Pergamon

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POLYACRYLAMIDE AS AN ORGANIC NITROGEN SOURCE FOR SOIL MICROORGANISMS WITH POTENTIAL EFFECTS ON INORGANIC SOIL NITROGEN IN AGRICULTURAL SOIL

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Summary—Linear polyacrylamide (PAM) is gaining considerable acceptance as an effective anti-erosion additive in irrigation water. The potential effects of repeated PAM application on soil microbial ecology and the potential for biotransformation of this polymer in soils are not completely known. Untreated and PAM-treated soils (coarse-silty, mixed, mesic Durixerollic Calciorthids) were collected from agricultural fields near Kimberly, ID. Soils were analyzed to determine the effects of PAM treatment on bacterial counts and inorganic N concentrations and the potential for PAM biotransformation. Culturable heterotrophic bacterial numbers were significantly elevated in PAM-treated soil for the plot planted to potatoes; this effect was not observed in the plot planted to dry pink beans. Total bacterial numbers, determined by AODC, were not altered by PAM treatment in any of the soils sampled. Polyacrylamide-treated soil planted to potatoes contained significantly higher concentrations of NO_3^- and NH_3 (36.7 ± 2.20 and 1.30 ± 0.3 mg kg^{-1}, respectively) than did untreated soil (10.7 ± 2.30 and 0.50 ± 0.02 mg kg^{-1}, respectively). For bean field soil there was no difference between treated and untreated soil inorganic N concentrations. Enrichment cultures generated from PAM-treated and untreated soils utilized PAM as sole N source, but not as sole C source. While the monomeric constituents of PAM, acrylamide and acrylic acid, both supported bacterial growth as sole C source, the PAM polymer did not. Enrichment cultures that used PAM for N exhibited amidase activity specific for PAM as well as smaller aliphatic amides. Utilization of PAM for N, but not for C, indicates that ultimately PAM may be converted into long chain polyacrylate, which may be further degraded by physical and biological mechanisms or be incorporated into organic matter.

INTRODUCTION

Linear polyacrylamide (PAM) is currently being added to irrigation water to control soil erosion associated with flood-furrow and sprinkler irrigation. Applied at 10 mg l^{-1} to irrigation water early in the set (0.7–3.0 kg ha^{-1}), PAM has reduced furrow sediment loss in Idaho by an average of 94%; soil stabilization is due to physical binding of PAM to soil aggregates (Lentz *et al.*, 1992). The type of PAM that is most effective in reducing erosion has a very high molecular weight ($1–2 \times 10^7$ MW) and a linear, anionic configuration. It is a copolymer made up of approximately 82 mol% acrylamide subunits and 18 mol% acrylate subunits (Lentz *et al.*, 1992), which exists as an anionic species in an environment of pH 6 or above. The anionic characteristic is responsible for the soil stabilizing effect via complex electrostatic interactions with soil aggregates (Fig. 1).

Little is known about microbial biotransformation of the PAM polymer, the effects of PAM on the microbial ecology of agricultural soils or the potential effects of biotransformation products on the system. There are very few reports of PAM biodegradation (Azzam *et al.*, 1983; Senft, 1993; Grula *et al.*, 1994). An increase in growth response of aerobic bacteria (Nadler and Steinberger, 1993) and increased microbial biomass (Steinberger *et al.*, 1993) in soil treated with PAM have been reported, but the cause of these effects are not known. Perhaps the major reason for this lack of information is the physical nature of the chemical; once it is added to soil it appears to bind irreversibly (Nadler *et al.*, 1992) and cannot be extracted in its intact form. This technical difficulty prohibits direct non-isotopic biodegradation studies in soil and hence restricts investigations to indirect measures of *in situ* environmental effect or biodegradation in alternative media.

