



An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters

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Abstract

Acid mine drainage pollution may be associated with large water volume flows and exceptionally long periods of time over which the drainage may require treatment. While the use and role of sulphate reducing bacteria has been demonstrated in active treatment systems for acid mine drainage remediation, reactor size requirement and the cost and availability of the carbon and electron donor source are factors which constrain process development. Little attention has focussed on the use of waste stabilisation ponding processes for acid mine drainage treatment. Wastewater ponding is a mature technology for the treatment of large water volumes and its use as a basis for appropriate reactor design for acid mine drainage treatment is described including high rates of sulphate reduction and the precipitation of metal sulphides. Together with the co-disposal of organic wastes, algal biomass is generated as an independent carbon source for SRB production. Treatment of tannery effluent in a custom-designed high rate algal ponding process, and its use as a carbon source in the generation and precipitation of metal sulphides, has been demonstrated through piloting to the implementation of a full-scale process. The treatment of both mine drainage and zinc refinery wastewaters are reported. A complementary role for microalgal production in the generation of alkalinity and bioadsorptive removal of metals has been utilised and an Integrated 'Algal Sulphate Reducing Ponding Process for the Treatment of Acidic and Metal Wastewaters' (ASPAM) has been described.

Introduction

Pollution of surface waters with acid mine drainage (AMD) follows geochemical trauma induced by mining operations and the resulting impact on the environment of ferric oxide 'Yellow Boy' precipitates has been known at least since Roman times (Wildeman et al. 1991). The biological and physico-chemical processes giving rise to pyrite oxidation, acid formation and heavy metal solubilisation have been well described and are comprehensively reviewed elsewhere (Andrews 1989; Silver 1989; Kuenen & Robertson 1992; Pronk & Johnson 1992; Robb 1994; Johnson 1995).

Biological approaches to AMD treatment have been reviewed by Kuenen & Robertson (1992); Gadd & White (1993); Barton (1995) and Johnson (1995)

and applications of the sulphate reducing bacteria (SRB) have been of particular interest, given their role in the generation of insoluble metal sulphides and the neutralising effect of the sulphate reducing reaction (Barton & Tomei 1995). The biology of these organisms has been the subject of comprehensive treatment by Postgate (1984), Widdel & Hansen (1992), Odom & Singleton (1993) and Barton (1995).

The use of wetlands for the treatment of AMD is a, so-called, passive technology which has developed rapidly in recent years (Johnson 1995; Robinson & Robb 1995; Van Zyl 1996; Younger et al. 1997) and provides a low operational cost approach to long-term management of the problem. Drawbacks include the large surface area requirement for higher AMD flows and concerns relating to the diffuse spread and long term stability of the metals deposited.

Active biological AMD treatment systems rely in the main on high SRB bioreactor growth rates and the precipitation of metal sulphides. Numerous SRB reactor design studies have been reported including anaerobic filters (De Walle et al. 1979; Chian & De Walle 1983), mixed reactors (Maree & Hill 1989), packed bed anaerobic bioreactors (Riviera 1983; Maree et al. 1987) fluidised bed systems (Umita et al. 1988; van Houten et al. 1994), sequencing batch reactors (Herrera et al. 1991), the upflow anaerobic sludge bed (Buisman et al. 1989; Barnes et al. 1991) and the baffle reactor (Grobicki & Stuckey 1992). Scaled-up applications of active AMD treatment technologies have been limited but the successful operation of a SRB process effecting a geohydrological containment function at the Budelco zinc refinery, The Netherlands, has been reported (Scheeren et al. 1993).

Notwithstanding the type of biological AMD process technology used, the singular factors constraining the biological treatment approach are the reactor configuration used, the cost of construction and the availability and cost of the carbon source and electron donor for the microbial reduction processes. Among the complex biota which establishes in wetlands plantings of *Sphagnum sp.*, *Typha latifolia* and *Phragmites australis* have been used and may provide a carbon source to the system of up to 40 ton-ha⁻¹.yr⁻¹ (Wieder 1993). Among many other carbon sources which have been evaluated for active SRB production are sewage sludge (Butlin et al. 1956; Pipes 1960; Burgess & Wood 1961; Conradie & Grutz 1973), animal waste slurries (Ueki et al. 1988); straw, hay and lucerne (Bechard et al. 1993); lactate and cheese whey (Olezkiewicz & Hilton 1986; Herrera et al. 1991) molasses (Maree & Hill 1989), ethanol and methanol (Postgate 1984; Braun & Stolp 1985; Swezyk & Pfenning 1987) and producer gas (Du Preez et al. 1992; van Houten et al. 1994).

