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Bacterial growth on *N,N*-dimethylformamide: implications for the biotreatment of industrial wastewater

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The degradation of *N,N*-dimethylformamide (DMF) by bacterial consortia was investigated under aerobic, fermentative and nitrate-reducing conditions and a variety of salt concentrations (0.2%, 4% and 7% NaCl w/v) and pH values (5 and 7). Optimization of degradation conditions was studied to provide information and recommendations for large-scale biological treatment processes. Under aerobic conditions, mineralization of DMF (200 mg l^{-1}, 2.7 mM) was achieved under all combinations of salinity and pH. The rate of bacterial growth decreased with increasing salinity. Changes in the salt concentration and pH still resulted in mineralization and unchanged yield of bacterial cells. At 0.2% NaCl and either pH 5 or 7, growth occurred on DMF in the range 0.2–1 g l^{-1}. However, cell yield decreased with increasing concentrations of DMF. Under conditions of 0.2% NaCl, pH 7 and 4% NaCl, pH 5, growth on DMF at 5 g l^{-1} resulted in the production of an intermediate that was detected using gas chromatography (GC). It is proposed that the intermediate was dimethylamine, and its persistence in growth media was attributed to suppressed growth as a result of an increase in pH. A culture capable of degrading DMF under nitrate-reducing conditions was obtained at 0.2% NaCl and pH 7, but not at more saline and acidic conditions. Growth and degradation of DMF were considerably slower under these conditions compared with aerobic conditions. Fermentative degradation of DMF was not observed. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 8–16.

Keywords: *N,N*-dimethylformamide; salinity; pH; nitrate-reducing; denitrifying; effluent

Introduction

N,N-Dimethylformamide (DMF) [$(CH_3)_2NCHO$] is a common industrial chemical, with a worldwide production of ca. 300,000 tons in 1991 [9]. It is mainly employed in the textile and pharmaceutical industries as a versatile solvent, being miscible in both water and organic solvents. Its widespread use means that it is commonly found in industrial effluents. DMF is biodegradable and bacterial cultures that use it under aerobic conditions as a sole source of carbon and energy have been isolated [3,4,11,12]. Two pathways have been proposed for its degradation [3]: one pathway uses DMFase activity to produce dimethylamine which is then converted to monomethylamine; the other pathway converts methyl groups to formaldehyde with methylformamide and formamide as intermediates [3]. Despite evidence of its degradability, degradation of DMF in treatment plants of industrial effluents is variable [11].

Most studies of biodegradation of xenobiotic chemicals are carried out under conditions of low salinity and neutral pH, and activated sludge systems are usually protected from high salinity and high pH by pre-treatment of wastewater entering the aeration tank. Pre-treatment incurs costs. Consequently, the treatment of DMF-containing wastewater under conditions of low pH and elevated salinity, which would dispense with the need for pre-treatment, would have obvious benefits. As part of a study on the degradation of DMF in a model effluent (containing DMF, toluene, aniline, *o*-dichlorobenzene, an azo dye, methanol, sodium acetate and isopropanol), degradation of DMF was investigated under a range of salinities and pH values. As biological wastewater treatment processes can contain aerobic and anaerobic phases, a variety of environmental conditions was investigated to determine under what conditions DMF could be degraded effectively.

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Materials and methods

Materials

Unless otherwise stated, all chemicals were obtained from Merck, Sigma or Aldrich and were of Analar or equivalent grade.

Enrichment cultures

Enrichment cultures were set up using DMF as the sole carbon and nitrogen source at a concentration of 2.74 mM (200 mg l^{-1}). Degradation of DMF was investigated at 27°C under the following salt and pH conditions: 0.2% NaCl (w/v), pH 7; 4% NaCl, pH 7; 7% NaCl, pH 7; 0.2% NaCl, pH 5; 4% NaCl, pH 5 and 7% NaCl, pH 5.

For each test condition, a series of test cultures was set up containing different terminal electron acceptors: oxygen (O_2 — aerobic conditions), anaerobic with no added electron acceptor (fermentative conditions) and anaerobic with added nitrate (NO_3^- — denitrifying/nitrate-reducing conditions). To provide fermentative and nitrate-reducing conditions, oxygen was removed from the inoculum and media under a vacuum, and the culture vessels were then sparged with oxygen-free nitrogen. Aerobic test cultures were agitated on an orbital shaker at 100 rpm, whereas other test cultures remained static.

Inoculum

Enrichment cultures were set up using one of two inocula — these contained materials from diverse sources differing in pH, salinity

and availability of oxygen to maximise the chances of obtaining DMF-degrading microbial cultures.

(i) Inoculum A was used for the aerobic test cultures and contained the following (ml): oil-field-produced water (200); marine mud (100); acid peat bog water (100); acidic peat soil (from two sources; 2×100); acidic iron water from an iron-bearing moorland stream (100); river water and mud from the river Aire at Beal Weir (100 of each); activated sludge from a laboratory reactor treating a model effluent at 0.2% NaCl (w/v), pH 7 (10); activated sludge from a laboratory reactor treating a model effluent at 4% NaCl (w/v), pH 7 (10). After thorough mixing, the inoculum was divided and pH was adjusted to either 5 or 7 using 1 M NaOH or 1 M HCl.