The potential for biotransformation of PAM and the effects of PAM application on soil bacterial

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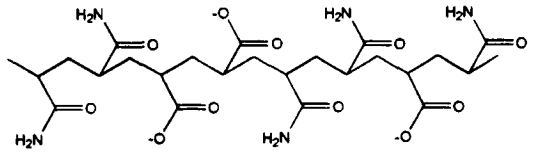

Fig. 1. Molecular structure of a small segment of the PAM polymer used as an anti-erosion irrigation additive.

communities and nutrient cycling were addressed in our study.

MATERIALS AND METHODS

Field site

The PAM experimental field site located at the USDA Agricultural Research Service (ARS) in Kimberly, Idaho, consists of a total area of 2 ha. The soil is Portneuf silt loam (coarse-silty, mixed, mesic, Durixerollic Calciorthid), with a pH of approximately 7.8 and a 2–8% $CaCO_3$ equivalent in the A horizon.

Two PAM experimental fields, identified as "managed-inflow" and "long-term", had been established at the time of field sampling. Furrows were spaced 56 cm apart and 179 m long in the "long term" field and were 91 cm apart and 114 m long in the "managed-inflow" field. Only alternate furrows were irrigated. Furrows were assigned treatments in random order with three replicate furrows for each treatment.

Treatments in the "managed inflow" study consisted of low-inflow PAM (22.6 l min^{-1}), high-inflow PAM (45.1 l min^{-1}) and paired non-treated controls. This field was planted to potatoes (*Solanum tuberosum* cv Russet Burbank) and will be referred to hereafter as the potato field.

The "long-term" study, which was established in 1992, included two treatments, PAM, and a petroleum distillate-PAM emulsion, along with paired non-treated controls. The plot was planted to dry beans (*Phaseolus vulgaris* L. cv Viva Pink) and will be referred to hereafter as the bean field.

The potato field received one application of N fertilizer as a urea-ammonium-nitrate mix (89.6 kg ha^{-1}, solution 32) at the beginning of the season; the bean field received no N fertilizer. In both experimental fields PAM-treated irrigation water contained 10 mg l^{-1} PAM during the initial advance, followed by delivery of untreated water for the remainder of the irrigation set (usually 12 h duration). During the growing season the potato field was generally irrigated twice a week, while the bean field was irrigated at 10 to 14 d intervals. The total amount of PAM applied during the growing season was 5.6 kg ha^{-1} in the bean field, 13.0 kg ha^{-1} in the low-flow potato plot and 19.91 kg ha^{-1} in the high-flow potato plot.

The total amounts of amide-N applied to each of the test plots in the form of PAM were approximately 896 g N ha^{-1}, 2080 g N ha^{-1}, and 3190 g N ha^{-1} for the bean plot, low-flow potato plot and high-flow potato plot, respectively.

Soil collection

Soil (approximately 100 g) was collected from the upper 3 cm of each sampled furrow bottom, at distances of 0.5 m, 1.0 m and 1.5 m from the irrigation inlet in August of 1994. Samples were combined, sieved (4 mm), and stored for no more than 24 h at 4°C. Triplicate composite samples were obtained for each crop type and each treatment.

Chemicals

Polyacrylamide (PAM), E-4103, (Cytec Industries, Stamford, CT) was used in enrichment cultures. The polymer preparation had a molecular weight of $1–2 \times 10^7$, 18 mol% anionic charge, and was free of N-containing contaminants. This polymer is comparable to the commercially-available Magnifloc 836A, described in more detail by Lentz *et al.* (1992), which was the formulation used in the field applications. The nitrogen content in this PAM preparation is approximately 164 g N kg^{-1} of PAM. Cytec Industries also provided the following PAM preparations of reduced molecular weight for use in enrichment cultures: E-4101 (200 000 MW), E-4100 (12 000-15 000 MW) and E-4099 (3 000-4 000 MW).

Inorganic nitrogen determination, bacterial enumeration, and enrichment cultures

Soil (10 g) was extracted with 100 ml of 2 M KCl by the method of Keeney and Nelson (1982) and then refrigerated for no longer than 1 week prior to analysis. Nitrate-N concentration in each extract was determined by UV spectroscopy as described by Keeney and Nelson (1982). Ammonia-N in the extracts was quantified using an ammonia specific electrode (Orion, model 95-12).