Boshoff et al. (1996) have investigated the anaerobic fermentation of waste-grown microalgal biomass produced in waste stabilisation ponds and the linked production of sulphide by SRB.

Early suggestions for engineering algal removal systems include the description of meanders treating AMD in which algal growth occurs together with benthic flora and other vegetation (Gale & Wixson 1979; Jennet et al. 1979; Mann & Fyfe 1988). Filip et al. (1979) have reported metal removal by sand filter immobilised algae and Brady et al. (1994) have described a membrane immobilization process for removing metals by filtration. Immobilisation of algal

biosorbants has been commercialised as a silica bead preparation marketed as AlgaSORBTM (Greene & Bedell 1990).

Integration of algal and SRB processes

While waste stabilisation pond (WSP) technology has been developed over the past 40 years for a wide range of wastewater treatment applications (Mara & Do Monte 1987; Mara et al. 1996) little attention has apparently focussed on the use of these systems for AMD remediation. Successful operation of WSP involves the large-scale application of algal photosynthesis, and the role of SRB in the anaerobic compartments of these systems has been reported (de Pauw & Salamoni 1991). Principal advantages of using the WSP as a basic design concept around which to develop AMD treatment processes are that:

- the linkage between the treatment of large volumes of wastewaters and mass algal production has been firmly established as a mature and widely utilised operational technology (Mara et al. 1996);
- the earthwork pond provides a reactor at least an order of magnitude less costly to construct than steel-reinforced vessels (Oswald 1995);
- renewable algal biomass as a potential carbon source for SRB may be reliably produced in large amounts (up to 50 tons.ha⁻¹.yr⁻¹) in separately optimised high rate systems (Oswald 1988);
- the ability of algal biomass to adsorb heavy metals and thereby reducing metal concentrations to residuals in the ppb range has been demonstrated and commercialised (Wilde & Benneman 1993);
- anaerobic ponds may support high levels of SRB activity (Pescod 1996).

This study reports the development of an Integrated Algal Sulphate Reducing Ponding Process for Acidic and Metal Wastewater Treatment (ASPAM) utilising the basic reactor design approach proposed by Oswald (1991) for the operation of Integrated Wastewater Ponding Systems. The investigation was based on a development of high rate algal ponding for the treatment of tannery wastewaters (Rose et al. 1996), and previous preliminary reports of algal biomass used in metal bioremediation, and as a carbon source for sulphate reduction (Boshoff et al. 1996; van Hille & Duncan 1996). Figures 1 and 11 illustrate the fully configured process which, in its simplest form, may

involve the settling of metal sulphide sludges in the anaerobic compartment of a facultative pond treating some source of organic waste material in a co-disposal function. In addition to providing algal biomass as an independent carbon source, and the final polishing of metals from the waste stream, the algal high rate pond may also be used to provide control of sulphide release with the recycle of an oxygen-rich cap to the surface of the facultative pond. We have found that this basic approach may be expanded to achieve a potentially high level of control in the pre-settlement of heavy metal loads from large wastewater flows.

Materials and methods

Sulphates and sulphides were analysed according to Standard Methods (APHA 1985). Chemical oxygen demand (COD) was analysed using a Merck Spectroquant Kit. Chlorophyll a. (chl.a) was extracted into 100% acetone and quantified according to Lichtenthaler (1987). Borosilicate glassware was used in the metal binding experiments and the metal solutions were prepared using distilled water. Metals were analysed on a GBC 909AA Atomic Absorption Spectrophotometer linked to a GBC integrator. Total organic carbon (TOC) was analysed using a Dohrmann 180 Total Organic Carbon Analyser.

Metal binding and removal studies were performed in 250ml Erhlenmeyer flasks. Metal removal was controlled against pure metal solution precipitation at the same pH and at the various metal concentrations tested. Reported results represent the difference between control and experimental readings. A ten hour settling period was allowed for removal of metal precipitate.

An eight litre upflow anaerobic reactor was fed media with the following composition ($\text{g}\cdot\text{l}^{-1}$): NH_4Cl 0.5; K_2HPO_4 1.0; $\text{mgSO}_4\cdot 7\text{H}_2\text{O}$ 0.2; $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ 0.1; $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ 0.1; Na_2SO_4 0.5. Dried *Spirulina* sp. was used as the organic substrate. The reactor was seeded with sludge from a methanogenic reactor treating raw sewage. Gas production was monitored as well as sulphate reduction between inlet and overflow ports.

