produced in biological waste treatment. Biomass yield studies are now proven to be excellent tools for studying microbial energetics and metabolical efficiency.

In the present paper the effect of Ni(II) ions on the biomass yields of mixed cultures of microorganisms, continuously cultured in a once-through activated sludge, will be discussed. The details of the dilute-out studies and calculated biokinetic constants maximum specific growth rate (μm), half-substrate saturation coefficient (K_s) etc., have already been published in a previous paper (Yetis and Gokcay, 1989).

Biomass yield is expressed as an increase in microbial biomass at the expense of a growth-limiting substrate; Pirt (1975) conceptualizes microorganisms as requiring energy for both growth and maintenance purposes. Accordingly, the observed substrate change is not solely associated with growth but additional substrate expenditure for microbial functions other than growth is also valid. For example, endogenous metabolism is conceived as a process where microorganisms continually oxidize some of the cellular matter at a constant specific rate so as to turn over their macromolecular components (Herbert, 1958), such as proteins (Pine, 1972) and peptidoglycan (DeBoer *et al.*, 1981). Metabolic functions such as maintaining solute gradients across cellular membranes (Huetings *et al.*, 1979) and motility also consume energy and some portion of the substrate is evidently utilized at rates proportional to the growth rate to fulfil these requirements (Tempest and Neijssel, 1984).

The addition of protonophorous uncouplers, i.e. 2,4-dinitrophenol (DNP), greatly enhances respiration by microbes while biomass synthesis is almost completely halted (Neijsel, 1976). The lipophilic DNP, here, is

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thought to cause leakage of protons hence diminishing the proton gradient. However, how ammonia or sulphate-limited cultures dissipate their surplus respiratory energies remains to be explored.

Tempest and Neijssel (1984) envisage several possible mechanisms by which microorganisms dispose of their excess metabolic energies. These may be grouped as: the direct involvement of an ATPase, futile cycles, metabolites acting as uncouplers, modification of respiratory chain components and/or activities, mechanisms that bypass substrate-level phosphorylation steps and thereby metabolically uncouple glycolysis. It is, however, not certain to what extent such "energy spilling reactions" account for the maintenance uptake. Gommers *et al.* (1988) and others (Tempest and Neijssel, 1984) indicated that growth is essentially energy-limited. In the case of highly reduced substrates such as glycerol (Heuting and Tempest, 1977), methanol (Jones, 1977), ethane (Katz and Rognstad, 1978) or when the microorganism is furnished with two substrates (Gommers *et al.*, 1988) e.g. one designed to fulfil its carbon requirement and the other, for example methanol, for energy; then growth may be carbon-limited rather than energy, and the observed biomass yield may approach near maximum. However, 100% carbon-carbon conversion has never been demonstrated. The reason was recently explained by Gommers *et al.* (1988) in terms of obligatory CO_2 production during biosynthesis. These workers have also calculated that above a heat of combustion of 550 kJ/mol carbon, substrates approach carbon limitation and below this value it is basically energy-limited growth.

Several equations have been proposed to describe the effect of maintenance on substrate utilization by microbes. The initial model proposed by Pirt (1965), Eq. 1 and 2, assumes a constant rate of maintenance others suggest two components for maintenance, one that is growth-rate dependent and one that is constant (Pirt, 1982). The latter equation(s) are not fully defined in practice and hence will not be dealt with here.

$$q/\mu = q_g/\mu + q_m/\mu \qquad (1)$$

or substituting, $Y = \mu/q$

$$1/Y = 1/Y_g + q_m * 1/\mu \qquad (2)$$

where q_g and q_m are the specific substrate uptake rate constants for growth and maintenance respectively, Y is apparent or observed biomass yield and Y_g is biomass yield for growth (also known as the true yield, Y_T, or the maximum yield Y_m) respectively. Finally q and μ are the observed substrate uptake and growth rate constants respectively. Occasionally the growth-independent q/m is replaced by the symbol m and referred as the maintenance rate or ration. The Eq. 2 may then be re-written in the form:

$$1/Y = 1/Y_g + m/\mu \qquad (3)$$

where, m is the specific rate of maintenance. Conversely, some workers prefer to use the"microbial decay" concept, introduced by Herbert (1958), rather than the "maintenance". The decay concept assumes that a portion of the synthesized biomass is lost due to endogenous respiration