Soil bacteria were enumerated by the heterotrophic plate count method on soil extract agar (Wollum, 1982) and by the acridine orange direct count (AODC) method (Schmidt and Paul, 1982).

Soil samples (500 mg) from PAM-treated and untreated furrows were inoculated into 25 ml of mineral salts medium consisting of KH_2PO_4 (940 mg), $Na_2HPO_4 \cdot 7H_2O$ (17.74 g), $CaCl_2$ (14.7 mg), $MgSO_4 \cdot 7H_2O$ (240 mg), and 1 ml of a trace metals solution in 1.0 l of deionized water, at a pH of 7.6. The trace metals solution consisted of H_3BO_3 (2.85 g), $MnCl_2 \cdot 4H_2O$ (1.8 g), $FeSO_4 \cdot 7H_2O$ (1.35 g), $CoCl_2 \cdot 6H_2O$ (40 mg), $CuCl_2 \cdot 2H_2O$ (30 mg), $Na_2MoO_4 \cdot 2H_2O$ (30 mg), and $ZnCl_2$ (20 mg), in 1.0 l of deionized water. Polyacrylamide, acrylamide (AMD) or acrylic acid (AA) were added separately to growth flasks (final concentration 0.05%) to

determine whether enrichment cultures could be established with the ability to utilize these compounds as a sole C or N source. When AMD, AA or PAM was added as the sole C source, the medium was supplemented with 0.2% NH_4NO_3. When AMD or PAM was added as the sole N source, the medium was supplemented to contain 0.1% glucose or 0.05% acetate + 0.05% mannitol.

Enrichment cultures were incubated at 30°C on a reciprocal shaker, transferred every 5 d, a least 7 times prior to use in experiments. Growth curves were generated via absorbance at 520 nm.

To assess the possibility of abiotic release of NH_4^+-N during incubation, mineral salts medium supplemented with PAM, mannitol and acetate as described above with no added inoculum were shaken at 30°C for 72 h. Samples were removed at 24 h intervals, acidified, and then analyzed for NH_4^+-N using ion chromatography.

Amidase determination

Amidase activity was determined in enrichment cultures derived from PAM-treated soil and able to use PAM for N. Subcultures were grown in 25 ml of mineral salts medium supplemented with either NH_4NO_3 (0.024%), propionamide (0.04%) or PAM (0.05%) as a N source and 0.05% mannitol + 0.05% acetate for C. The flasks were inoculated with 2 ml of enrichment culture and shaken at 25°C for 48 h. Cells and culture supernatant were separated via centrifugation at 10 000 × g for 10 min at 4°C; the supernatant was collected and maintained on ice. Cells were washed twice in 75 mM potassium phosphate buffer at pH 7.6, and cell-free extracts were obtained by sonication followed by centrifugation.

The cell-free extract and the culture supernatant were pooled and used as enzyme source in the amidase assay procedure described by Friedrich and Mitrenga (1981). The substrates used were formamide, propionamide and PAM, at concentrations of 100 mM, 100 mM, and 0.025%, respectively. The assay mixtures were incubated at 30°C for 2 h, then maintained on ice. Amidase activity was determined immediately by quantifying the concentration of NH_4^+-N released from the test amide by ion chromatography. Controls containing only buffer + enzyme or buffer + amide were also analyzed for NH_4^+. Protein determinations for the enzyme preparations were conducted using the method described by Lowry *et al.* (1951).

Ion chromatography

A Dionex 100 ion chromatograph equipped with a CS12 column, a cation self-regenerating suppressor, and a conductivity detector was used to quantify NH_4^+-N. Methane sulfonic acid (MSA) at 20 mM concentration was the eluent used at a flow rate of 1.0 ml min^{-1} (lower limit of detection = 10 $\mu g\ l^{-1}$).