(ii) Inoculum B was used for anaerobic enrichments under nitrate-reducing and fermentative conditions and contained inoculum A supplemented with anaerobic digester sludge (500 ml) from the Dewsbury Sewage Treatment Works.

Mineral media

Enrichment cultures were supplemented with one of two media.

(i) Inoculum A was supplemented with synthetic sewage mineral salts (SSMS) medium which contained the following (mg l^{-1}); KH_2PO_4 (5000); $CaCl_2{\cdot}2H_2O$ (22); $MgSO_4{\cdot}7H_2O$ (40); $FeCl_3{\cdot}6H_2O$ (7.5); $Na_2MoO_4{\cdot}2H_2O$ (1.1); $ZnSO_4{\cdot}7H_2O$ (0.7); $MnSO_4{\cdot}4H_2O$ (0.6); $CuSO_4{\cdot}5H_2O$ (0.6); $CoCl_2{\cdot}6H_2O$ (0.6). Sodium chloride was added to a final concentration of 0.2%, 4% or 7% (w/v) and the pH of the medium was adjusted to pH 5 or 7 using 1 M NaOH or 1 M HCl.

(ii) For nitrate-reducing conditions, inoculum B was supplemented with SSMS medium, $NaHCO_3$ (840 mg l^{-1}) and $NaNO_3$ (850 mg l^{-1}).

Test culture vessels

Aerobic test cultures (250 ml culture media) were grown in 500 ml conical flasks, with cotton wool bungs. Fermentative and nitrate-reducing test cultures were grown in 160 ml serum bottles. Bottles were sealed with butyl rubber septa which were held in place with aluminium seals (Pierce and Warriner Ltd, UK).

Experimental procedure

Bacterial growth on DMF as the sole carbon and nitrogen source was measured as optical density (540 nm) using a spectrophotometer (Pye Unicam SP6-200). Plots of $\log_{10}(OD_{540})$ *vs.* time were used to calculate the mean generation time. Biomass was determined using standard methods [1]. GF/F glass microfiber filters (0.7 μm, Whatman, UK) were used for greater retention of bacterial biomass. Yield could then be calculated and expressed as biomass generated (dry weight) per unit test substrate utilized. DMF and possible intermediates were analyzed using gas chromatography (GC). Mineralization of DMF results in the release of ammonia. Ammonia was analyzed using the indophenol method [5]. The concentration of nitrate in nitrate-reducing test cultures was determined using the brucine sulfate colorimetric method [7].

For routine cultivation, DMF was used as the sole carbon and nitrogen source at 0.2 g l^{-1}, but in some experiments, higher concentrations (0.5, 0.75, 1, 2.5, 5, 7.5, 10 or 20 g l^{-1}) were used.

Growth on DMF in the presence of additional sources of nitrogen

Unless otherwise stated, DMF was used as the sole source of carbon and nitrogen. However, industrial wastewater is likely to contain other available sources of nitrogen. Consequently, the 0.2% NaCl, pH 7 aerobic test culture was grown on DMF in the presence of NH_4Cl (850 mg l^{-1}).

The effect of salinity and pH changes on bacterial growth

Two methods were used to study the effect of salinity and changes in pH on bacterial growth.

Method (1): 0.2% NaCl, pH 7 test culture: Samples from the 0.2% NaCl pH 7 aerobic test culture were used to inoculate (10% v/v) fresh mineral medium containing 0.2% NaCl, pH 7 or 4% NaCl, pH 5. Growth on DMF (200 mg l^{-1}) was measured for a period of 24 h, after which a sample of the culture (corresponding to 10% v/v inoculum) was transferred to the original growth conditions (i.e., 0.2% NaCl, pH 7). Growth on DMF (200 mg l^{-1}) was measured at OD_{540}.

Method (2): 4% NaCl, pH 5 test culture: Three samples from the 4% NaCl, pH 5 aerobic test culture (corresponding to 10% v/v) were removed and the bacteria harvested at 27,000×*g* for 10 min. The supernatants were discarded and the pellets were resuspended in a small volume of fresh mineral salts media (0.2% NaCl, pH 7; 4% NaCl, pH 5 and 7% NaCl, pH 5). The bacterial suspension was then used to inoculate fresh mineral salts media (0.2% NaCl, pH 7; 4% NaCl, pH 5 and 7% NaCl, pH 5). Discarding the supernatant minimised the transfer of NaCl, NH_3-N and carbon into the fresh mineral salts medium. Growth on DMF (200 mg l^{-1}) was measured and after DMF was completely removed from the growth medium, a sample of the culture was used to inoculate fresh 4% NaCl, pH 5 medium (i.e., the original growth conditions).

Bacterial growth on alternative carbon sources

Samples were taken from three test cultures (0.2% NaCl, pH 7 and 4% NaCl, pH 5 aerobic; 0.2% NaCl, pH 7 under nitrate-reducing conditions) and the bacteria were harvested by centrifugation at 27,000×*g*. Each pellet was resuspended in fresh growth medium (at the appropriate NaCl concentration and pH), which was then used to inoculate fresh growth medium (at the appropriate NaCl concentration and pH), containing one of the following carbon sources: DMF, dimethylamine (DMA), monomethylamine (MMA), methylformamide (MF), formamide, formic acid, formaldehyde, ethanol, methanol or acetic acid. The first seven consist of DMF and known intermediates in its aerobic degradation [3]. Although it contains three C atoms, DMF does not contain a C–C bond, and consequently, its degradation can be similar to C_1 compounds. Ghisalba *et al.* [3] found that DMF-degrading microorganisms could be characterised on the basis of their growth on simple C_1 or C_2 compounds. Consequently, growth on methanol, ethanol and acetic acid was investigated.