$$(dx/dt)_g = (dx/dt)_T - (dx/dt)_E \qquad (4)$$

where (dx/dt) represents the rate of growth, and subscripts g, T and E indicate observed growth, total growth and endogenous decay respectively. After the necessary substitutions and re-arrangements, the following equation is obtained (Benefield and Randall, 1980):

$$q = 1/Y_T \, \mu + k_d/Y_T \qquad (5)$$

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where k_d is the specific decay rate coefficient. The constants k_d and Y_T may be evaluated from the slope and the intercept of the straight line drawn by plotting q versus μ. Although the two equations, Eqns. 3 and 5, differ significantly in concept, they are mathematically identical. It follows that

$$m = k_d/Y_T \tag{6}$$

MATERIAL AND METHODS

A synthetic wastewater was used throughout the experiments. The chemical composition of the synthetic wastewater is given in Table 1. Proteose peptone (Oxoid) was added to the medium to give 1221.7 mg l^{-1} peptone or 650 mg l^{-1} protein concentration for source of organic carbon and nitrogen. The phosphate salt was introduced to the medium to provide both buffer action and a source of phosphorus to the microorganisms. The culture was carbon-limited in all experiments.

A once-through completely mixed activated sludge unit was simulated in the laboratory by using a Gallenkamp-500 series modular fermenter. The working liquid volume was 1 litre in the fermenter. Inflow and outflow to the reactor were maintained by a dual function, nutrient/harvest, peristaltic pump. Aeration was supplied by an aerator module. Effective mixing in the reactor was achieved by the magnetic stirrer module and by sparged air under the impeller. A temperature controller module was used to keep the temperature constant at 25°C throughout the experiments.

Table 1. Composition of synthetic wastewater

Constituent	Concentration (mgl^{-1})
Proteose-pepton	1221.7
NaCl	407.4
Na_2SO_4	44.6
K_2HPO_4	44.6
$MgCl_2.6H_2$	3.7
$FeCl_2.2H_2O$	3.7
$CaCl_2.2H_2O$	3.7
$MnSO_4$	$57*10^{-3}$
H_2MoO_4	$31*10^{-3}$
NaOH	$8*10^{-3}$
$ZnSO_4$	$46*10^{-3}$
$CoSO_4$	$49*10^{-3}$
$CuSO_4$	$76*10^{-3}$

The mixed liquor suspended solids, MLSS, determined by filtering samples through 0.45 µm pore size membrane filters and drying at 105°C. Substrate concentrations were determined in centrifuged aliquots either by measuring protein according to the Folin-Ciocalteu method (Lowry *et al.*, 1951) or by standard COD analysis (APHA 1975). During the use of the Folin-Ciocalteu method, a 30% bacto bovine albumin (Difco) was used as reference. Protein concentrations were converted to COD values according to the ratio COD/protein = 2 as determined in this study. This ratio was more or less constant in the influent and effluent and ranged between 2.04 and 2.09.

EXPERIMENTAL RESULTS

The base-line studies

The base-line studies were carried out by using a synthetic wastewater devoid of Ni(II). The dilute-out curve obtained by varying the dilution rate, D, of the reactor by changing influent flow rate is summarized in Fig.1. In order to calculate the mathematical constants expressing growth yield, i.e. Y_T (or Y_m) and k_d, the computed q was plotted versus the D according to Eq. 5, as depicted in Fig. 2. The values of these constants were then determined respectively from the slope and intercept of the straight line obtained in this figure.

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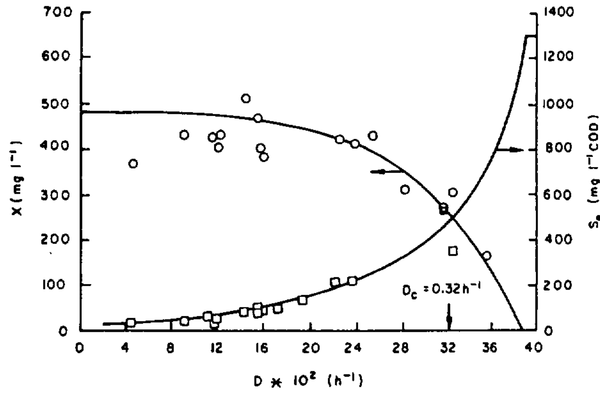

Figure 1. Dilute-out curve for synthetic wastewater devoid of Ni(II).

