

Final Report

Prepared for Boojum Research

Bioremediation of Mine Area Groundwater Inorganic Contamination (*BioMAGIC*)

F.G. Ferris
Department of Geology
University of Toronto

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INTRODUCTION

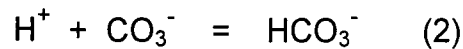
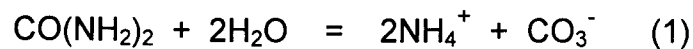
The occurrence of metal-contaminated acid mine drainage (AMD) is one of the most problematic situations facing the mining industry today (Hutchinson and Ellison 1992; Ledin and Pedersen 1996). Sources of AMD include the walls of underground and open pit mining sites, waste rock and ore stock piles, tailings waste, and spent heap leach piles from leaching operations. If left uncontrolled and untreated, AMD has the potential to contaminate surface and groundwater broadly enough to adversely affect regional water quality, as well as indigenous fish and wildlife populations (Hutchinson and Ellison 1992; Drabkowski 1993).

The chemical composition of AMD is determined principally by mine waste mineralogy, and the amount of water (meteoric and/or groundwater) infiltrating and percolating through the waste (Filion and Ferguson 1990). Iron sulfide minerals, such as pyrite, begin to undergo chemical oxidation almost immediately upon exposure to atmospheric oxygen and water (Evangelou and Zhang 1995). In the absence of neutralizing agents (e.g., carbonates or clays), these chemical reactions produce acid and liberate various metals whose concentrations will vary as a function of the composition of the ore and waste rock (Blowes and Ptacek 1994). As pH decreases, bacteria and ferric iron catalyze the overall process to a rate 10 to 20 times the abiotic chemical rate (Stumm and Morgan 1996; Okereke and Stevens 1991). Rainfall, snow melt, and other hydrological processes then leach the acid solutions from the waste sites into downstream

and groundwater environments. Typically, AMD exhibits pH values well below 5.6 (the pH of meteoric water in equilibrium with atmospheric carbon dioxide) that are accompanied by high concentrations of dissolved sulfate, ferrous iron, and metals such as Al, Cr, Ni, Cu, Zn, Ag, Au, and Pb (Hutchinson and Ellison 1992; Drabkowski 1993).

The primary goal of AMD treatment is to prevent acid generation at new sites, and to abate acid drainage where it occurs at existing facilities. Two basic approaches are used. These are (i) control of acid generation and leachate migration, and (ii) collection and treatment of AMD (Ledin and Pedersen 1996). Technologies currently employed to control acid generation and leachate migration include liming or carbonate treatment, application of bactericides, inundation (i.e., flooding) with water to maintain anaerobic conditions that prohibit sulfide mineral oxidation, and installation of dry covers to prevent water infiltration. Collection and treatment of AMD contamination in surface waters is accomplished using alkaline recharge trenches or wetland systems (natural or engineered, for example, the Boojum ARUM [acid reduction using microbiology] treatment). Treatment protocols for *in situ* **Bioremediation of Mine Area Groundwater Inorganic Contamination (BioMAGIC)** are not presently available, but are urgently required as field studies have demonstrated that infiltration of AMD into groundwater is common at mine sites (Ledin and Pedersen 1996).

This study is focused on assessing the feasibility of an *in situ BioMAGIC* treatment process to mitigate the potential adverse impact of AMD contamination along groundwater flow paths. The concept is based on using urea-degrading bacteria to increase the pH and alkalinity of acidic groundwater containing high concentrations of dissolved metals. Specifically, the enzymatic hydrolysis of urea by bacteria is apt to be of considerable benefit as the reaction consumes protons and promotes the development of alkaline conditions according to the following reactions:



The intention is not only to reduce the extent of acidification that normally accompanies the hydrolysis of dissolved metals in mine drainage, particularly ferric iron, but also to initiate the removal of dissolved metals through mineral precipitation and sorption reactions (Stumm and Morgan 1996; Chapman et al. 1983; Herbert 1994; Herbert 1996). Anticipated benefits include restoration of normal pH values and substantially reduced metal-loading in water that is apt to move off mine-site property. Moreover, urea is produced as a bulk commodity agricultural fertilizer that is readily available (Tisdale et al. 1985).

The target site for this investigation is the South Bay mine site in northwestern Ontario. Boojum Research has identified a plume of AMD

contaminated groundwater that ultimately discharges into a small lake located approximately 800 m from the nearest surface source of AMD. A detailed site description is found in Appendix A. The basic premise for *BioMAGIC* at South Bay is that urea would be introduced into the contaminated groundwater system (most likely through installation of infiltration galleries across the groundwater flow path) to stimulate the activity of indigenous urea-degrading bacteria to a point where a significant increase in groundwater pH can be recognized.

The approach used to evaluate the potential use of urea-degrading bacteria for BIOMAGIC involved both geochemical modeling and microbiological investigations. Flow-through column work has also been undertaken to learn about the potential impact of bacteria on groundwater flow in porous geological media. Construction of the geochemical model using the MINEQL+ program was based on South Bay groundwater chemistry provided exclusively by Boojum Research. The objective of the modeling exercise was to determine (i) the extent to which groundwater pH would change in response to urea degradation, (ii) the minimum amount of urea that must be degraded to restore near-neutral pH, and (iii) the impact of anticipated pH changes on the aqueous chemistry and solubility of dissolved metals. Microbiological studies encompassed a survey of South Bay groundwaters to determine whether any urea-degrading bacteria existed in the contaminated aquifer, and to identify specific locations with conditions favorable to their growth. Subsequent work involved single colony isolation of South Bay urea-degrading bacteria to compare their activity with that of a reference bacterial

culture (*Bacillus pasturii*) from the American Type Culture Collection (ATCC).

This microbiological information is needed to identify areas where *BioMAGIC*, is most likely to be implemented successfully, and whether natural populations of bacteria might be used or, alternatively, whether a need exists to add urea-degrading bacteria to the groundwater system.

METHODOLOGY

Geochemical Modeling

The geochemical modeling program MINEQL+ (Westal et al. 1976; Schecher and McAvoy 1994) was used to evaluate how urea-degrading bacteria would be expected to influence the pH and aqueous chemistry of the South Bay AMD contaminant plume. A brief description of the description of the procedures used in the modeling exercise follows. For a more detailed account on how the geochemical modeling was executed, consult Appendix A.

The aqueous geochemical data from 25 peizometers for the modeling work was supplied directly by Boojum Research, and used as received. After selecting the elementary chemical components for the modeling, total analytical concentrations were entered into the tableaux of the MINEQL+ program. Preliminary modeling runs were used to calculate the relative proportions of different redox species (e.g., $\text{Fe}^{2+}/\text{Fe}^{3+}$) at measured field pH and Eh values.

This permitted calculation of the proton condition, which was a necessary prerequisite as total H^+ concentrations must be taken into account in the MINEQL+ mass balance computations to permit calculation of pH (Bethke 1996).

After calibrating the models to match measured field and computed pH values, multiple runs (titrations) were undertaken in which increasing concentrations of the products of bacterial urea hydrolysis (i.e., NH_4^+ and CO_3^-) were added simultaneously in a 2:1 mole ratio corresponding to equation (1). Titrations were performed for all 25 piezometers to determine the amount of urea that would need to be hydrolyzed *in situ* by bacteria to achieve a pH of 8.0. Finally, to determine whether additional urea hydrolysis might be needed to prevent acidification if the groundwater Fe^{2+} was completely oxidized to Fe^{3+} (e.g., upon discharge into Mud Lake), titrations were run at high Eh instead of measured field Eh as a fixed boundary condition.

The output of the geochemical models included saturation index (SI) values that extend from the following relationship:

$$SI = \log (IAP / K_{so})$$

where IAP represents the ion activity product of a solid mineral phase, and K_{so} is the corresponding equilibrium solubility product (Stumm and Morgan 1996).