Gas chromatography

Samples taken from test cultures were analyzed by GC (Pye Unicam 204 GC) following direct injection of aqueous under-

Table 1 DMF enrichment cultures

Conditions	NaCl (% w/v)	pH	MGT (h)	μ (h^{-1})	Yield ($g\ g^{-1}$)	Ammonia produced (mM)	Notes
Aerobic, shaken	0.2	7	14.02±2.98 (6)	0.051±0.01 (6)	0.52±0.12 (6)	2.45±0.49 (4)	mixed bacteria
	4	7	*	*	0.4	–	mixed bacteria
	7	7	*	*	0.32	–	mixed bacteria
	0.2	5	9.76±1.78 (5)	0.073±0.013 (5)	0.48±0.1(6)	2.66±0.12 (4)	mixed bacteria
	4	5	21.02±3.7(7)	0.034±0.006 (7)	0.58 (1)	2.5±0.43 (4)	mixed bacteria
	7	5	33.8 (1)	0.021 (1)	–	–	mixed bacteria
NO_3^--reducing, static	0.2	7	52.8	0.013	–	–	–
	4	5			unsuccessful		
	7	5			unsuccessful		
Fermentative, static	0.2	7			unsuccessful		
	4	5			unsuccessful		
	7	5			unsuccessful		

*Growth occurred, but as the result of bacterial aggregation, growth rates could not be calculated.
MGT = mean generation time. Yield ($g\ g^{-1}$) is expressed as biomass generated (dry weight) / unit test substrate utilized. μ is the specific growth rate. These values are averages, with the value in parentheses denoting the number of samples.

ivatized samples. A 10% Carbowax 20 M on Unisorb AW DMCS 80/100 mesh column was used (Jones Chromatography). Separation was achieved using the following conditions: oven temperature, 140°C; detector and injector temperature, 200°C; carrier (nitrogen) flow rate, 40 ml min^{-1}; air flow rate, 440 ml min^{-1}; and hydrogen flow rate, 44 ml min^{-1}. The following retention times were recorded (min): formaldehyde, 0.27; monomethylamine (MMA), 0.3; dimethylamine (DMA), 0.4; DMF, 1.0; and methylformamide, 2.9. Formic acid and formamide could not be detected by GC.

Results and discussion

Isolation of cultures degrading DMF

Results from the enrichment cultures are shown in Table 1. Under aerobic conditions, it was possible to obtain DMF-degrading cultures at all the combinations of pH and salt concentrations tested. However, growth at 7% NaCl, pH 5 was often unsuccessful, growth sometimes failing after a few successful subcultures. Under these circumstances, a sample of the 4% NaCl, pH 5 test culture was successively subcultured into increasingly saline media (of 1% NaCl increments). Although the degradation of DMF has previously been reported [3,4,11,12], this is the first study to report its degradation under saline and/or acidic conditions.

Degradation of DMF under anaerobic conditions

Several attempts were made to obtain a culture capable of degrading DMF under fermentative conditions. However, this was not successful. Only one culture was obtained under nitrate-reducing conditions (0.2% NaCl, pH 7). Attempts were made to gradually increase the salinity and decrease the pH of this nitrate-reducing culture in small steps (0.5% NaCl, or 0.5 pH units). However, this was met with limited success — cultures would not grow consistently under the new conditions and were termed *unstable*. In cultures capable of degradation of DMF under nitrate-reducing conditions, DMF removal was paralleled by growth and removal of nitrate (data not shown).

Although degradation of DMF occurred under nitrate-reducing conditions, it took considerably longer than under aerobic conditions at the same salinity and pH (the growth rate, μ, being

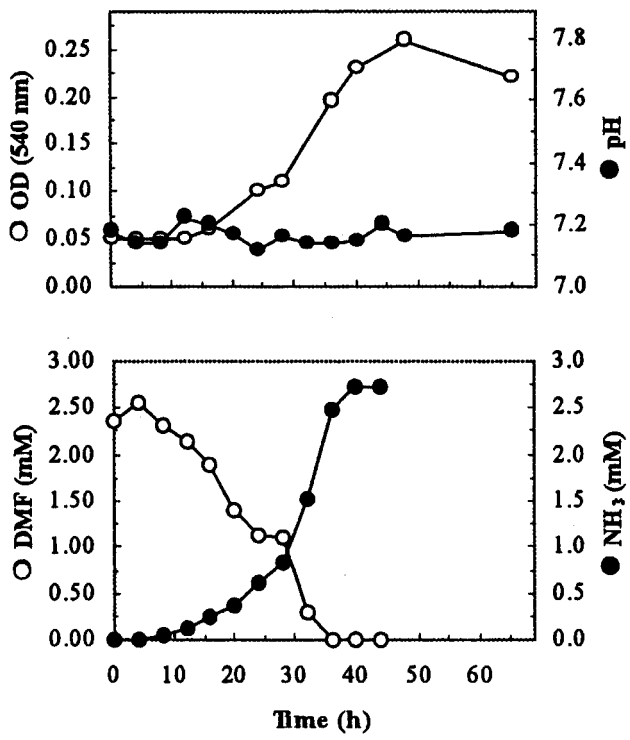

Figure 1 Growth characteristics of the 0.2% NaCl, pH 7 aerobic test culture on DMF at 200 mg l^{-1} (2.74 mM).