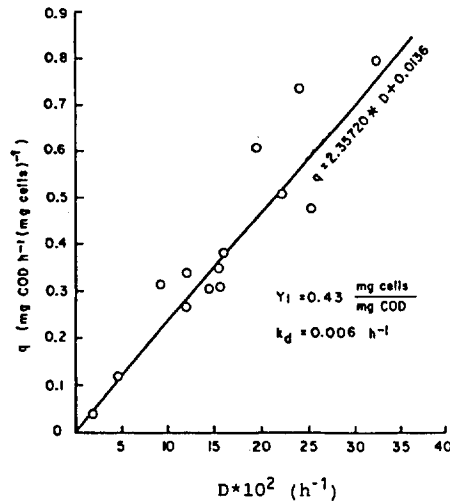

Figure 2. Influence of D on the specific rate of COD consumption of activated sludge fed with a synthetic wastewater devoid of Ni(II).

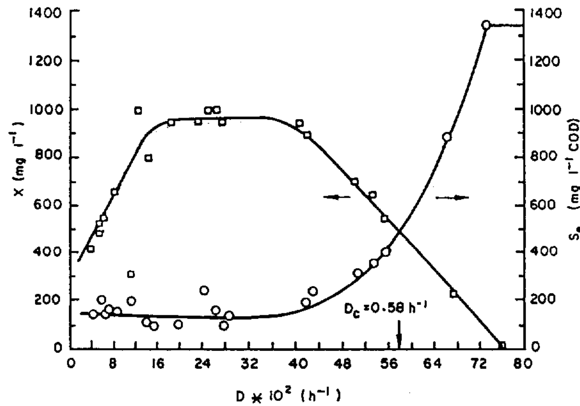

Figure 3. Dilute-out curve for synthetic wastewater containing 5.0 mg l^{-1} Ni(II).

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Effect of 5.0 mg l^{-1} Ni(II)

Prior to the experiments with 5.0 mg l^{-1} Ni(II), the activated sludge culture was acclimatized to nickel for two months. During acclimatization the nickel dose in the feed was gradually increased in 0.5 mg l^{-1} increments until 5.0 mg l^{-1} was reached. The continuous feeding of 5.0 mg l^{-1} nickel after acclimatization was over did not adversely affect the culture, but on the contrary stimulated its performance. As can be seen from Fig. 3, the presence of 5 mg l^{-1} Ni(II) caused mixed liquor suspended solids (MLSS) to double in concentration between the D's 0.18 h^{-1} and 0.36 h^{-1} as compared to the base-line study. In parallel to the Y, the Y_t was calculated from Fig. 4 and was found to have also risen from the earlier 0.43 value in the base-line study to 1.25 mg cells/mg COD in this range of D's.

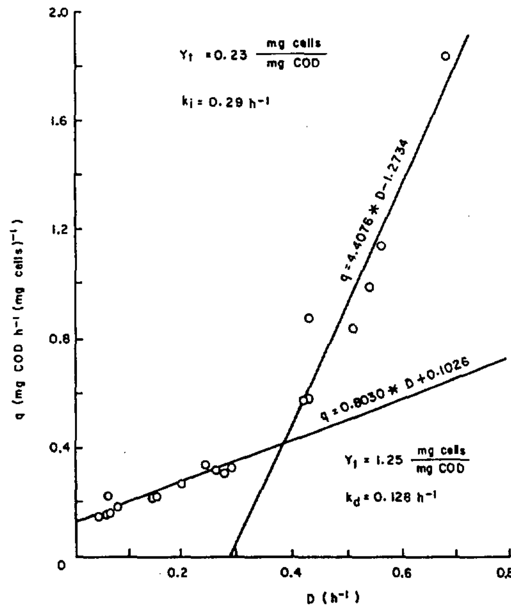

Figure 4. Influence of D on the synthetic rate of COD consumption of activated sludge fed with a synthetic wastewater containing 5.0 mg l^{-1} Ni(II).