RESULTS AND DISCUSSION

Soil microbial populations and inorganic N concentrations

There were no significant differences in heterotrophic plate counts between PAM-treated and untreated soil in the bean field (Fig. 2). However, for the potato field, there were significantly larger populations of culturable heterotrophs in PAM-treated soil (Fig. 3). Total bacterial numbers were not affected by PAM treatment in any of the soils tested, although the soil treated with a petroleum distillate-PAM emulsion in the bean field exhibited elevated AODC counts (Figs 2 and 3). The effects of PAM application to soils has been reported to have no predictable effect on quantified culturable bacteria by Nadler and Steinberger (1993), or on microbial biomass by Steinberger *et al.* (1993). Our results also indicate that PAM effects on bacterial numbers are likely to be site-specific and difficult to predict.

The effects of PAM application on inorganic N concentrations also appear to be site-specific (Figs 4 and 5). There were significant differences in inorganic N concentrations between PAM-treated and

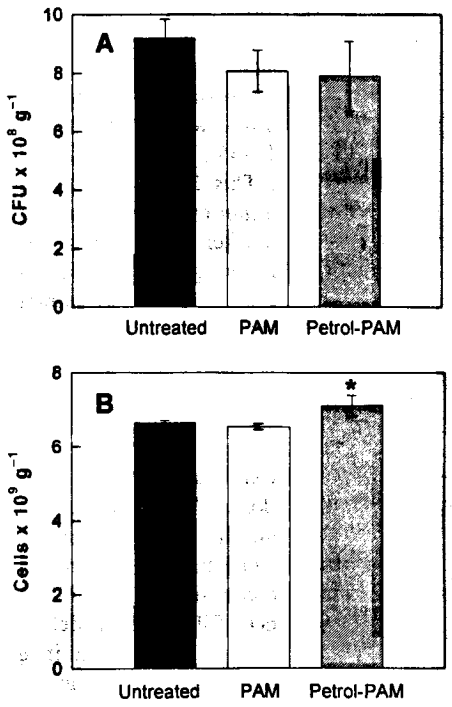

Fig. 2. Culturable heterotrophic bacteria (A) and total bacteria by AODC (B) in PAM-treated and untreated bean field soils. Error bars indicate ± SE for triplicate determinations. Significant difference from the control at $P < 0.05$ is denoted by an asterisk, as determined by one-way ANOVA.

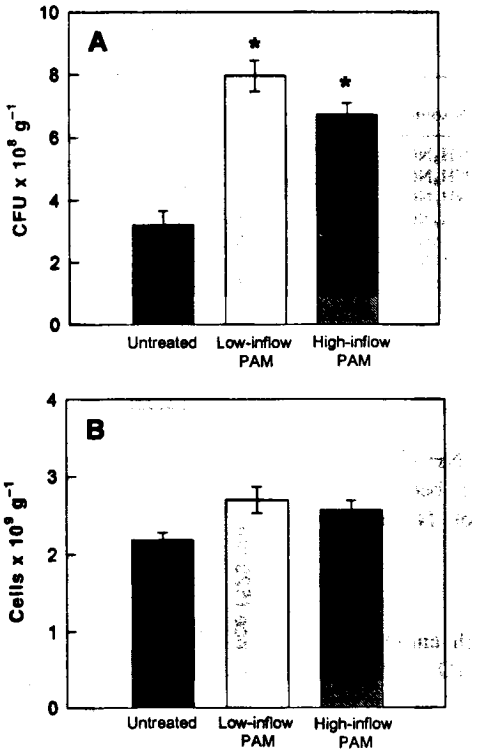

Fig. 3. Culturable heterotrophic bacteria (A) and total bacteria by AODC (B) in PAM-treated and untreated potato field soils. Error bars indicate ± SE for triplicate determinations. Significant difference from the control at $P < 0.05$ is denoted by an asterisk, as determined by one-way ANOVA.