Table 2 Growth parameters obtained from aerobic DMF test cultures

(a) Growth of the 0.2% NaCl, pH 5 and 0.2% NaCl, pH 7 aerobic test culture on 0.2 to 1.0 g l^{-1} DMF

Parameter	0.2% NaCl, pH 5 DMF g l^{-1} (mM)				0.2% NaCl, pH 7 DMF g l^{-1} (mM)			
	0.2 (2.7)	0.5 (6.9)	0.75 (10.3)	1.0 (13.7)	0.2 (2.7)	0.5 (6.9)	0.75 (10.3)	1.0 (13.7)
MGT (h)	10.7	8.8	8.8	10.3	10.9	8.3	8.9	8.2
Specific growth rate, μ (h^{-1})	0.065	0.079	0.079	0.067	0.064	0.084	0.078	0.085
DMF removal (%)	100	100	100	100	100	100	100	100
DMF-N released as NH_3 (%)	100	89	84	96	100	100	100	87
Yield (g g^{-1})	0.43	0.43	0.34	0.32	0.49	0.34	0.33	0.26

(b) Growth of the 0.2% NaCl, pH 7 test culture on 0.2 to 20 g l^{-1} DMF

Parameter	0.2% NaCl, pH 7 DMF g l^{-1} (mM)						
	0.2 (2.7)	1.0 (13.7)	2.5 (34.3)	5.0 (68.5)	7.5 (103)	10 (137)	20 (274)
MGT (h)	–	–	–	10.24	14.13	13.7	11.8
Specific growth rate, μ (h^{-1})	–	–	–	0.068	0.049	0.051	0.059
DMF removal (%)	100	100	100	100	100	100	100
Intermediate production	ND	ND	ND	T	T	T	T
pH range	7	7–7.8	7–8.6	7–8.6	7–9.2†	7–9.2†	7–9.2†
Yield (g g^{-1})	0.62	0.26	0.21	0.18	–	0.13	0.16

(c) Growth of the 4% NaCl, pH 5 test culture on 0.2 to 20 g l^{-1} DMF

Parameter	4% NaCl, pH 5 DMF g l^{-1} (mM)						
	0.2 (2.7)	1.0 (13.7)	2.5 (34.3)	5.0 (68.5)	7.5 (103)	10 (137)	20 (274)
MGT (h)	53.9	–	–	59.6	–	76	–
Specific growth rate, μ (h^{-1})	0.013	–	–	0.012	–	0.009	–
DMF removal (%)	100	100	100	100	100	100	100
Intermediate production	T	T	T	T	T	P	P
pH range	5.3–5.7	5.3–6	5.3–6.5	5.3*	5.3*	5.3*	5.3*
Yield (g g^{-1})	–	–	0.34	0.24	0.2	0.15	0.13

MGT=mean generation time. Yield (g g^{-1}) is expressed as biomass generated (dry weight)/unit test substrate utilized. μ is the specific growth rate. These values are averages, with the value in parentheses denoting the number of samples. ND=not detected, T=transient. An intermediate was produced but was utilized during the experiment. P=persistent. An intermediate was produced and was not utilized during the experiment. When the pH reached 8* or 9†, it was adjusted to pH 5 or 7, respectively, with 1 M HCl.

only about a quarter of that observed under aerobic conditions; Table 1). We believe this to be the first report of anaerobic degradation of DMF coupled to nitrate reduction. Anaerobic degradation of DMF under methanogenic conditions was implied by COD removal data presented by Stronach et al. [10] but was not proved by specific analysis. The observation of DMF mineralization linked to denitrification means that anaerobic treatment of this compound may be feasible. However, the low growth rates observed under nitrate-reducing conditions suggest that aerobic treatment would probably be preferred when treating DMF-containing wastewater.

Degradation of DMF by selected aerobic microbial cultures

During growth on DMF, the following measurements were taken: growth measured at OD_{540}, DMF removal, ammonia production and changes in pH. There are two known pathways for aerobic degradation of DMF [3]. The end product of both pathways is ammonia, and an assay for ammonia was used to give an indication of mineralization. An example of growth on DMF (0.2% NaCl, pH 7, aerobic conditions), with reference to these parameters, is shown in Figure 1.

Each aerobic test culture was composed of a consortium of bacterial strains. The mean generation time increased with an increase in salinity (Table 1). In all stable test cultures, DMF (at 2.74 mM) was completely removed from the growth media and no intermediates were detected using GC. The yield was generally ca. 0.4–0.5 g biomass generated/g DMF utilized, and under three conditions (0.2% NaCl, pH 5; 0.2% NaCl, pH 7; and 4% NaCl, pH 5), 72–100% DMF-derived nitrogen was released as ammonia (Table 1). At this concentration, the amount of ammonia produced suggested that the three aerobic test cultures completely mineralized DMF. Bacterial aggregation during growth was evident in some cultures, which caused sampling problems. Furthermore, measurement of culture turbidity was difficult and unreliable for these cultures. Consequently, the mean generation times could not be obtained for all test cultures.