Curiously, the Y_t value for the culture decreased to 0.23 mg cells/mg COD at D's greater than 0.36 h^{-1}. The k_d, also changed with respect to 0.36 h^{-1}. The k_d value was calculated as 0.127 h^{-1} for $D<0.36$ h^{-1} which was far greater than the 0.006 h^{-1} value obtained for the base-line study. For $D>0.36$ h^{-1} the k_d value was computed as -0.29 h^{-1} from Fig. 4. It was unique for the authors to experience a minus sign for the decay rate coefficient. Owing to the fact that the decay process is itself a negative process, the $-k_d$ coefficient is substituted by "k_i"; and tentatively termed here as the "coefficient of induction". In an attempt to fit a curve to Fig. 4, quadratic regression for up to the ninth degree were tried without a successful fit. Explicitly, the fit obtained by the two intersecting straight lines, as shown in Fig. 4, was much better than what would be obtained with a curvature going through these data points.

Effect of 10.0 mg l^{-1} Ni(II)

Experiments with 5.0 mg l^{-1} Ni(II) were followed by 10.0 mg l^{-1} Ni(II). After the completion of the usual acclimatization procedure, the shape of the dilute-out curve was found to have reverted to the base-line, as shown in Fig. 5. From this figure it is readily seen that the MLSS concentration is slightly higher than the base-line values and it was constant for the most part of the dilute-out curve as it was for the base-line study. The q versus D plot in Fig. 6 revealed -0.01 h^{-1} for the k_d, and 0.5 for the Y_T. The minus sign before the k_d value was again interpreted as induction. As can be seen from these values the performance of the culture, in

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terms of absolute growth, was almost back to the base-line study, though Y_T was somewhat higher, i.e. 0.5 in 10 mg l^{-1} Ni(II) versus 0.43 in the base-line. The k_d, which was 0.006 h^{-1} in the base-line study, was calculated as -0.01 h^{-1} for the 10 mg l^{-1} nickel case.

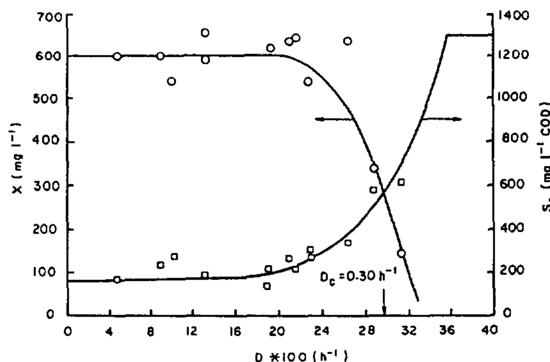

Figure 5. Dilute-out curve for synthetic wastewater containing 10.0 mg l^{-1} Ni(II).

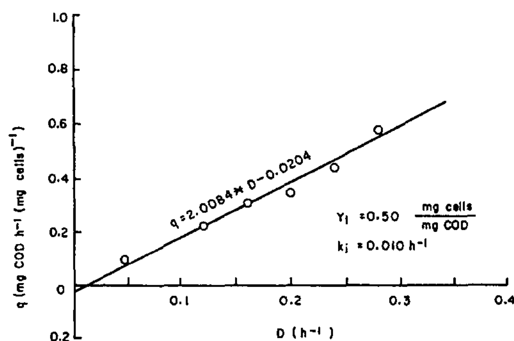

Figure 6. Influence of D on the specific rate of COD consumption of activated sludge fed with a synthetic wastewater containing 10.0 mg l^{-1} Ni(II).

Effect of 25.0 mg l^{-1} Ni(II)

Finally, the Ni(II) concentration in the influent was increased to 25.0 mg l^{-1} and the culture was acclimatized in the usual manner. However, the culture could not be maintained at a truly steady-state condition as the effluent COD and MLSS concentrations started to fluctuate at a random manner. Curiously, the unit could be kept at a constant D at 0.14h^{-1} for over one month without completely washing out, but with random wash-out and no-wash-out cycles.

DISCUSSION AND CONCLUSION

From the foregoing presentation it is apparent that the maintenance or the decay concepts do not hold in the case of 5.0 and 10.0 mg l^{-1} influent Ni(II) concentrations. Moreover, considering the antagonism, a minus k_d value was tentatively substituted here with an "induction coefficient". Conceptually, an induction of this nature is difficult to explain, if at all possible. For example, induction of yield in accordance with the "maintenance" concept would mean that cells create additional substrate rather than consume for maintenance. Alternatively, considering the decay concept, then induction would mean creation of additional biomass over the maximum possible. Clearly, both arguments are most unconventional in terms of thermodynamics. A possible explanation is speculated below.