The *Spirulina* sp. culture for the metal binding studies was isolated from a tannery WSP, grown and maintained in Zarouk's media (Zarouk 1966) at a constant temperature of 28°C under cold white light with a light/dark cycle of 18:6 hours. Cells were harvested by filtration through a GF/C filter or a nylon mesh with a pore size of 50 microns.

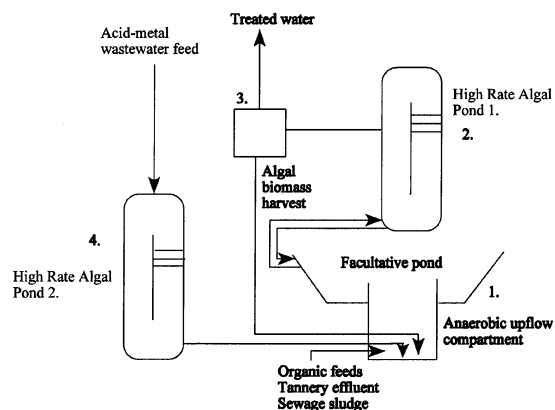


Figure 1. Flow diagram of the principal unit operations involved in the algal sulphate reducing high rate ponding process. The fully integrated model is described in Figure 11. The effluent may pass directly to the anaerobic compartment or alternatively through the high rate algal pond 2 pretreatment step 4.

Metal binding to *Spirulina* was measured in a culture harvested and resuspended in either Zarouk's media or water. The cultures were placed on a shaker at 60 rpm and metal of varying concentrations added. Samples were removed at time intervals and filtered through a 0.45 micron nylon membrane filter. The filter was digested with $200\mu\text{l}$ concentrated HNO_3 and analysed for metals as described above.

Results and discussion

Tannery effluent

The use of tannery effluent as a source of waste organic matter to provide the carbon and electron donor for the sulphate removal and sulphide production unit operations of the process was investigated. In addition to its high organic load, tannery effluent also contains high levels of sulphate. Tannery wastewater treatment plants are a rich source of adapted SRB, making the system a useful research model for the study of SRB sulphide production processes.

A 1.5 m^3 upflow anaerobic reactor was operated at a tannery producing automotive upholstery leather. The reactor was fed a stream of the tannery's mixed effluent drawn prior to treatment and mixed with a sulphate solution to produce a final concentration around $2000\text{ mg}\cdot\text{l}^{-1}\text{ SO}_4$ and a $\text{COD}:\text{SO}_4 < 0.5$. This mixture would simulate the flow, in Figure 1, of a metal-sulphate solution entering directly to the anaerobic compartment of the facultative pond. An hydraulic retention time (HRT) of 3 days was sustained in the

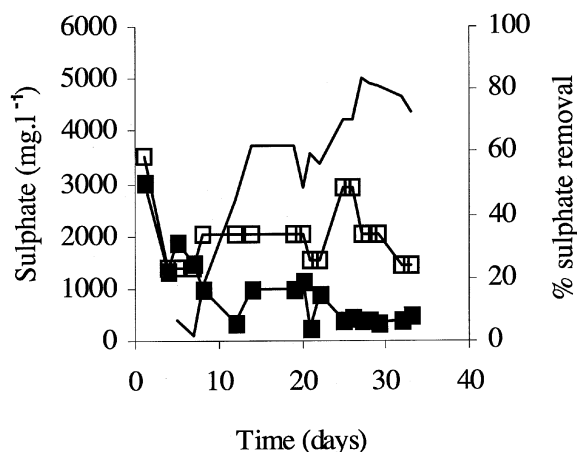


Figure 2. Sulphate reduction in an upflow anaerobic digester fed tannery effluent blended with a synthetic mine water solution. Open square = inlet sulphate concentration; closed square = outlet sulphate concentration; line = % sulphate removal.

reactor for the 60 day duration of the experiment and no methane production was observed. Figure 2 shows the start-up period of this study, the achievement of an optimum sulphate reduction in the reactor around 80% and a conversion rate of over $500 \text{ mg SO}_4 \cdot \text{l}^{-1} \text{ reactor volume} \cdot \text{day}^{-1}$. Figure 3 reports the blending of reactor overflow liquor with a synthetic mine water solution to effect metal sulphide precipitation ($2000 \text{ mg} \cdot \text{l}^{-1} \text{ SO}_4$; $200 \text{ mg} \cdot \text{l}^{-1} \text{ Fe}$; final pH 7.2) and shows the removal of iron at levels about 8 times anticipated stoichiometric removal (as monosulphide).