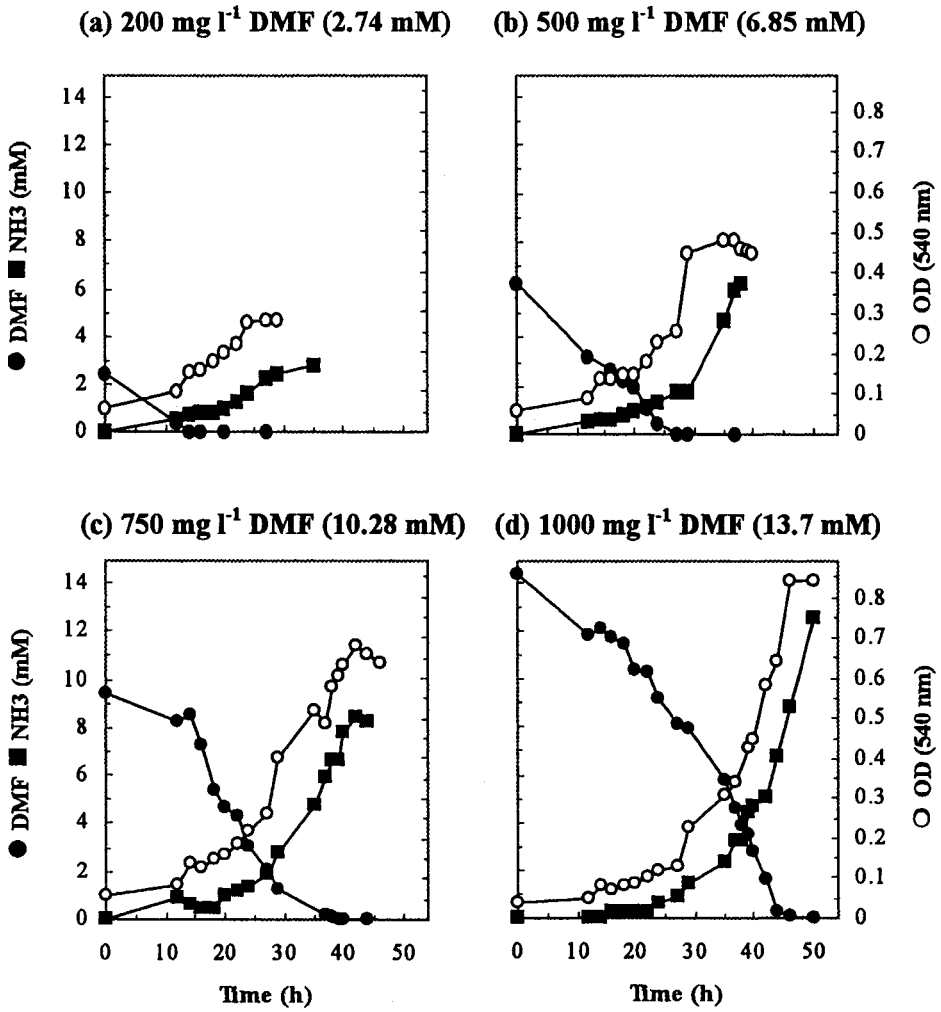

Figure 2 Effect of DMF concentration on growth (0.2% NaCl, pH 7, aerobic test culture).

Further experiments were performed using three of the aerobic test cultures. The 0.2% NaCl, pH 7 test culture was used as a control system (industrial wastewater is traditionally diluted and neutralized prior to biological treatment) to be compared with the more extreme conditions of 4% NaCl, pH 5 and 7% NaCl, pH 5 (used to reflect possible industrial wastewater conditions prior to biological treatment).

Growth on DMF in the presence of additional sources of nitrogen

Growth of the 0.2% NaCl, pH 7 aerobic test culture was not adversely affected by the presence of an additional nitrogen source. The mean generation time (19.5 h) when no additional nitrogen source was provided was not significantly different from that obtained (18.4 h) when NH_4Cl was added to the medium.

The effect of DMF concentration on bacterial growth

Growth at a range of DMF concentrations was investigated, and the results are shown in Table 2 and Figure 2 (Table 2a; Figure 2, 0.2–1.0 g l^{-1}; Table 2b, 0.2–20 g l^{-1}). For concentrations of DMF in the range 0.2–1 g l^{-1}, a high proportion of DMF nitrogen (84–100%) was released as ammonia (Figure 2(ii)). However, the initial rate of DMF removal was greater than the rate of ammonia production (Figures 1 and 2), suggesting the production of a nitrogenous intermediate(s) that was later converted to ammonia. This was shown to be the case when growth at higher concentrations (e.g., 5 g l^{-1}) resulted in production of an intermediate which was observed by GC. The intermediate was either transient or persistent. An example of growth on DMF (5 g l^{-1}), DMF removal, ammonia production and the production of an intermediate are shown in Figure 3. Although test cultures were buffered, bacterial growth was accompanied by an increase in pH. Generally, an intermediate was not detected when the growth media remained within a pH range of 7–8.6. When the pH increased above 8.6 (with a starting pH near neutral), an intermediate was detected, and its presence appeared to be transitory. However, above pH 9, the presence of the intermediate was more persistent, and the pH had to be adjusted to neutral (using 1 M HCl) before the intermediate was completely removed from the growth medium (Table 2b). When the starting pH of the growth medium was 5 but increased to 8 during growth, the intermediate appeared to be more