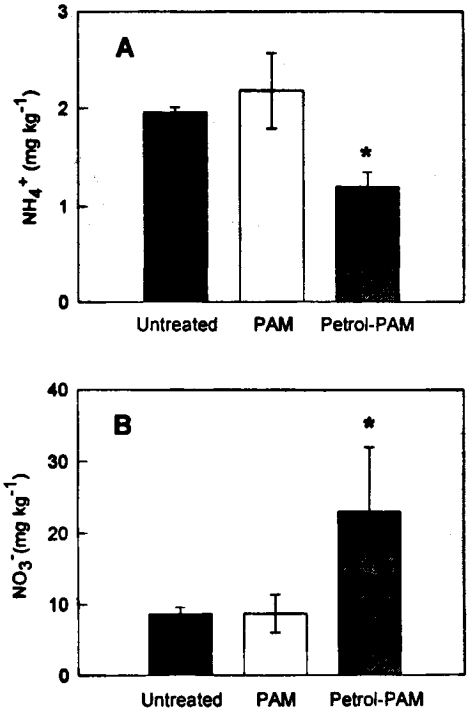

Fig. 4. KCl extractable NH_4^+–N (A) and NO_3^-–N (B) in PAM-treated and untreated bean field soils. Error bars indicate ± SE for triplicate determinations. Significant difference from the control at $P < 0.05$ is denoted by an asterisk, as determined by one-way ANOVA.

untreated soils for the potato field. Both high and low-inflow PAM-treated soils exhibited elevated amounts of KCl extractable NO_3^-–N compared to untreated samples (Fig. 5), indicating that PAM treatment may effect the physical-chemical retention of NO_3^- or rates of microbial NO_3^- transformation. Abdelmagid and Tabatabai (1982) reported that application of AMD resulted in an increase in NO_3^- and NO_2^- in soil due to biotransformation of the monomer. It is possible that a similar mechanism may be active in PAM-treated soils.

Concentrations of KCl-extractable NH_3–N were higher in the high-inflow PAM-treated soil than in the untreated soil, while no significant increase was observed for the low-inflow PAM-treated soil (Fig. 5). The data indicate that the increase in NH_3–N was not likely due to the flow rate itself (data not shown) but may be due to a larger PAM dose that was applied to the high-flow furrows (19.9 kg ha^{-1} vs 13.0 kg ha^{-1} in the low-flow plot).

Bean field soil treated with the petroleum distillate-PAM emulsion exhibited amounts of inorganic N significantly different from those of untreated bean field soil, whereas the PAM treatment alone had no such effects (Fig. 4). This suggests that the petroleum distillate component of the emulsion was responsible for the effects. Any effect of non-emul-

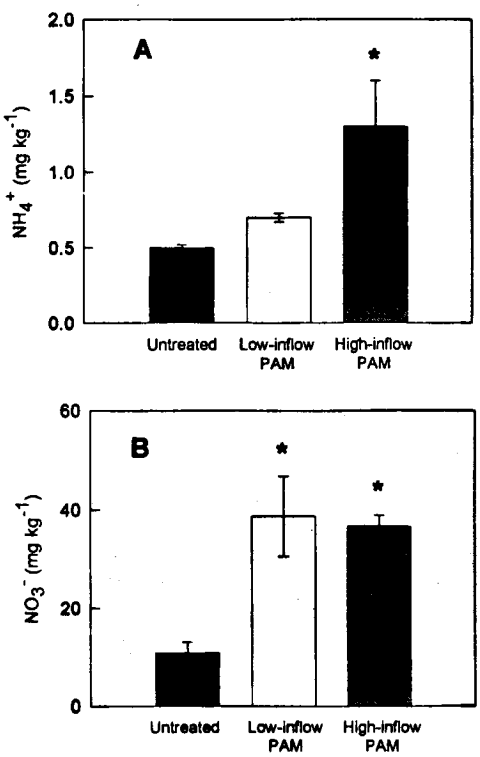

Fig. 5. KCl extractable NH_4^+–N (A) and NO_3^-–N (B) in PAM-treated and untreated potato field soils. Error bars indicate ± SE for triplicate determinations. Significant difference from the control at $P < 0.05$ is denoted by an asterisk, as determined by one-way ANOVA.