When SI values are less than zero, solutions are undersaturated with respect to

the mineral phase in question. Conversely, solutions are oversaturated with respect to a given mineral phase when SI values are greater than zero. It is instructive to note, however, that SI values only provide an indication of the degree to which aqueous solutions depart from true thermodynamic equilibrium and do not necessarily indicate that a mineral will actually dissolve in undersaturated conditions, or precipitate in oversaturated conditions.

Contour plots of output data from the geochemical models were constructed using the statistics computer program STATISTICA (v. 5.0). Isopleths were drawn for an 1100 x 240 m grid covering the 25 piezometers evaluated in the geochemical models using a distance weighted least squares fitting routine.

Microbiology

Microbiological culturing was undertaken to determine the presence or absence of different physiological groups of bacteria in groundwater samples collected and submitted for analysis by Boojum Research. The bacteria that were actively sought included those capable of urea hydrolysis, as well as nitrate-reducing bacteria and sulfate-reducing bacteria. Standard selective minimal media were utilised for urea degrading and nitrate-reducing bacteria (Difco), while Postgate medium was employed to culture sulfate-reducers (Chapelle 1993). In addition, isolation of bacteria from South Bay groundwater

was accomplished using standard single colony isolation techniques on trypticase soy agar. The isolated bacteria were subsequently screened for their ability to hydrolyse urea in trypticase soy broth (TSB) amended with 2.0 % (w/v) urea.

In order to determine whether urea-degrading bacteria from South Bay would be capable of raising the pH of AMD contaminated groundwater, cultures were first grown to mid-exponential phase in TSB. As a comparative positive control, the known urea-degrading bacterium *Bacillus pasteurii* (ATCC) was included in the experiments. Samples of pH 3.0 groundwater (50 mL) were then amended with 10^{-3} M urea (an amount anticipated from geochemical modeling to raise groundwater pH to a value near 8.0), and inoculated with 1.0 mL of the TSB cultures. The groundwater-urea cultures were then incubated 2 weeks at room temperature, and the final pH was recorded. Solids that accumulated in response to bacterial growth in the urea amended groundwater cultures were subsequently examined by scanning electron microscopy and energy dispersive X-ray spectroscopy.

Rates of bacterial urea hydrolysis were evaluated using *B. pasturii* grown in half-strength and quarter-strength TSB. The different concentrations of TSB were used in these experiments to evaluate the importance of nutrient concentration and bacterial growth rate on urea hydrolysis. *B. pasturii* was initially grown to mid-exponential phase in TSB, and then inoculated (10 % by

volume) into lower strength TSB containing 10^{-4} , 10^{-3} , or 10^{-2} M urea. The cultures were then incubated at room temperature for 2 weeks. At 5 day intervals, bacterial growth was measured spectrophotometrically by taking optical density readings at 600 nm (OD_{600}) and culture pH was determined to follow the extent of urea hydrolysis.

Column Studies

Flow-through column work was undertaken to learn about the potential impact of bacteria, as well as effects of mineral precipitation induced in response to urea hydrolysis, on groundwater flow in a porous sand media similar to the AMD contaminated aquifer at South Bay. These studies were done using vertical up-flow columns that were maintained at a constant hydraulic head. This configuration avoided formation of air pockets and ensured full saturation of the columns. Moreover, at a constant hydraulic head, differences in flow rates between columns revealed changes in the hydraulic conductivity of the porous medium, as anticipated from Darcy's Law (Freeze and Cherry 1979).

Three different series of experiments were run. The first involved columns packed with clean acid washed silica sand. The second incorporated sand that was coated with hydrous ferric oxide (HFO), while the third set consisted of HFO coated sand mixed with bacteria. To coat the sand with HFO, 200 mL volumes of sand were stirred overnight in 400 mL of a 10^{-2} M $Fe(NO_3)_3$ solution at pH 2.0.

The pH of the sand suspension was then adjusted using 1.0 N NaOH to pH 7.0 to precipitate the Fe^{3+} on the sand grains as HFO. After washing with ultrapure water, the wet HFO coated sand was packed into columns for experimental work. To incorporate bacteria into the columns, cultures of the *Shewanella* alga BrY (this is a common iron-reducing bacterium that is often found in groundwater environments) were grown to mid-exponential phase in TSB (Lovley 1991). The bacteria were then washed by centrifugation and subsequently mixed into the HFO coated sand before being packed into the columns. The columns were subsequently saturated with an up-flow of ultrapure water, and the volume of discharge was measured over known time intervals to determine flow rates.

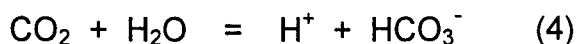
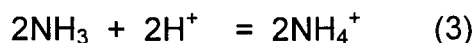
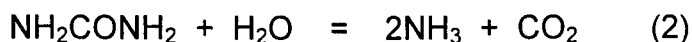
RESULTS AND DISCUSSION

Geochemical Modeling

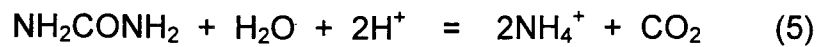
The predictive geochemical models that were developed apply directly to piezometers along the hydraulic gradient and contamination plume that extends between the tailings and Mud Lake. Among the computational results (Appendix A), two key findings stand out. First, the $\text{Fe}^{2+}/\text{Fe}^{3+}$ couple dominates the redox chemistry of the contaminant plume with Fe^{3+} concentrations decreasing, and Fe^{2+} concentrations increasing down gradient (Fig. 1). The highest Fe^{3+} concentrations are found near the tailing area, while Fe^{2+} dominants elsewhere.

The decrease in Fe^{3+} concentration and production of Fe^{2+} is likely the result of *in situ* anaerobic bacterial respiration (Lovley 1991; Chapelle 1993). At the same time, calculations suggest that SO_4^{2-} is the most thermodynamically stable sulfur species, implying that S^{2-} is highly prone to oxidation. It can be inferred from these results that groundwater emerging in Mud Lake is highly charged with Fe^{2+} that can be expected to undergo rapid oxidation and hydrolysis upon exposure to the atmosphere (Stumm and Morgan 1996). This, in turn, contributes to the generation of low pH acid conditions because the alkalinity of the water is so poor.

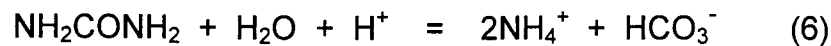
The second key finding from computational work with the geochemical models is that only mM concentrations of urea need to be degraded to bring about and sustain a substantial increase in groundwater pH – even after oxidation of emergent reduced groundwater (Appendix A). From contour plots, the least amount of urea degradation required to raise pH is in the core of the plume (Fig. 2), a condition symptomatic of the poor buffering capacity of the water. Conceptually, this situation can be illustrated by the coupled reactions for urea hydrolysis, the protonation of ammonia to form ammonium, and the deprotonation of carbonic acid to form bicarbonate.



At low pH where very little buffering is provided by dissolved carbon dioxide, reactions 2 and 3 combine to remove 2 moles of H⁺ per mole of urea:



When bicarbonate begins to become the dominant species of dissolved inorganic carbon at more neutral pH values, combining equations 4 and 5 shows that only one mole of H⁺ is removed per mole of urea:



Thus, smaller amounts of urea are needed to bring about large changes in pH when the initial pH of groundwater is relatively low.

The most important conclusion that can be drawn from modeling the impact of urea hydrolysis products on groundwater pH that, in geochemical and thermodynamic terms, ***BioMAGIC is highly feasible***. Moreover, because urea is very soluble, introduction of appropriate concentrations to recognize the benefit of *BioMAGIC* is unlikely to be a problem for field application (Swensen and Bakken 1998).