Metal removal from two zinc refinery waste streams utilising tannery-fed sulphate reducing digester liquor was also evaluated and Figure 4 reports total metal removed from a wastewater pond and from a slimes dam seepage sump collection on the site. Zinc recovery levels of $2640 \text{ mg} \cdot \text{l}^{-1}$ and $438 \text{ mg} \cdot \text{l}^{-1}$ were recorded in each case indicating the relatively large amounts of metals which can be removed using this approach.

High rate pond technology for the treatment of tannery wastewater was scaled up at a tannery in Wellington, South Africa, with the construction of a 20 Ml facultative pond reactor followed by a 2500 m^2 HRAP supporting a near monoculture of the cyanobacterium *Spirulina sp.* (Rose et al. 1996). Steps 1 and 2 in Figure 1 indicate the flow path of the tannery effluent fed directly to the facultative pond. The COD and sulphate/sulphide removal function of the system are reported in Table 1 which shows nearly 100% sulphate reduction in the anaerobic compartment, and

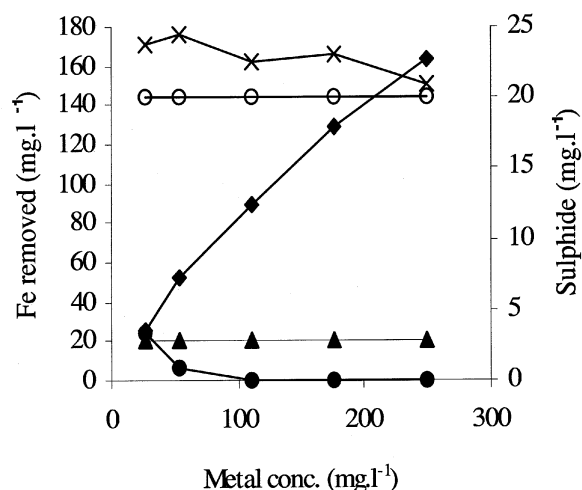


Figure 3. Iron removal from a synthetic mine water solution with the addition of a sulphide-rich tannery effluent-fed anaerobic sulphate reducing reactor overflow liquor. Cross = total sulphide; open circle = dissolved sulphide before addition of iron; closed circle = dissolved sulphide after addition of iron; triangle = anticipated stoichiometric metal removal; diamond = total metal removal obtained during the experiment.

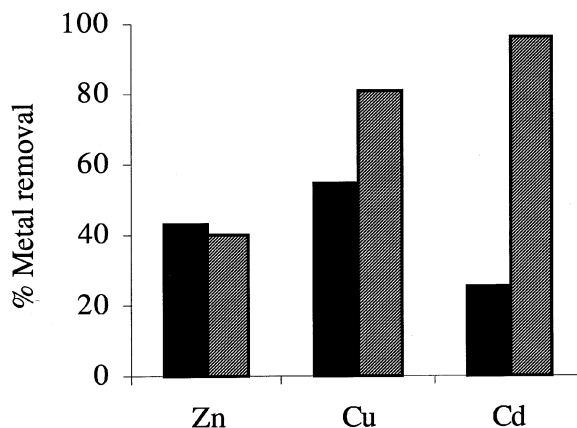


Figure 4. Percentage removal of metals from zinc refinery wastewater pond and slimes dam seep effluents using sulphide-rich overflow liquor from a tannery-fed sulphate reducing digester. Closed column = pond effluent ($\text{Zn } 6000 \text{ mg} \cdot \text{l}^{-1}$; $\text{Cu } 97.8 \text{ mg} \cdot \text{l}^{-1}$; $\text{Cd } 14 \text{ mg} \cdot \text{l}^{-1}$); striped column = seep effluent ($\text{Zn } 996 \text{ mg} \cdot \text{l}^{-1}$; $\text{Cu } 28.6 \text{ mg} \cdot \text{l}^{-1}$; $\text{Cd } 4.7 \text{ mg} \cdot \text{l}^{-1}$).

a 92% reoxidation of sulphide produced in the aerobic facultative zone, which caps the facultative pond, thereby controlling sulphide emissions, followed by nearly 100% final oxidation in the HRAP. An oxy-pause was established at a depth of 0.5 m from the surface of the facultative pond. An 84% reduction in COD load was achieved across the system. The metal removal function of the system was monitored and Table 2 shows the influent metal load from the tan-

Table 1. Performance of an operating scale 20MI facultative and 2500 m² high rate algal pond (HRAP) system treating tannery effluent showing sulphate reduction, sulphide oxidation and COD removal in the various stages of the process.