Table 1. Growth responses, measured by absorbance at 520 nm, of enrichment cultures derived from PAM-treated and untreated soils following incubation for 5 d with various C and N sources (+, growth; 0, no growth)

C source	N source	PAM treated soil inoculum	Untreated soil inoculum
None	NH_4NO_3	0	0
PAM	NH_4NO_3	0	0
AMD	NH_4NO_3	+	+
AA	NH_4NO_3	+	+
Glucose	none	0	0
Glucose	PAM	+	+
Glucose	AMD	+	+
Glucose	AA	0	0
None	none	0	0
PAM	PAM	0	0
AMD	AMD	+	+
AA	AA	0	0

sion PAM treatment on NO_3^-–N or NH_3–N concentrations in these soils may have been overwhelmed by a relative abundance of N due to symbiotic, leguminous N fixation.

Biotransformation of PAM

Table 1 shows results obtained with enrichment cultures supplemented with PAM, AMD or AA as the sole source of N or C. Soil inoculum from PAM-treated furrows yielded the same results as inoculum from untreated furrows in all cases. Successful enrichments were established with AA as sole C source, AMD as sole C and N source, and PAM as sole N source. Growth curves generated for successful enrichments derived from treated and untreated soil inocula are shown in Figs 6 and 7. In most cases similar curves were derived for PAM-treated and untreated soils. The culture generated from PAM-treated soil with AMD for C appeared to exhibit more rapid growth relative to the enrichment culture from untreated soils (Fig. 6(B)), and the opposite pattern was observed for enrichments utilizing AA as a C source (Fig. 7(B)). This indicates the possibility of different communities in the various enrichments.

Nitrogen utilization

Both AMD and PAM were able to support bacterial growth as the sole N source in enrichment cultures (Table 1). To verify that the growth observed in the enrichment cultures with PAM as the sole N was due to biological hydrolysis of the PAM molecule, agitated uninoculated medium was assayed for free NH_4^+. No NH_4^+ was released due to agitation, although a very small amount of NH_4^+ ($< 0.35\ \mu g\ l^{-1}$) was detected as an initial contaminant. Since it is unlikely that this concentration of N could sustain the bacterial growth observed, the mechanism by which the organisms accessed the N in PAM is presumably a biotic one.

Amidase (EC 3.5.1.4) enzyme activity is one of the mechanisms by which amides can be used by bacteria as a source of N via the following

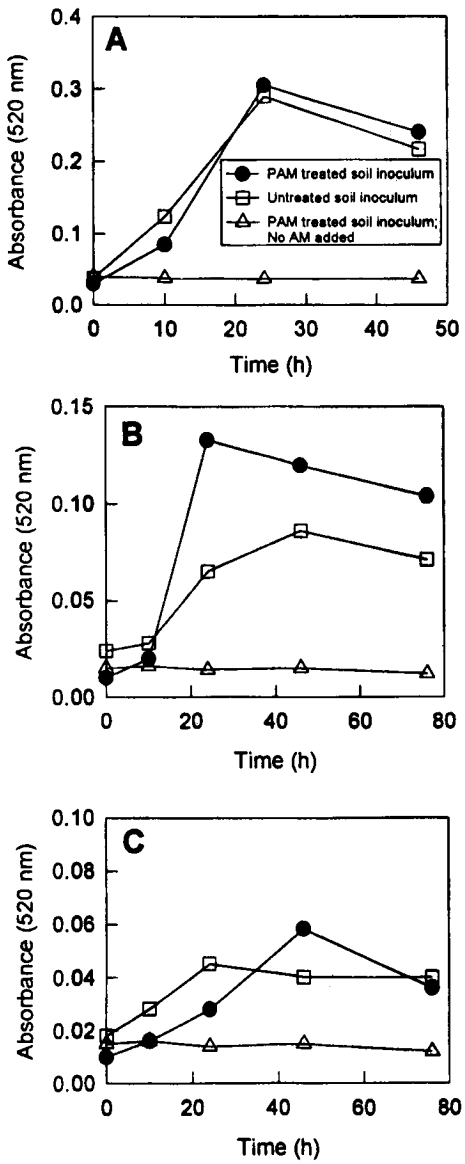

Fig. 6. Growth response of enrichment cultures generated from PAM-treated and untreated soils in which AMD served as sole N source (A), sole C source (B), or sole N and C source (C).