In addition to the increase in pH that is expected to accompany bacterial urea-degradation in South Bay groundwater, the geochemical modeling exercise revealed large potential changes in mineral saturation states (Appendix A). Although it is difficult to ascertain which minerals will precipitate, based on thermodynamic data alone (i.e., the computational basis for geochemical modeling), kinetic constraints arising from differences in surface free energies anticipate, according to the Ostwald Step Rule, that the least soluble phase among a suite of related minerals is likely to precipitate first (Stumm and Morgan 1996). In the case of iron oxides such as hematite, goethite, lepidocrocite, and ferrihydrite (or HFO as it often called), the later is the least thermodynamically stable, yet generally precipitates first in most aquatic systems (Schwertmann and Murad 1983; Cornell and Schwertmann 1996). If deemed necessary, this could be can be verified in laboratory and field experiments.

The degree to which mineral precipitation occurs in *BioMAGIC* is an important consideration for two reasons. On one hand, substantial mineral precipitation underground can dramatically decrease aquifer permeability and thus reduce water flow. Certainly, contour plots for ferrihydrite (HFO) indicate that groundwater in the area of the contaminant plume at South Bay is oversaturated at field pH, and becomes highly oversaturated at pH 8.0 (Figs. 3 and 4). Column experiments confirmed that the hydraulic conductivity of sand coated with HFO and bacteria, alone and mixed together, is less than clean sand (Table 1).

A reduction in hydraulic conductivity along the contaminant plume at South Bay would tend to lengthen the transit time of the water, and could increase dilution of contaminants beneficially through greater mixing with uncontaminated meteoric water. On the other hand, mineral precipitation generates solid phases that can both concentrate metals directly into their crystal lattice and capture dissolved metals through surface complexation (i.e., sorption) reactions (Stumm and Morgan 1996). While this offers an effective way to remove contaminant metals from solution, considerable differences exist in the metal scavenging ability of different minerals. However, this aspect of the *BioMAGIC* process was beyond the scope of the present investigation and needs to be confirmed and quantified in laboratory and field experiments.

Urea-Degrading Bacteria at South Bay

The microbiological survey of South Bay groundwater determined that urea-degrading bacteria, as well as nitrate-reducing bacteria and sulfate-reducing bacteria, are indigenous to the aquifer. This is not unexpected because urea-degrading and anaerobic respiratory bacteria are very common in soils (Chapelle 1993; Swensen and Bakken 1998; Nielsen et al. 1998; Klose and Tabatabai 1999). Fungi also grew in the culture media, and must be considered to be important members of the groundwater ecosystem. The distribution of microorganisms, however, was not uniform as samples from peizometers with

more acidic groundwater appeared to be well suited for fungi, and less hospitable for bacteria (Table 2).

The apparent preference of urea-degrading bacteria for more neutral pH conditions, as observed in the initial microbiological survey, provides potentially useful insight on where to initiate *BioMAGIC* in the field. Acid charged waters infiltrate into the aquifer in the immediate vicinity of the tailings, but become more neutral down gradient as the groundwater approaches Mud Lake. This is because of mixing with uncontaminated meteoric water, and because of *in situ* anaerobic bacterial processes, most likely Fe³⁺-reduction as inferred from the geochemical modeling (Fig. 1). In the down gradient area the groundwater, while at a somewhat higher pH, is still very acidic with poor buffering capacity. This is apt to be the best spot to initiate *BioMAGIC* – just before the contaminated groundwater emerges in Mud Lake. The slightly elevated pH is supportive to the activity of urea-degrading bacteria that, in turn, could generate enough alkalinity to prevent further acidification of Mud Lake. Direct application of urea to the sediment of Mud Lake might also be considered, although some experimental work ought to be undertaken before this later option is explored in the field.

In experiments testing for urea consumption in pH 4.0 South Bay groundwater, the ATCC *B. pasturii* culture and bacterial isolates from South Bay managed to increase groundwater pH to near 8.0 over a period of two weeks. These experiments employed urea in mM concentrations, as inferred from

computational work with the geochemical models. These tests provide important corroborative experimental evidence in support of the geochemical modeling effort, and the intrinsic feasibility of *BioMAGIC*.

As equations 5 and 6 indicate, the kinetics of bacterial urea hydrolysis can be anticipated to follow second or third order rate laws depending on pH and urea concentration. Above pH 5.6, the pH of meteoric water in equilibrium with atmospheric $p\text{CO}_2$, bicarbonate (HCO_3^-) is the dominant species of dissolved inorganic carbon in solution. Thus, according to equation 6, a second order rate law likely applies for urea hydrolysis:

$$\partial[\text{urea}] / \partial t = -k [\text{urea}] [\text{H}^+] \quad (7)$$

Similarly, the rate law for the change in proton concentration (or pH, $-\log [\text{H}^+]$) is:

$$\partial[\text{H}^+] / \partial t = -k [\text{urea}] [\text{H}^+] \quad (8)$$

Because the rate laws are similar, the change in proton concentration (i.e., pH) with respect to time can be used to provide an indication of the extent of urea hydrolysis. The differentiated form of equation 8 is:

$$[\text{H}^+]_t / [\text{H}^+]_0 = [\text{H}^+]_0 - [\text{urea}]_0 / [\text{H}^+]_0 - [\text{urea}]_0 e^{-kt([\text{H}^+]_0 - [\text{urea}]_0)} \quad (9)$$

At neutral pH values, where the proton concentration is quite small in comparison to mM urea concentrations, equation 9 simplifies to:

$$[\text{H}^+]_t / [\text{H}^+]_0 = 1 / e^{kt[\text{urea}]_0} \quad (9)$$

Taking logarithms yields:

$$\text{pH}_t - \text{pH}_0 = \Delta\text{pH} = 2.303 k t [\text{urea}]_0 \quad (10)$$

It can be seen from equation 10 that the change in pH with respect to time owing to bacterial urea hydrolysis should be directly proportional to $2.303 k [\text{urea}]_0$, where k is the second order rate constant and $[\text{urea}]_0$ is the initial concentration of urea. This type of rate relationship is generally referred to as a pseudo first order rate law, and commonly arises in high order reactions where one or more of the reactants is present in excess (Stumm and Morgan 1996). Thus, it follows that $2.303 k [\text{urea}]_0$ corresponds to a first order constant k' , and equation 10 simplifies to:

$$\Delta\text{pH} = k' t \quad (11)$$

The observed change in pH over time accompanying urea hydrolysis in cultures of *B. pasteurii* grown in different concentrations of TSB is shown in Figures 5 and 6. Corresponding growth curves for *B. pasteurii* are shown in

Figures 7 and 8. A linear increase in ΔpH with respect to time was observed in all of the cultures, starting from an initial pH of approximately 6.5, as anticipated from equation 11. The cultures also exhibited exponential growth, as is commonly observed with bacteria (Chapelle 1993). Table 3 provides a compilation of the rate constants obtained from regression analyses of the experimental data for ΔpH and bacterial growth ($\log [\text{O.D.}_{600}$ at time t / initial O.D._{600}]) at different TSB and urea concentrations. The very strong correlation coefficients obtained from the regression analyses indicate that from 86 to greater than 99 % of the variance in the observed ΔpH and $\log [\text{O.D.}_{600}$ at time t / initial O.D._{600}] values can be attributed to a linear function of time, as expected for first order rate laws.

The growth rate constants of *B. pasteurii* were similar in quarter-strength and half-strength TSB at a urea concentration of 10^{-4} M, as shown in Table 3. In quarter-strength TSB, the growth rate constants decreased from around 0.020 hr^{-1} to 0.012 hr^{-1} with increasing urea concentration, suggesting perhaps that under nutrient limiting conditions, higher concentrations of urea, or its breakdown products (i.e., NH_4^+ or HCO_3^-), inhibit growth somewhat. At intermediate urea concentrations (i.e., 10^{-3} and 10^{-2} M) in half-strength TSB, an increase in growth rate constants from around 0.020 hr^{-1} to 0.025 hr^{-1} was observed, indicating that an improved supply of nutrients promotes tolerance of higher urea concentrations. However, even in half-strength TSB, a lower growth rate

constant was observed with 10^{-1} M urea, further emphasizing the importance of nutrient supply for bacterial growth in the presence of urea.