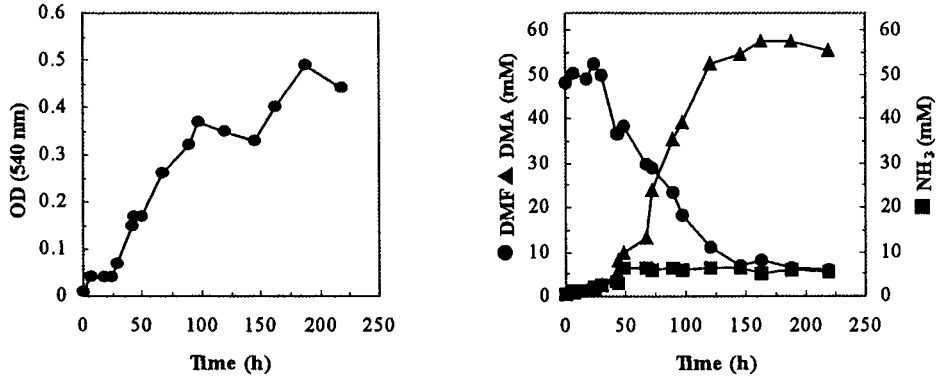

Figure 3 Growth of the 0.2% NaCl, pH 7 aerobic test culture on DMF at 5 g l^{-1} (68.5 mM). The data shown assume that the intermediate was dimethylamine (DMA).

persistent, and growth was interrupted. When the pH of the media was returned to pH 5 (using 1 M HCl), growth resumed and the concentration of the intermediate decreased (Table 2c).

When the 0.2% NaCl, pH 7 test culture was exposed to a DMF concentration of 5 g l^{-1}, DMF was completely removed from the system. However, there was relatively little bacterial growth, suggesting that there was little carbon available for growth. Furthermore, low ammonia production (corresponding to ca. 13% DMF-derived nitrogen) also suggested that little of the DMF had been completely mineralized and that the intermediate was probably nitrogenous. The retention time of the intermediate suggested that the compound was either dimethylamine or monomethylamine. From GC calibrations of dimethylamine and monomethylamine and stoichiometric calculations, it was tentatively suggested that the intermediate was more likely to be dimethylamine (Figure 3). Other possible intermediates, such as formaldehyde, methylformamide and formic acid, were inconsistent with the observed results.

A sample of the 0.2% NaCl, pH 7 (5 g l^{-1}) test culture containing the intermediate was diluted with fresh/mineral salts medium (0.2% NaCl, pH 7) without an added carbon source (1 vol test culture:4 vol mineral salts medium). As the bacterial cells were not harvested, carbon from the original growth medium (i.e., the intermediate detected by GC) was also transferred into the fresh medium and was used as the sole source of carbon and nitrogen. Bacterial growth on this intermediate — decrease in intermediate concentration and increase in ammonia concentration — is shown in Figure 4. No other intermediates were detected using GC. If the intermediate was dimethylamine, it represented a concentration of ca. 8.5 mM. This calculation seemed feasible given the turbidity of the bacterial culture and the absence of any further intermediates (as shown by GC). Furthermore, assuming it was DMA, ca. 93% organic nitrogen in the intermediate was released as ammonia, which was feasible and could indicate mineralization.

The 7% NaCl, pH 5 test culture was not stable at the time of the experiment, and consequently, its ability to grow at higher concentrations of DMF was not investigated.

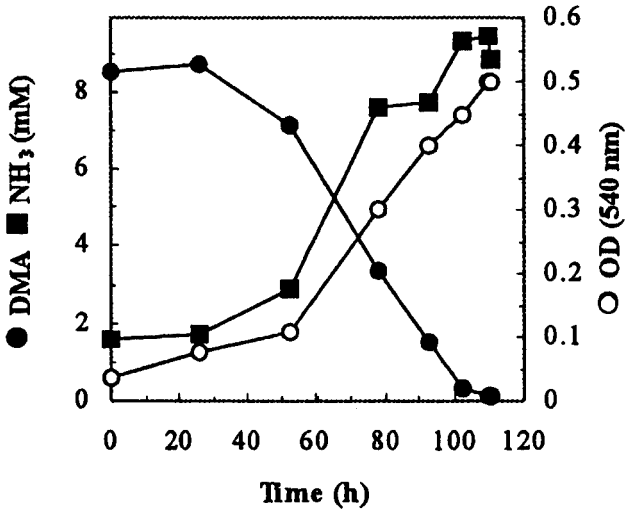

Figure 4 Growth of the 0.2% NaCl, pH 7 aerobic test culture on a DMF intermediate. No additional source of carbon or nitrogen was added to the growth medium. It was assumed that the intermediate was dimethylamine (DMA).