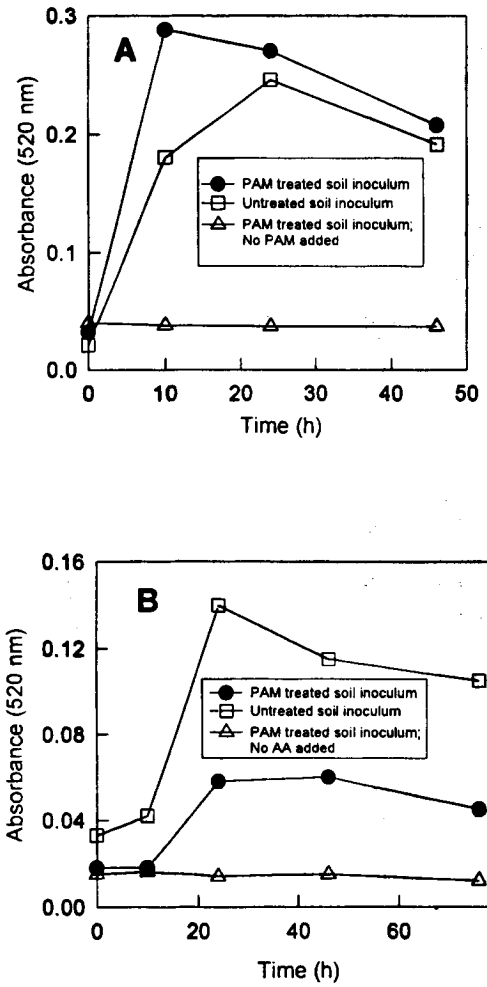

Fig. 7. Growth response of enrichment culture generated from PAM-treated and untreated soils in which PAM served as sole N source (A), and AA served as sole C source (B).

reaction:

$$RCONH_2 + H_2O \longrightarrow RCOOH + NH_3.$$

Shanker *et al.* (1990) documented that amidase activity was responsible for N release from AMD by soil microbes. In our study an enrichment that utilized PAM as the sole N source exhibited amidase activity when subcultured with various amide substrates. Amidase activity was not detected when the enrichment culture was supplied with NH_4NO_3 (Table 2). When the PAM-utilizing culture was grown on propionamide, most of the amidase activity measured was specific toward propionamide. There was little activity toward PAM, and no activity was observed toward formamide. When subcultured with PAM as the sole N source, there was substantial amidase activity toward PAM, and activity toward the two smaller substrates (Table 2). Therefore, it appears that different amidases or amidase isozymes were induced in the presence of propionamide or PAM. This phenomenon may contribute to the changes in soil inorganic N concentrations (Figs 4 and 5) observed for the potato field in response to PAM application. It is possible that this mechanism may be operating in the soil planted to beans but that the effect is relatively insignificant compared to active N fixation.

Taken together, the successful growth of enrichment cultures with PAM as sole N source and the detection of PAM-specific amidase activity in these cultures indicate that indigenous soil microorganisms are capable of utilizing PAM as a sole source of N, and that the most probable mechanism for this biotransformation is amidase activity. The effect of deamination of PAM already bound to soil, by soil biota is unknown. The degree of charge density on anionic-PAM influences the adsorption of the polymer to soils (Malik and Letey, 1991). Consequently, deamination of PAM in soil could potentially play a role in the observed reduction in anti-erosion activity of PAM following initial addition.