Although increasing concentrations of urea appeared to have an adverse impact on bacterial growth, an increase in the ΔpH rate constants was observed with increasing urea concentrations in both quarter-strength and half-strength TSB (Table 3). These results are consistent with recent studies on urea hydrolysis in soils which show that rates of bacterial growth and urea decomposition are generally unrelated (Swensen and Bakken 1998). At the same time, total bacterial biomass in soil tends to be significantly correlated with urea degradation rates (Klose and Tabatabai 1999). This is because urease, the enzyme that catalyses the hydrolysis of urea, retains its activity even when dead bacterial cells break open (i.e., lyse) and release their cytoplasmic contents.

As noted previously, the rate constants for ΔpH shown in Table 3 are properly pseudo first order rate constants that are valid for a fixed amount of bacterial biomass at a specific concentration of urea and unique initial pH. Conversion of the k' values to second order urea hydrolysis rate constants (corresponding to equation 7) is accomplished according to:

$$k = k' / 2.303 [\text{urea}]_0 \quad (12)$$

This transformation yields an average k value of $26 \text{ L mole}^{-1} \cdot \text{hr}^{-1}$ from 10^{-4} to 10^{-2} M urea, which compares quite favorably to k values that range from 27 to $100 \text{ L mole}^{-1} \text{ hr}^{-1}$ for soils incubated with $10^{-2.5}$ M urea (Swensen and Bakken 1998). The relatively close agreement between the mean laboratory k value obtained with *B. pasteurii* and those from soils incubated in the presence of urea is quite remarkable given that order of magnitude differences in urease enzyme affinity constants (K_m) have been reported for soil bacteria and pure bacterial cultures (Nielsen et al. 1998; Swensen and Kakken 1998).

The microbiological studies on rates of urea hydrolysis provide some key insight for the *BioMAGIC* process. First, enhanced rates of bacterial growth are not required to promote a more rapid increase in pH in response to urea hydrolysis. Instead, the supply of urea is critical as ΔpH rate constants increase progressively with increasing urea concentration. The implication is that an effective urea delivery system will be important to optimize the *in situ* performance of the *BioMAGIC* process. Second, laboratory derived second order rate constants for the hydrolysis of urea agree well with those measured for soils incubated with urea. While similar rates constants can be anticipated for urea hydrolysis in the South Bay aquifer, this remains to be verified through field experimentation. Upon verification, these rate constants might be usefully applied in chemical transport modeling to predict the actual field performance of the *BioMAGIC* process.

The onset of mineral precipitation from ground water that occurs in response to urea degradation was readily apparent in the groundwater laboratory experiments with the South Bay bacterial isolate and *B. pasturii*. Both cultures exhibited very dramatic colour changes – from dilute iron oxide orange to black and green, respectively. Chemical analyses of the culture fluids revealed high levels of dissolved Fe^{2+} , implying that Fe^{3+} -reduction accompanied urea degradation. This observation is consistent with the results of the geochemical modeling exercise which indicated that microbial Fe^{3+} reduction probably occurs in the South Bay contaminant plume (Fig. 1) (Lovley 1991). In addition, fluffy solid precipitates developed in both cultures. Examination of the precipitates by scanning electron microscopy and energy dispersive X-ray spectroscopy showed that they developed directly on bacterial cell surfaces (i.e., the bacteria served as heterogeneous nucleation templates for mineral precipitation) (Ferris 1993; Warren and Ferris 1998), and were comprised largely of iron, implying an oxide mineralogy, probably ferrihydrite. Further studies employing a combination of X-ray diffraction, and high resolution transmission electron microscopy and selected area electron diffraction are needed to confirm the mineralogy. The precipitates were also found to contain detectable concentration of zinc, implying that the metal was very efficiently removed from solution by the solid phase precipitates (Stumm and Morgan 1996). Note that the detection limit for energy dispersive X-ray spectroscopy is in the 1.0 weight percent range. Additional work is needed for quantification of solid phase metal partitioning as a consequence of

the *BioMAGIC* process. This scavenging of metals could be one of the most important side benefits of *BioMAGIC*.

CONCLUSIONS AND RECOMMENDATIONS

Based on computational results from geochemical modeling establishing the feasibility of BIOMAGIC, and microbiological studies confirming that urea-degrading bacteria are active in South Bay groundwater, future development work ought to proceed along three parallel lines of investigation. These include (i) implementation of exhaustive field testing; (ii) quantitative assessment of the impact of urea-degradation on solid phase metal partitioning (i.e., mineral precipitation and metal sorption); and (iii) construction of a reactive transport model – integrating groundwater flow parameters and rates of biogeochemical processes (from field and laboratory investigations) – to predict performance under realistic field conditions, and develop guidelines for full implementation. With respect to the general conclusions and recommendations outlined above:

- Microbiological investigations suggest that the BIOMAGIC field test should be conducted somewhere between the tailings area and Mud Lake where urea-degrading bacteria are likely to be more active.

- An infiltration gallery delivery system, for example a trench with a porous screen filled with fertilizer grade urea, needs to be designed and installed. It should probably extend into the contaminant plume.
- Close spaced monitoring wells (multi-level peizometers) may need to be installed down gradient of the urea delivery system to measure changes in groundwater chemistry on a regular basis within a reasonable time frame (week to month travel time of water between closest and farthest spaced monitoring wells). This would be an area with steepest possible gradient of hydraulic heads between wells. At least one full season of regular field sampling (weekly to biweekly) ought to be anticipated.
- The use of injection of dissolved urea solutions, followed by pumping to measure extent of urea degradation and groundwater chemistry changes, could be done on a wider scale at existing wells around the site. These tests could employ different lengths of time between injection and pumping to assess the combined influence of dilution by groundwater advection and bacterial urea-degration.
- The impact of urea additions to Mud Lake sediment and water ought to be investigated in laboratory microcosms, and macrocosms constructed in the field. If favorable results are obtained, Mud Lake might be restored to a more neutral pH level in a timely fashion.

- Studies on the products, and rate and extent of solid phase metal partitioning, that accumulate in response to bacterial urea-degradation should focus primarily on reduced groundwater from the contaminant plume, and Mud Lake water. These are places where BIOMAGIC is apt to be initiated with the least amount of difficulty.
- Solid phase metal partitioning induced by bacterial urea-degradation should be studied in solution, as well as sand filled microcosms, to ascertain whether any differences in process performance can arise because of attachment of bacteria to solid surfaces.

A standard computer code for reactive transport modeling should be immediately identified, and input parameters clearly defined to establish what information is already available, and what additional information is needed, to construct and validate a predictive model.

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Table 1: Mean hydraulic conductivity of clean silica sand, and sand coated with hydrous ferric oxide (HFO), bacteria, and HFO-bacteria.