	Tannery effluent	Facultative pond anaerobic compartment	Facultative pond aerobic cap	HRAP
Sulphate as SO ₄ ²⁻ (mg.l ⁻¹)	975	<1	989	809
Sulphide as Na ₂ S (mg.l ⁻¹)	285	1100	76.5	0.1
COD (mg.l ⁻¹)	2474	1216	1216	394

Table 2. Heavy metal removal from tannery effluent by sulphide precipitation in the anaerobic compartment of a 20 MI facultative pond feeding a 2500 m² high rate algal pond. Removal efficiency is measured by subsequent heavy metal adsorption by algal biomass in the HRAP shown before and after the commissioning of the sulphide producing anaerobic unit operation in the flow path. Percentage total metal removal includes both biomass adsorption and metal sulphide precipitation.

	Biomass metal concentration before sulphide treatment (mg.Kg ⁻¹)	Biomass metal concentration after sulphide treatment (mg.Kg ⁻¹)	Total metal removed (%)
Cadmium	5.96	< 1	> 99.9
Chromium	25.8	< 1	> 99.9
Cobalt	22.4	3.3	85
Iron	2012	795	60
Lead	219	2.3	99
Nickel	49.2	17.5	64
Zinc	218.5	22.5	90

ning operation adsorbed by the microalgal biomass in the HRAP before and after commissioning the anaerobic sulphate reducing stage of the operation. The metal reduction of final treated water to < 1mg.l⁻¹, achieved by the joint action of sulphide precipitation followed by biomass adsorption, provides a demonstration of the AMD polishing quality which may be anticipated in the ASPAM system. A 90% COD removal was recorded and methane formed 19% of free gas produced by the system. At an operating pH of 8.3 no free sulphide release was detected above the pond surface. Figure 5 reports the results of an investigation of the sulphide tolerance of the *Spirulina* sp. culture which established in the HRAP described above. It was found, in a series of sealed flask studies (headspace flushed with nitrogen gas to exclude oxygen), that after an initial adaptation, growth was sustained even at the daily addition to the culture of a 300 mg.l⁻¹ sodium sulphide. The complete oxidation of the sulphide load by microalgal activity provides an indication of the efficiency that can be expected from the capping of the facultative pond with the HRAP recycle liquor.

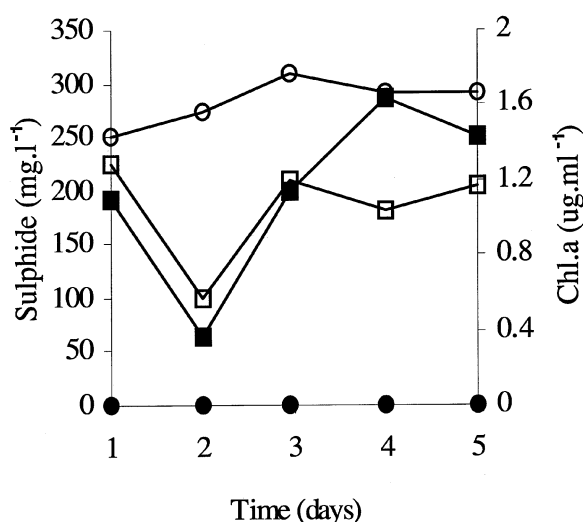


Figure 5. Effect of sulphide addition to a *Spirulina* culture. Open circle = sodium sulphide daily feed to the culture pH9, closed circle = residual dissolved sulphide in the growth medium, open square = chlorophyll a. concentration in sulphide fed culture medium, closed square = chlorophyll a. concentration control culture without sulphide feed.

Table 3. Metal removal capacity of an anaerobic sulphate reducing digester overflow liquor from a digester fed a slurry of *Spirulina* as sole carbon and electron donor source. Results are reported as percentage metal ion removed over a concentration range 500 to 3000 mg.l⁻¹ and standard deviations are reported in brackets.