Carbon utilization

Successful enrichment cultures were derived with the monomeric constituents of the polymer, AA or AMD, present as sole C source, but no cultures could use the PAM polymer as a sole C source (Table 1). One important aspect of these results is related to the environmental fate of the AMD monomer. Acrylamide is a potent neurotoxin (Anonymous, 1988). Commercially-available PAM preparations may contain no more than 0.05% AMD monomer (Barvenik, 1994). Our data indicate that indigenous soil microbial communities are capable of rapidly degrading AMD to access both N and C. This conclusion supports the findings of Shanker *et al.* (1990) who reported complete degradation of 500 mg AMD kg^{-1} applied to soil within 5 d and Lande *et al.* (1979) who reported half-life

Table 2. Specificity of amidase activity exhibited by PAM utilizing enrichment culture, grown with various N sources

N source in growth medium	Amidase specific activity toward test amide (units mg^{-1} protein)		
	Formamide	Propionamide	PAM
NH_4NO_3	0	0	0
Propionamide	0	0.811 ± 0.030[a]	0.005 ± 0.001
PAM	0.819 ± 0.244	0.730 ± 0.068	0.512 ± 0.056

[a]Mean of triplicate determinations ± SE.

values of AMD in soil ranging from 18 to 45 h when 25 mg kg^{-1} was applied to various soils. An estimate of the amount of AMD applied per irrigation event using 10 mg PAM l^{-1} is approximately 5.0×10^{-4} mg kg^{-1}, assuming 15 cm of penetration.

Another issue related to the AMD monomer is its potential release from PAM upon biotransformation of the polymer in soil. Even though the chemical nature of PAM would likely prohibit AMD release, lingering concerns, especially within the lay community, are associated with agricultural PAM application. PAM does not release AMD monomer in soil enrichment cultures (Table 1). Since AMD was able to support bacterial growth by supplying C, release of AMD from PAM likely would have resulted in successful enrichment growth with PAM as sole C source. However, in no case did PAM support bacterial growth as sole C source.

Smith *et al.* (1996) have claimed that a polyacrylamide solution of unspecified MW and charge type spontaneously depolymerized to form acrylamide monomer upon incubation, although the data presented do not appear to strongly support this claim. Acrylamide was present initially at low concentrations in the incubation mixtures, presumably as a contaminant. Acrylamide did not increase in the majority of the incubations, and in the few instances where increases were noted, they were minuscule compared to the initial PAM concentration in the mixture. Furthermore, Smith *et al.* did not describe the composition of the incubation mixtures with respect to PAM formulation, cellular amendments or sterility of the media used. Therefore it is difficult to evaluate the experimental design and the results of their paper.

The inability of PAM to support bacterial growth as a C source is probably related to the large size of this polymer and the lack of exoenzymes able to depolymerize the polymer. Because UV and shear forces have been shown to reduce chain length (Tolstikh *et al.*, 1992; Randby, 1993), smaller PAM molecules and UV-treated PAM solutions were used in efforts to enrich for organisms capable of utilizing these molecules for C. Treatment with UV radiation did effectively reduce PAM chain length as determined by size-exclusion HPLC (data not shown). However, the smaller PAM molecules, of MW as low as 3 000–4 000, or the UV-treated PAM preparations were not able to support bacterial growth as sole C source (data not shown).

In conclusion, application of high MW, linear, anionic PAM to soil represents an unique opportunity to investigate the environmental fate of this amide containing heteropolymer. In our study, PAM effects on soil bacterial populations and inorganic N concentrations were site specific. Indigenous bacteria were capable of PAM biotransformation, utilizing the polymer as a source of N via inducible amidase activity. To our knowledge this is the first report of an amidase capable of transforming such a large amide-containing substrate. PAM did not serve as sole C source for enrichment cultures, even though the monomeric reactants used in its synthesis, but AMD and AA did. Utilization of PAM for N, but not for C, indicates that ultimately PAM may be converted into long chain polyacrylate, which may be further degraded by physical and biological forces or become incorporated into organic matter.

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