Growth on alternative carbon sources

Growth was tested on alternative carbon sources (200 mg l^{-1}) using the test cultures maintained at 0.2% NaCl, pH 7 (under both aerobic and nitrate-reducing conditions) and at 4% NaCl, pH 5 (under aerobic conditions) and was observed on most substrates (Table 3), including methanol, ethanol and acetic acid.

Possible pathways for DMF degradation

As mentioned earlier, there are two known pathways for aerobic degradation of DMF [3]: pathway 1 uses DMFase activity to produce dimethylamine (later converted to monomethylamine) and formic acid. This pathway is used by *Pseudomonas* spp., isolated from sewage sludge originating from an industrial sewage treatment works [3]. Pathway 2 converts methyl groups to formaldehyde, with methylformamide and formamide as intermediates. Formamide is then converted to formic acid and an ammonium salt. This pathway is used by *Pseudomonas* spp., isolated from an urban refuse deposit [3]. The end product of both pathways is ammonia. Of the test cultures studied (0.2% NaCl, pH 7 aerobic and nitrate-reducing and 4% NaCl, pH 5 aerobic), there was growth on substrates that are intermediates in both pathways, e.g., dimethylamine (pathway 1) and methylformamide (pathway 2). Consequently, degradation of DMF could be a result of both pathways operating, especially as each test culture consisted of a bacterial consortium, or alternatively, enzymes of some pathways

Table 3 Growth of DMF-degrading mixed cultures on alternative substrates

Substrate	Growth conditions		
	0.2% NaCl, pH 7 aerobic	4% NaCl, pH 5 aerobic	0.2% NaCl, pH 7 NO_3^--reducing
Dimethylformamide	+	+	+
Methylformamide	+	+	+
Formamide	+	–	+
Dimethylamine	+	+	+
Monomethylamine	+	+	–
Formaldehyde*	+	+	+
Formic acid*	+	–	+
Acetic acid*	+	+	+
Ethanol*	+	+	+
Methanol*	+	+	+

*Growth media were supplemented with NH_4Cl as a source of nitrogen at a concentration of 380 mg l^{-1}. In all other cases, the substrate was used as the sole source of nitrogen. "+" indicates growth (i.e., an increase in optical density), whereas "–" indicates no growth.
Substrates were used at a concentration of 200 mg l^{-1}.

may have a low specificity. However, the accumulation of DMA, in media with a high concentration of DMF, suggests that the DMFase pathway is probably predominant. Transitory accumulation of DMA during DMF degradation has been reported [6]. However, compounds that were not used as carbon sources by some cultures are intermediates in the later stages of the DMF degradation pathway (e.g., formamide, monomethylamine and formic acid). All three test cultures grew on methanol, ethanol and acetic acid. Overall, the results suggested that the three test cultures contained populations of facultative methylotrophs.

The effect of salinity and pH changes on bacterial growth

0.2% NaCl, pH 7 test culture: When the 0.2% NaCl, pH 7 culture was grown at 4% NaCl, pH 5 for 24 h, the results in Table 4 show that there was little effect on growth. The mean generation time was 13.9 h, which was comparable with that given by the control culture (kept at 0.2% NaCl, pH 7), and about 60% of that of the culture isolated and routinely maintained at 4% NaCl, pH 5. When a sample of the bacterial suspension was subcultured under the original growth conditions, i.e., 0.2% NaCl, pH 7, the mean generation time remained comparable to the 0.2% NaCl, pH 7 control (Table 4). In all cases, there was 100% DMF removal, 69–89% DMF-derived nitrogen was released as ammonia and no intermediates were detected using GC.

4% NaCl, pH 5 test culture: The 4% NaCl, pH 5 aerobic test culture was grown under the following conditions; 0.2% NaCl, pH 7, 4% NaCl, pH 5 and 7% NaCl, pH 5. When DMF was completely removed from the growth medium, a sample of the culture was returned to its original growth conditions, i.e., 4% NaCl, pH 5. The results are shown in Table 5. The 4% NaCl, pH 5 test culture represented the control system (i.e., growth was studied at 4% NaCl, pH 5 throughout). When the 4% NaCl, pH 5 test culture was grown under conditions of 0.2% NaCl, pH 7, the mean generation time decreased to 15.4 h, which was similar to the test culture routinely maintained at 0.2% NaCl, pH 7 (Tables 1 and 4). When comparing test cultures grown under conditions of 0.2% NaCl, pH 7 or 4% NaCl, pH 5, either routinely or over a short period, the yield was ca. 0.5 g g^{-1}, DMF was completely removed, and 74–100% DMF-derived nitrogen was released as ammonia (Table 5a and b). No intermediates were detected using GC. The 4% NaCl, pH 5 test culture grew more quickly under conditions of 0.2% NaCl, pH 7 and the ammonia data suggested that DMF was mineralized. From these data, it seemed that the 4% NaCl, pH 5 test

Table 4 Effects on the 0.2% NaCl, pH 7 test culture of a 24-hour change in salinity and pH to 4% NaCl, pH 5

Parameter	Growth conditions (% NaCl (w/v), pH) 0.2, 7 test culture			
	0–24 h, 0.2, 7 control	24–48 h, 0.2, 7 control	0–24 h, 4, 5	24–48 h, 0.2, 7
MGT (h)	12.9	12.5	13.9	15
Specific growth rate, μ (h^{-1})	0.054	0.055	0.050	0.046
DMF removal (%)	100	100	100	100
Intermediate production	ND	ND	ND	ND
DMF-N released as NH_3 (%)	70	82	69	89

*MGT of the 4% NaCl, pH 5 culture was in the range of 20–23 h.
The 0.2% NaCl, pH 7 test culture was grown at 0.2% NaCl, pH 7 or 4% NaCl, pH 5 for 24 h. After 24 h, a sample from each flask was used to inoculate (10% v/v inoculum) fresh medium (0.2% NaCl, pH 7). MGT=mean generation time. Yield (g g^{-1}) is expressed as biomass generated (dry weight)/unit test substrate utilised. μ is the specific growth rate. ND=not detected.