Coating on Silica Sand	Number of Columns	Mean Hydraulic Conductivity K
None	10	0.020
HFO	15	0.019
BrY	14	0.019
HFO, BrY	15	0.017

Table 2: Percentage distribution of different physiological groups of microorganisms at South Bay according to field pH values

Field pH range	Percent of Wells	Urease Positive	Nitrate Reducing	Sulfate Reducing	Fungi
3.0-3.4	2.6	0.0	3.7	0.0	16.7
3.5-3.9	5.3	0.0	0.0	0.0	16.7
4.0-4.4	10.5	3.8	0.0	0.0	33.3
4.5-4.9	2.6	3.8	3.7	0.0	0.0
5.0-5.4	5.3	7.7	7.4	10.0	0.0
5.5-5.9	34.2	34.6	37.0	25.0	16.7
6.0-6.4	23.7	30.8	29.6	40.0	0.0
6.5-6.9	13.2	19.2	18.5	25.0	0.0

Table 3: First order growth and DpH rate constants for *Bacillus pasteurii* grown in trypticase soy broth at different urea concentrations.

log [Urea M]	<u>Quarter-strength TSB</u>		<u>Half-strength TSB</u>	
	k growth	k' Δ pH	k growth	k' Δ pH
-4	0.021	0.019	0.021	0.013
-3	0.019	0.018	0.024	0.015
-2	0.019	0.031	0.024	0.023
-1	0.013	0.035	0.024	0.024

Figure 1

3D Contour Plot South Bay Ferrous Iron Fraction
Distance Weighted Least Squares

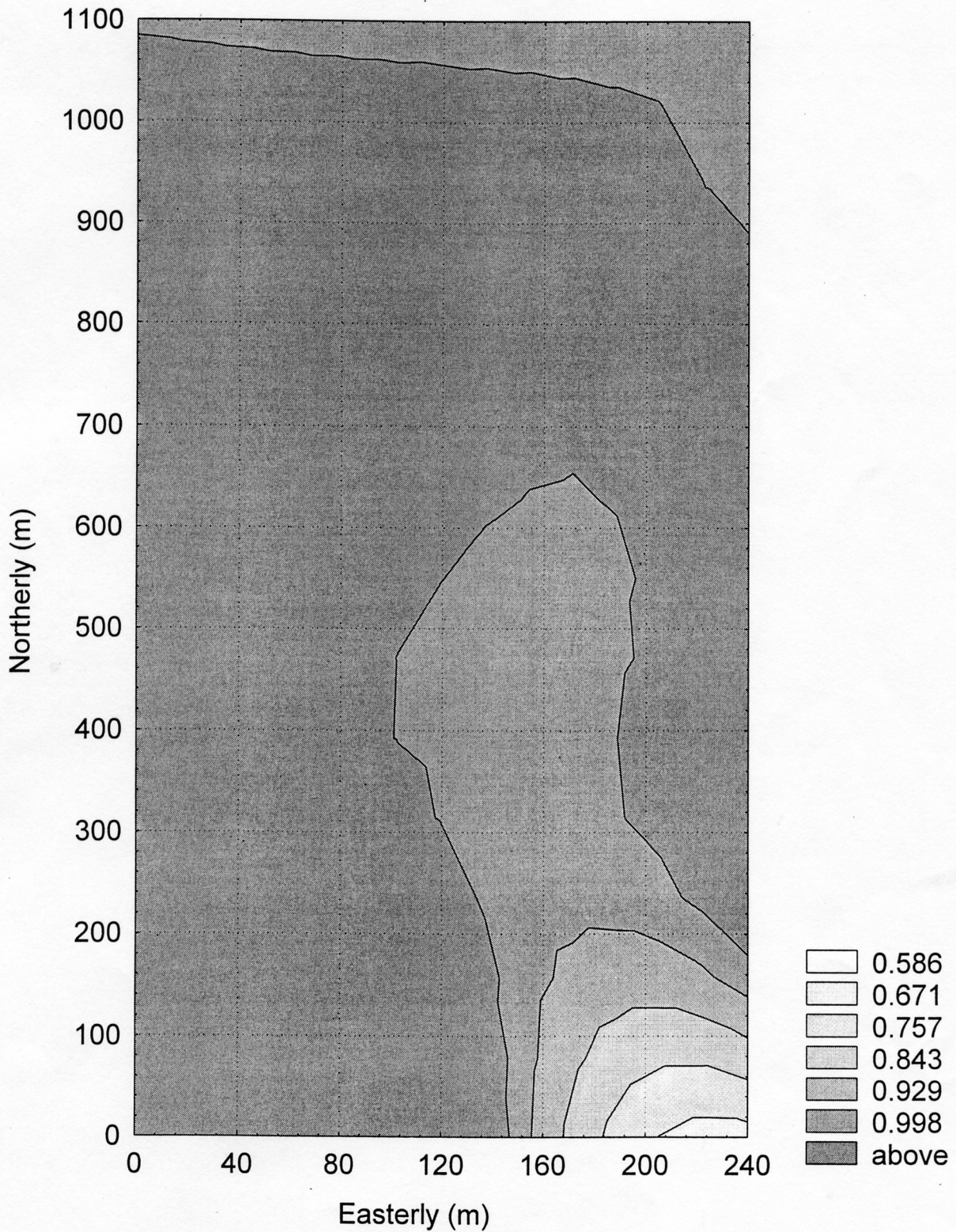


Figure 2

3D Contour Plot Urea Equivalents (M) to Increase pH to 8.0
Distance Weighted Least Squares