Metal	500 mg.l ⁻¹	1000 mg.l ⁻¹	1500 mg.l ⁻¹	2000 mg.l ⁻¹	2500 mg.l ⁻¹	3000 mg.l ⁻¹
Cu	79.2 (± 9.0)	77.4(± 19.0)	66.3 (± 14)	46.4 (± 4)	50.6 (± 15.8)	51.1 (± 8.6)
Zn	88.0 (± 13)	67.9(± 6.2)	38.9 (± 16)	32.6 (± 16)	38.0 (± 23)	36.7 (± 19)
Fe	100	51.0(± 17.0)	41.3 (± 58)	61.0 (± 4.9)	57.1 (± 4)	60.3 (± 6.7)

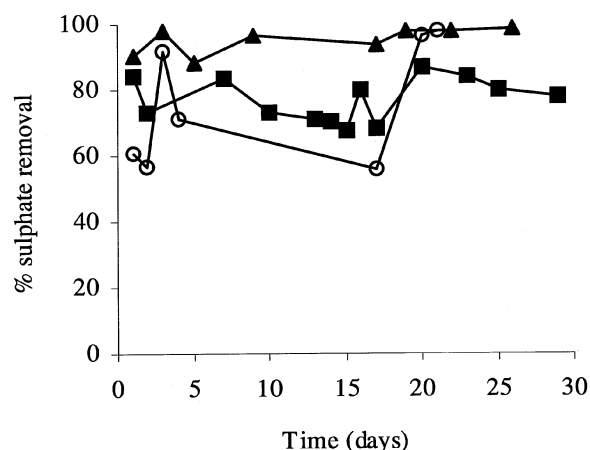


Figure 6. Sulphate removal in an upflow digester fed different concentrations of algal biomass as the sole carbon source for sulphate reducing bacteria. Results reflect the mean of three analyses. Triangle = 4 g.l⁻¹ algal biomass; square = 8 g.l⁻¹ algal biomass; open circle = 10 g.l⁻¹ algal biomass.

Currently the ASPAM system using primary sewage sludge to feed an integrated sulphate reducing high rate ponding process for the treatment of both AMD and a zinc refinery wastewater is under evaluation by the authors. While the tannery ponding system reported here was designed to treat an effluent load of 0.5 Ml.day⁻¹, ponding systems are operated at daily loading rates well over 200 Ml.day⁻¹ (Lawtry et al. 1995).

Algal biomass

The use of *Spirulina sp.* biomass produced in the above system was investigated as a carbon source for sulphate reduction. The objective was to utilise the rapidly biodegradable fraction for sulphide production and to retin in solution the more slowly degraded cell structural components to be utilised for the subsequent metal adsorption unit operation (see Figure 11). Biomass was harvested from the operating-scale HRAP described above, dried, resuspended in tap water and

fed to an 8l upflow anaerobic digester with an HRT of 1 day. After reactor stabilisation on the algal feed the slurry was fed in a synthetic sulphate effluent containing 800 mg.l⁻¹ SO₄, and at algal concentrations of 4, 8 and 10g.l⁻¹ for 40 days in each case. Figure 6 shows percentage sulphate removal over 20 days of stable operation for each feed rate with an average COD removal of approximately 25%, and a COD:SO₄ conversion efficiency for the utilised component of the 4g feed of 1.4:1. No methane production was observed over the study period. Metal uptake capacity of the reactor overflow was measured for Cu, Zn and Fe over a concentration range of 500 to 3000 mg.l⁻¹. Results of this study are reported in Table 3. which indicates that total metal removal levels are substantially higher than anticipated stoichiometric metal sulphide formation, which, in this study, would account for only about 5% of total metal ions removed. Metal binding to whole algal cells and cellular fragments has been well established and Crist et al. (1988) have identified a number of the chemical groups involved.

While current results indicate that the potential metal binding capacity of the SRB-digested algal biomass may be enhanced by passing through the digester, further work is being undertaken to characterise the nature, and to quantify the possible process significance, of this step. More complete digestion of algal biomass will, of course, occur at HRT longer than one day. Algal biomass production within the ponding process offers a degree of independence from external supply of carbon sources.

Alkalisation

The use of alkalinity produced by algal photosynthesis in the precipitation and recovery of heavy metals has not been widely reported. Our study has shown that in the ASPAM system the recycle of alkalinity (both hydroxide and carbonate) from the HRAP, and possibly also from the anaerobic compartment, could play an important role in achieving the successful pre-

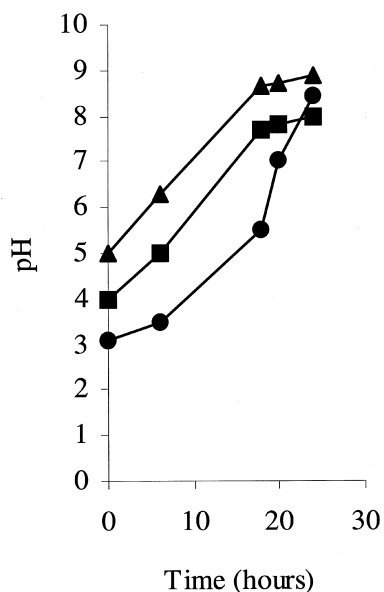


Figure 7. Re-alkalisation rate of a *Spirulina* culture where pH has been reduced to 3, 4 and 5 respectively. Triangle = pH5; square = pH4; circle = pH3.