Table 5 Effects on the 4% NaCl, pH 5 test culture of changing salinity and pH to 0.2% NaCl, pH 7 or 7% NaCl, pH 5

(a) Growth at 0.2% NaCl, pH 7

Parameter	Growth conditions	
	(i) 0.2% pH 7	(ii) 4% pH 5
MGT (h)	15.4 (2)	21.5 (2)
Specific growth rate, μ (h^{-1})	0.045 (2)	0.032 (2)
DMF removal (%)	100 (2)	100 (2)
DMF-N released as NH_3 (%)	100 (2)	84–100 (2)
Yield (g g^{-1})	0.49 (1)	0.49 (2)

(b) Growth at 4% NaCl, pH 5 (control)

Parameter	Growth conditions	
	(i) 4% pH 5	(ii) 4% pH 5
MGT (h)	20.4 (1)	22 (2)
Specific growth rate, μ (h^{-1})	0.034 (1)	0.031 (2)
DMF removal (%)	100 (2)	100 (2)
DMF-N released as NH_3 (%)	72–93 (2)	74–100 (2)
Yield (g g^{-1})	0.52 (1)	0.41 (1)

(c) Growth at 7% NaCl, pH 5

Parameter	Growth conditions	
	(i) 7% pH 5	(ii) 4% pH 5
MGT (h)	33.8 (1)	*
Specific growth rate, μ (h^{-1})	0.021 (1)	*
DMF removal (%)	100 (2)	100 (2)
DMF-N released as NH_3 (%)	–	100 (2)
Yield (g g^{-1})	0.63 (1)	0.51 (2)

*Growth occurred, but as a result of bacterial aggregation, growth rates could not be calculated.
The 4% NaCl, pH 5 test culture was grown under conditions of 0.2% NaCl, pH 7; 4% NaCl, pH 5; or 7% NaCl, pH 5 (termed growth condition i). After DMF was completely removed from the growth medium, the culture was used to inoculate fresh 4% NaCl, pH 5 medium (termed growth condition ii). MGT=mean generation time. Yield (g g^{-1}) is expressed as biomass generated (dry weight)/unit test substrate utilised. μ is the specific growth rate. The value in the parentheses denotes the number of samples.

culture was more likely to contain organisms that were halotolerant/acid-tolerant rather than halophilic/acidophilic.

When the 4% NaCl, pH 5 test culture was grown under 7% NaCl, pH 5, the mean generation time increased to 33.8 h (Table 5c). When a sample of this culture was returned to the original conditions, i.e., 4% NaCl, pH 5, bacterial aggregation was observed, which caused sampling problems, and the measurement of culture turbidity was unreliable. Consequently, the mean generation time could not be calculated. However, DMF was completely removed in ca. 50 h, no intermediates were detected using GC, 100% DMF-derived nitrogen was released as ammonia, and the yield was calculated to be 0.51 g g^{-1} (Table 5c). The values of these parameters were similar to those of the test culture routinely maintained at 4% NaCl, pH 5 (Table 5b and c). Although the observed aggregation caused sampling problems, it is possible that clumping of biomass could be advantageous in terms of sludge settling during the operation of a large-scale biological treatment process.

With two aerobic test cultures (0.2% NaCl, pH 7 and 4% NaCl, pH 5), bacterial growth and complete removal of DMF still occurred when the salinity and pH of the growth medium were changed. The rate of growth of the 0.2% NaCl, pH 7 test culture was not greatly affected when the growth conditions were changed to 4% NaCl, pH 5, and DMF was still mineralized. When the 4% NaCl, pH 5 test culture was grown at 7% NaCl, the mean generation time increased, which further suggested the presence of halotolerant rather than halophilic organisms.

Implications for degradation of DMF in biological effluent treatment plants

This study has shown that DMF can be degraded aerobically at elevated salinity and at relatively low pH. Furthermore, the results suggest that periods of salinity and pH fluctuation would still be accompanied by DMF degradation, although rates of DMF removal may change (generally decreasing with higher and increasing with lower salinity). Thus, it may be feasible to operate treatment of effluent for DMF degradation at neutral pH and at salinities up to 7% NaCl, and at pH 5 at salinities up to 4% NaCl. In the context of activated sludge treatment plants, the fact that growth rates on DMF (which are in any case low) decreased in more saline and acid conditions means that careful attention should be paid to operating

parameters such as the mean cell retention time (MCRT). Control of the MCRT is important if slow-growing populations are not to be lost from activated sludge [2,8]. Therefore, it is likely that operators of activated sludge plants will need to monitor MCRT during treatment of wastes containing DMF, especially if the wastes are of elevated salinity.

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