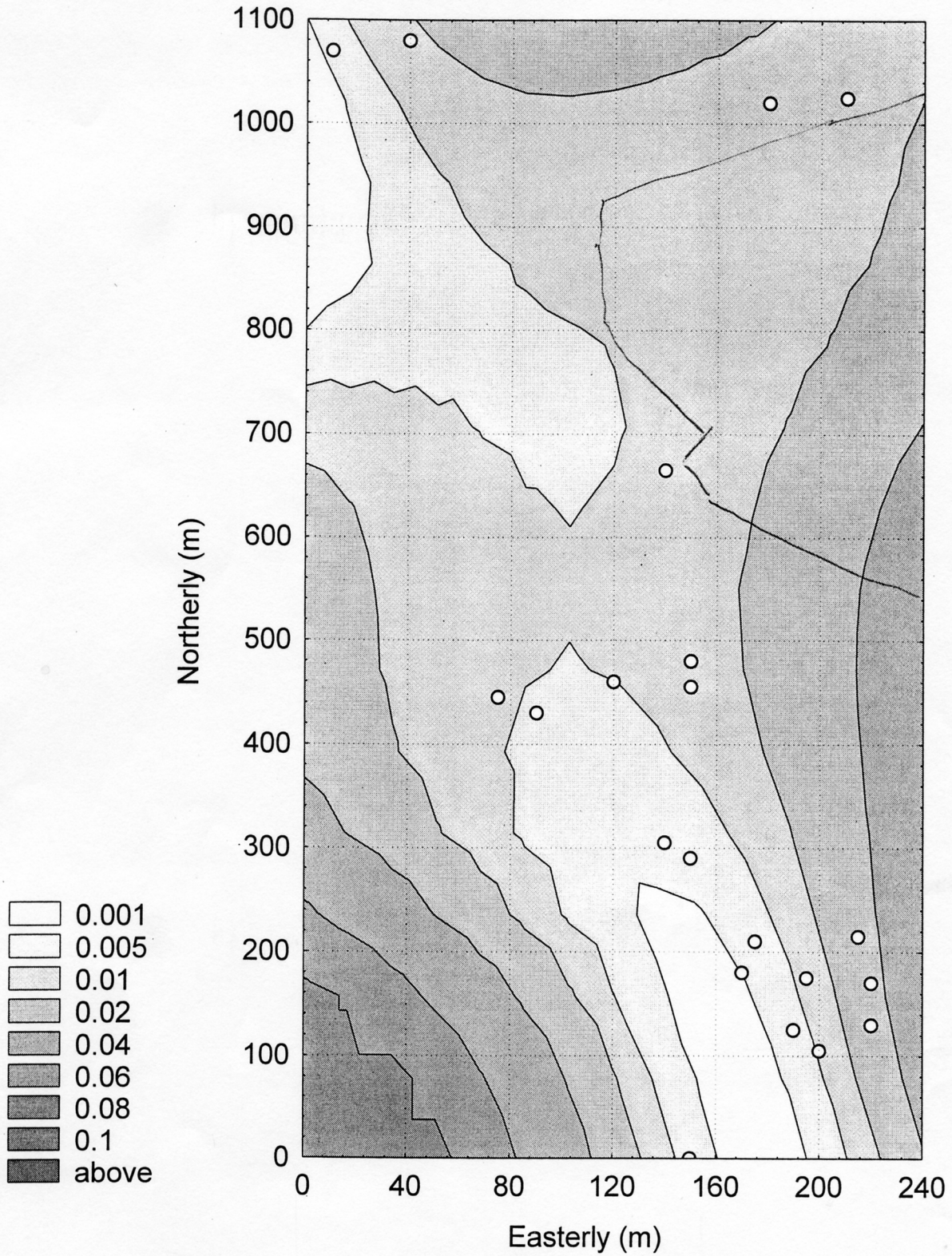


Figure 3

3D Contour Plot Ferrihydrite Initial SI
Distance Weighted Least Squares

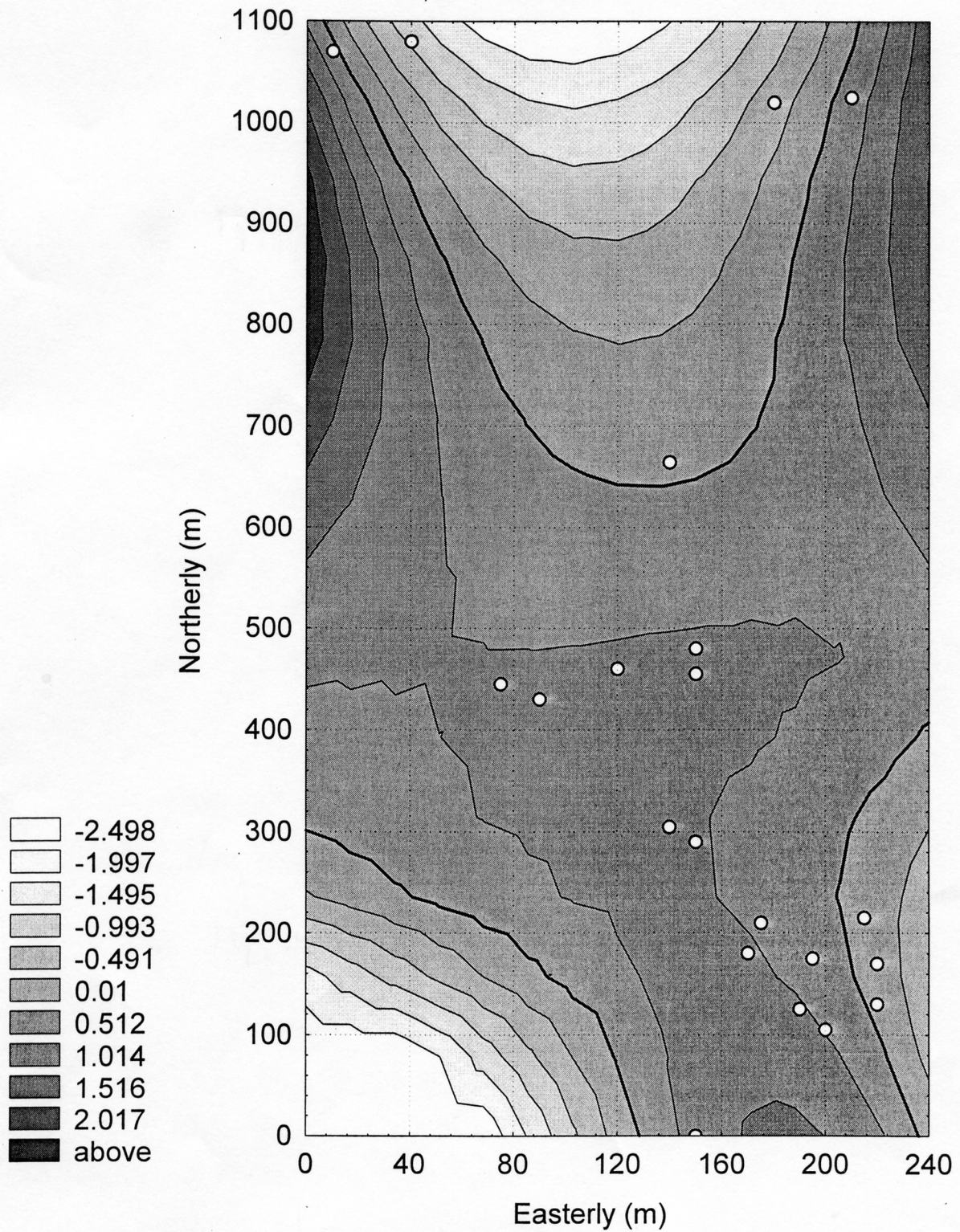


Figure 4

3D Contour Plot Ferrihydrite SI at pH 8.0
Distance Weighted Least Squares

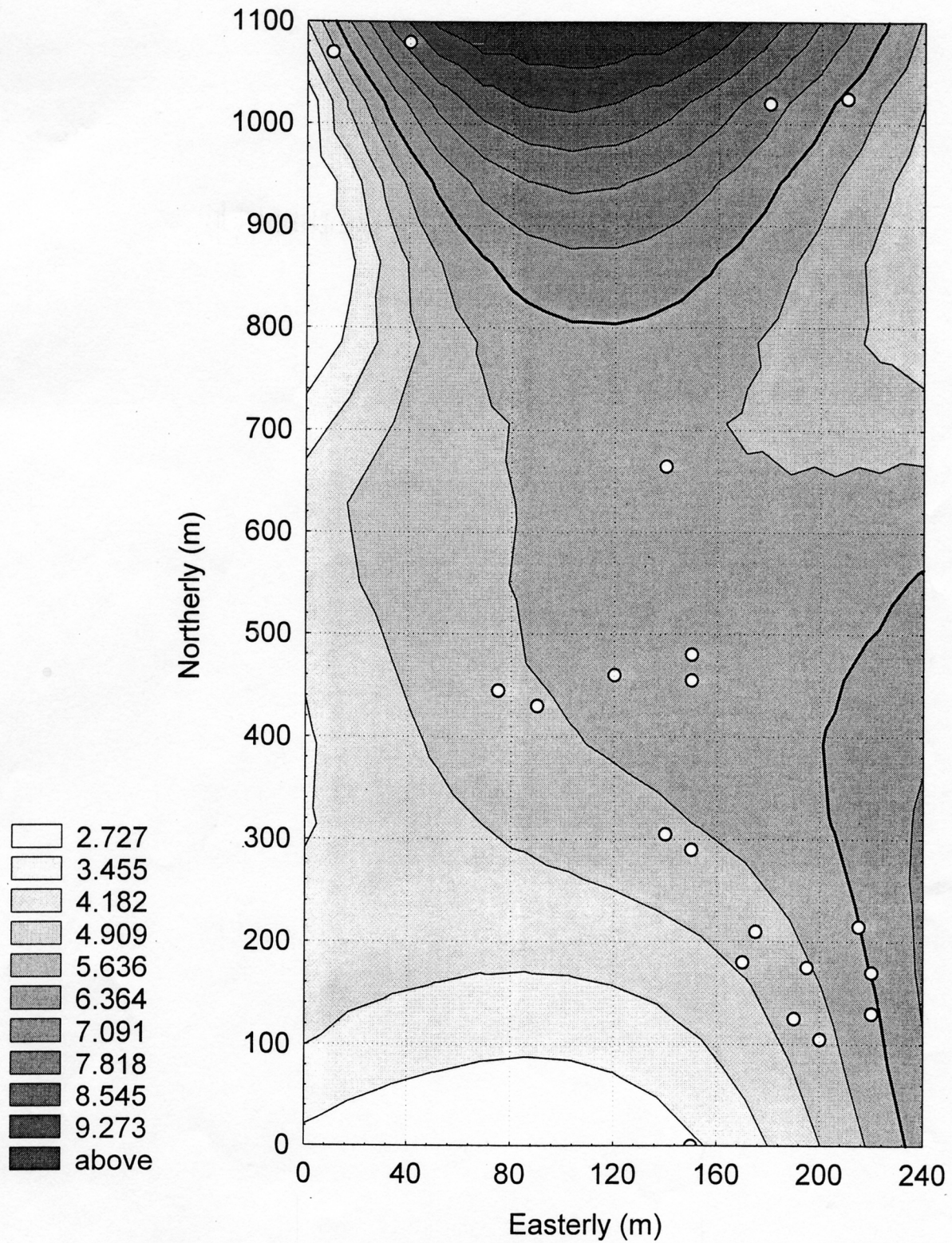


Figure 5: Change in pH as a function of time for *B. pastuerii* grown in quarter-strength TSB