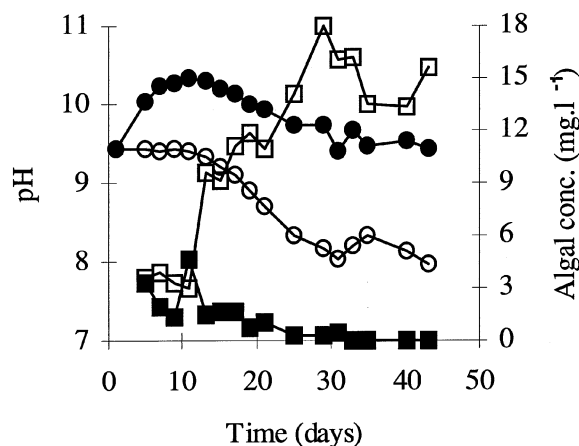


Figure 8. Response of a *Spirulina* culture to a continuous loading regime of AMD at 10% reactor volume-day⁻¹. Closed square = algal concentration of cultures fed 10% AMD-day⁻¹; open square = algal concentration of control cultures fed 10% growth medium-day⁻¹; closed circle = pH of algal culture fed 1-% AMD-day⁻¹; open circle = pH of AMD without algae.

precipitation of certain mixtures of heavy metals (van Hille et al. 1997). Formation and precipitation of metal sulphide/carbonate/hydroxide mixtures has been reviewed by Peterr et al. (1985), and would play an important role in reducing the major fraction of the metal load in the incoming AMD, prior to passing to the anaerobic compartment, and to HRAP1 (see Figure 11).

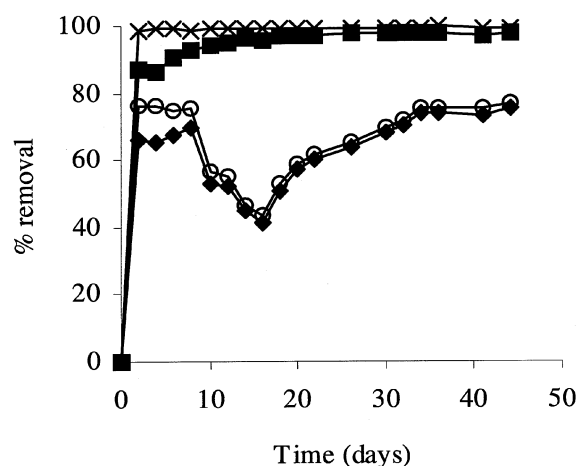


Figure 9. Cumulative removal of heavy metal component in a *Spirulina* culture under continuous loading of AMD at a rate of 10%·day⁻¹ tota; volume. Cross = Fe; square = Zn; open circle = Pb; diamond = Cu.

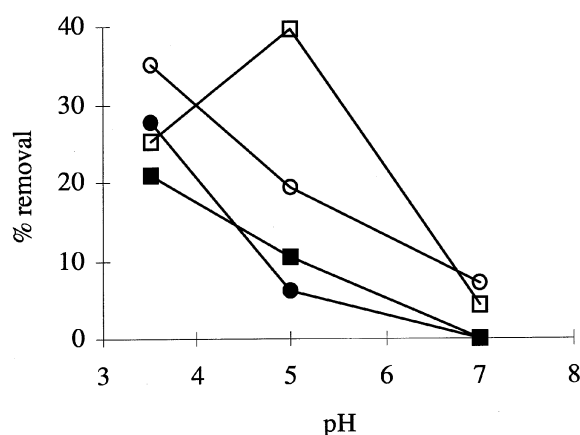


Figure 10. Metal removal capacity of extracellular metal complexing compounds released to the growth medium by live *Spirulina* cells. Open square = 1000 mg·l⁻¹ Cu solution; closed square = 100 mg·l⁻¹ Cu solution; open circle = 1000 mg·L⁻¹ Fe³⁺ solution; closed circle = 100 mg·l⁻¹ Fe³⁺ solution.