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In Table 2 the computed Y_T and Y_g values, as calculated from Eq. 6, are tabulated. It follows that the Y_t is a mathematical coefficient while Y_g is truly biological. It is seen from this table that the observed yield increased from 0.40 in the base-line study to 0.88 at 5.0 mg l^{-1} Ni(II) and dropped back to 0.58 (g dry biomass/g COD) at 10.0 mg l^{-1} Ni(II) case. According to Gommers *et al.* (1988), the observed maximum yield of microorganisms is a linear function of the heat of combustion of the growth-limiting substrate for up to 550 kJ/mole C, as depicted in Fig. 7 reproduced from their work.

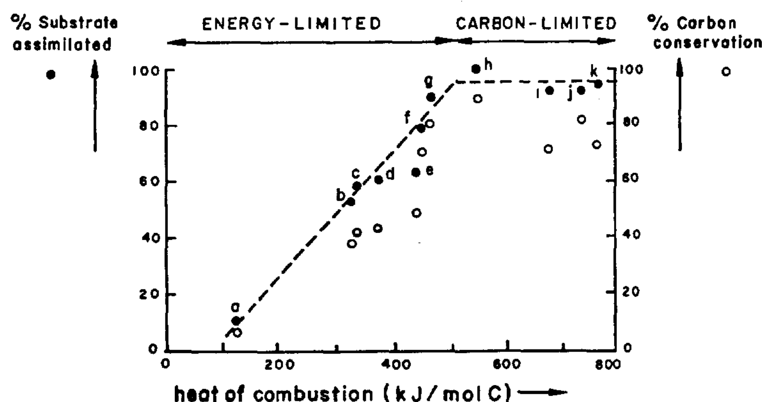

Figure 7. Amount of a single limiting carbon and energy source converted to biomass (expressed as percentage carbon conversion (open symbols) and as a percentage of substrate assimilated (closed systems) as a function of the heat of combustion of the substrate). The letters in the graph correspond with the substrate listed below: (a) oxalate, (b) malate, (c) fumarate, (d) succinate, (e) acetate, (f) lactate, (g) glucose, (h) glycerol, (i) ethanol, (j) methanol, (k) ethane (source: Gommers *et al.*, 1988).

Table 2. Summary of the biomass yield data

		Y_g	Y_T	Y_g	Y_T	k_d
		g dry biomass / g COD		g dry biomass / g substrate		h^{-1}
Base-line		0.38	0.43	0.40	0.46	0.006
5.0 mgl^{-1} Ni(II)	D>0.4 h^{-1}	0.83	0.23	0.88	0.25	-0.29
	D<0.4 h^{-1}	0.85	1.25	0.90	1.33	0.128
10.0 mgl^{-1} Ni(II)		0.54	0.50	0.58	0.53	-0.01

In an attempt to relate the observed yield values presented in Table 2 to the heat of combustion of the substrate, a homogenous sample from the Oxoid peptone, which was invariably used for the preparation of the feed solution in this study, was analyzed for its calorific value and for its carbon and hydrogen contents by using a bomb calorimeter and a C-H-N analyzer, respectively. On the basis of this analysis it was calculated that the Oxoid peptone contains 350 kJ/mol C. Consulting the "calorific value versus observed yield" figure in Table 3, it is seen that this corresponds to approximately 38% substrate assimilated, or 0.38 g/g observed yield, very close to that observed in the base-line study whereas, from the same figure, the heat of combustion corresponding to 88% substrate assimilation in the case of 5.0 mg l^{-1} Ni(II) is found close to 500 kJ/molC. It is, at the moment, uncertain how the extra 150 kJ/mole-C may be accounted for. Clearly, 5 mg l^{-1} Ni(II) by itself cannot account for the additional energy gained, as nickel in this state is not combustible to yield energy. It is the authors' speculation that in microbiological systems Ni ions, and perhaps other transitory metal ions too, at certain "critical" concentrations, catalyze "extended oxidation" of the substrate, producing energy of combustion in excess of that possible with oxygen at normal conditions; and that it is possible for the biological systems to harness this energy. Given the fact that induction of free radical and oxygen radical formation during combustion with oxygen in the presence of metals is a well known fact in metal chemistry.

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It is believed by these authors that such an "extended oxidation" may be well worth pursuing in research as a valuable opportunity to exploit such a fruitful phenomenon may exist in the field of environmental and/or process biotechnology in future.

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