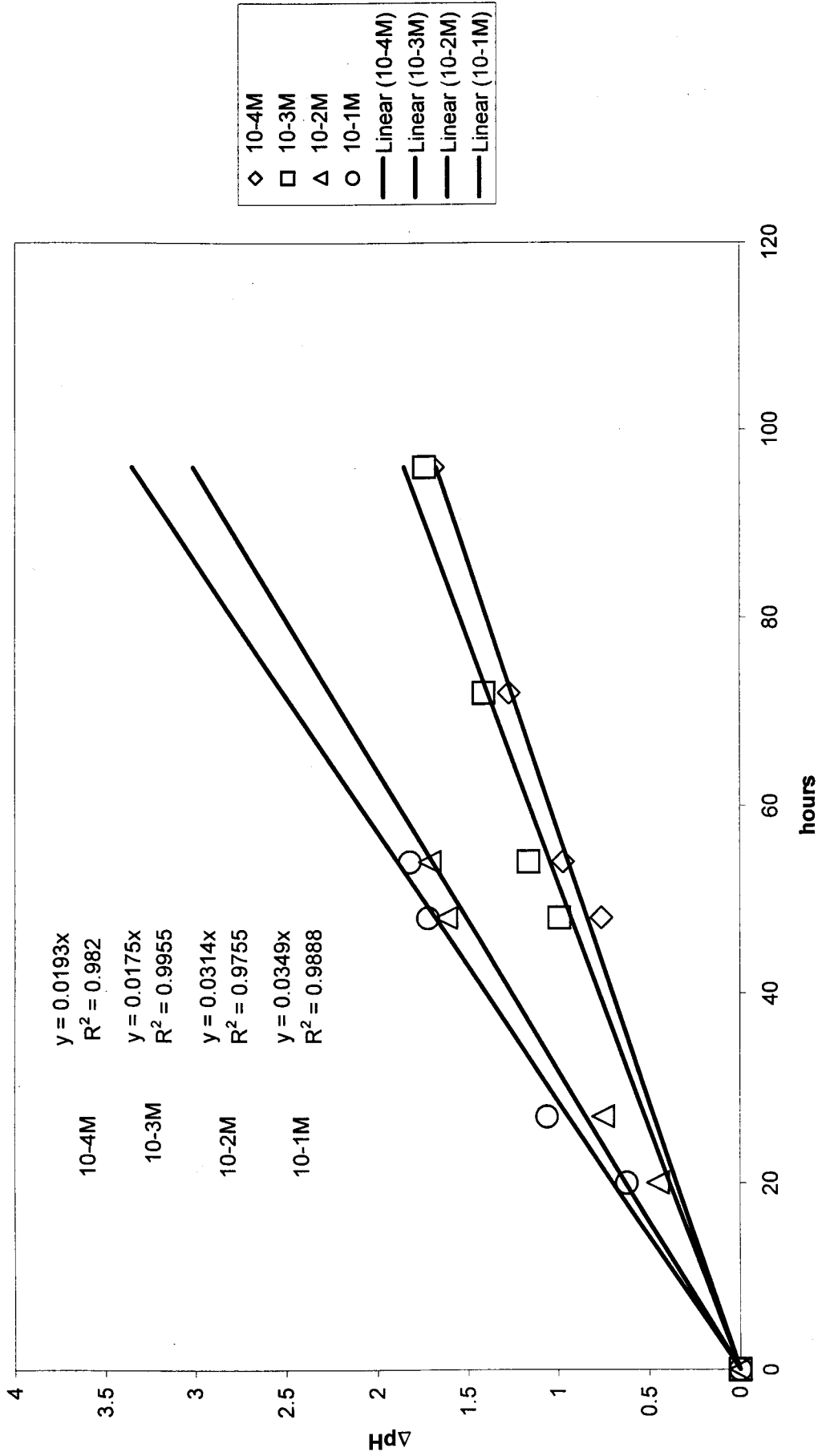


Figure 6: Change in pH as a function of time for *B. pastuerii* grown in half-strength TSB

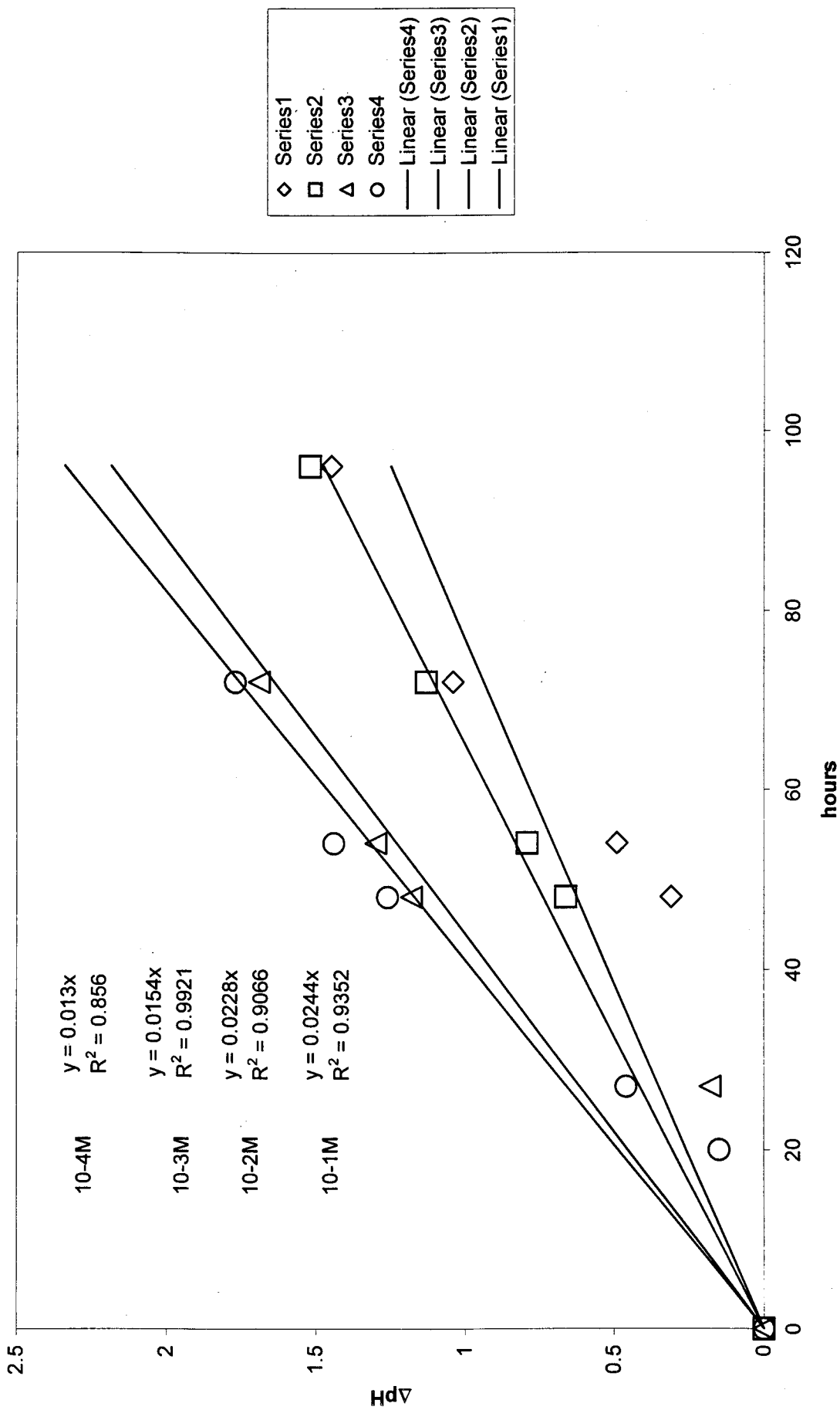


Figure 7: Growth of *B. pastuerii* in quarter-strength TSB

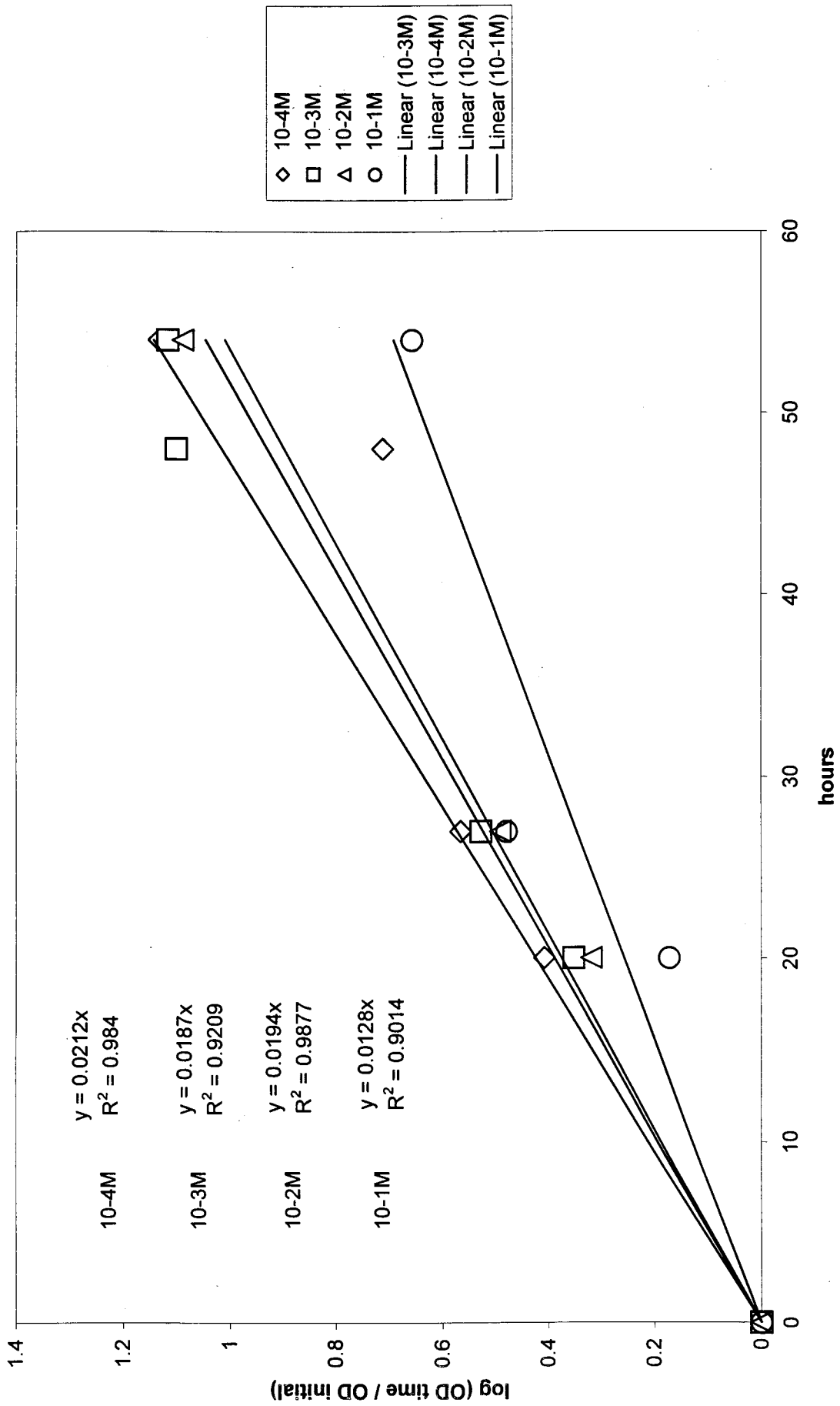
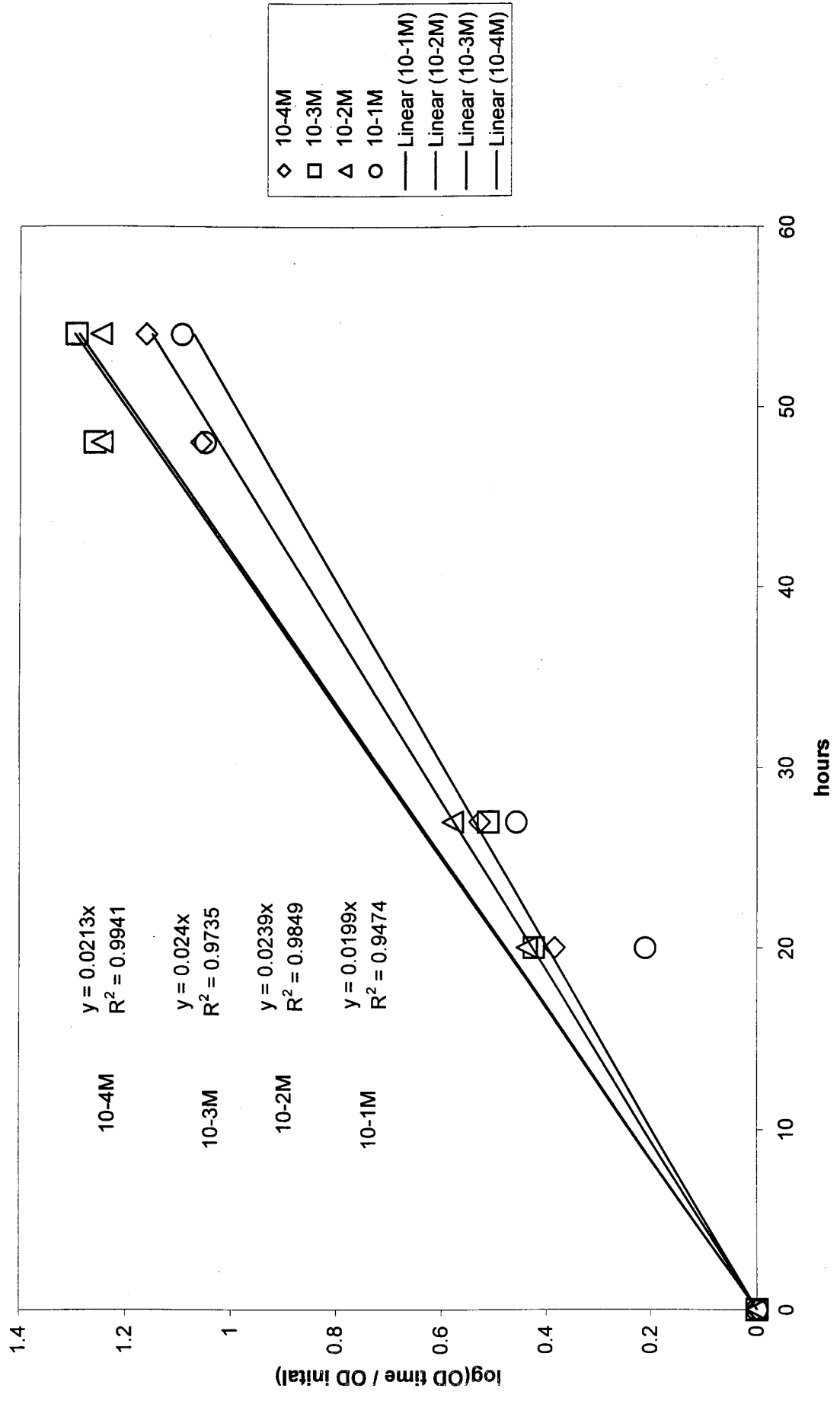


Figure 8: Growth of *B. pasteurii* in half-strength TSB



Appendix A