The ability of *Spirulina* cultures to rapidly re-alkalise their aquatic environment, following acidification with AMD, is illustrated in Figure 7 for a biomass loading of 3 µg·ml⁻¹ chlorophyll a. In practice a continuous loading regime would be established to ensure a stable and elevated operating pH. Figure 8 shows the survival of a *Spirulina* culture (1 µg·ml⁻¹ Chl a) together with its ability to sustain alkalinity production under a continuous loading regime with 10% AMD addition-day⁻¹ (pH 2 and metal concentration in mg·l⁻¹ Fe=95; Cu=1.08; Zn=2.2; Pb=1.52). Figure 9 shows the metal removal efficiency in this reactor for the different components of the partly treated AMD

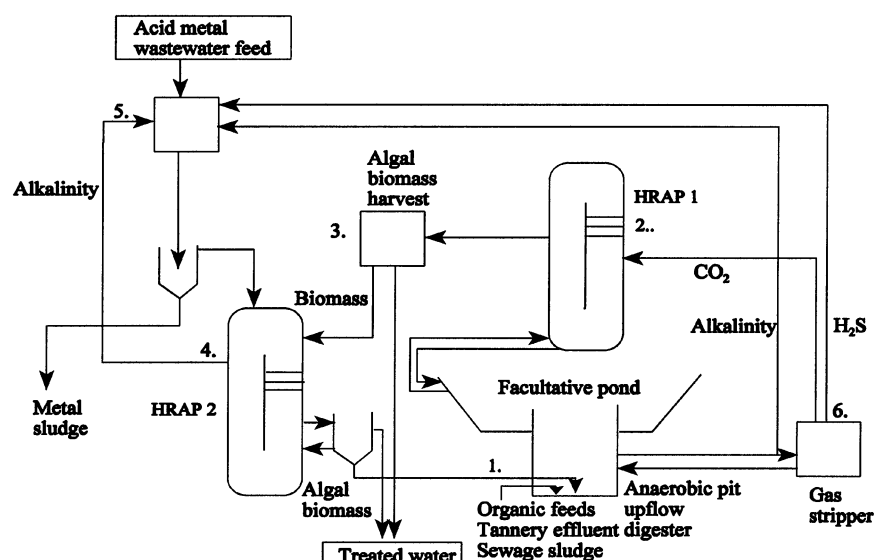


Figure 11. Flow diagram of the integrated unit operations of the sulphate reducing high rate algal ponding process ASPAM for the treatment of acid and metal pollution in mine drainage water. 1 = Facultative pond with anaerobic compartment; 2 = High Rate Algal Pond 1; 3 = Algal Biomass harvester; 4 = High Rate Algal Pond 2; 5 = Pretreatment mixer, 6 = gas stripping operation.

feed. The chlorophyll *a* level decline compared to control cultures, indicates stress conditions in this reactor and low growth rates which have, in turn, been shown to lead to increased production of extracellular metal complexing compounds by this organism (reported in the following section). Both sustained alkalinity production as an AMD neutralising step, together with an additional metal sequestering function, would play an important role in assisting a pre-treatment precipitation operation (HRAP 2, Figure 1) where the bulk of the incoming metal load may be recovered prior to entering the anaerobic compartment of the facultative ponds. Poor *Spirulina* production in this unit indicates the requirement for continuous biomass production under optimum conditions in a separate reactor – HRAP 1. This may also serve as the final polishing step for water leaving the system.

Metal complexing compounds

It is likely that the production of extracellular metal complexing compounds in the form of capsular polysaccharides, peptidoglycan and other fragments released from living algal cells plays some role as metal bioadsorbents in the metal removal functions noted above. Metal removal contributed by this fraction was estimated by measuring the metal binding capacity of the cell-free fraction of a *Spirulina* culture. The extracellular fraction was measured as total

organic carbon (TOC). Figure 10 reports percentage metal removal, at three pH values in the acid to neutral range, in the growth medium fraction from which the cells were removed by filtration through a GF/C glass fibre filter. The results show that the separable pre-digested extracellular fraction could account for between 20–40% of metal removal in the range evaluated and that this process operates best under acidic conditions. We have found production of this fraction to be linked to light stress with TOC yield increasing from $2.17 \text{ mg}\cdot\text{l}^{-1}$ to $44.39 \text{ mg}\cdot\text{l}^{-1}$ as light intensity was increased from 130 to $1000 \mu\text{moles}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$. Both high light and metal stresses may be manipulated in HRAP 2 to maximise production of the extracellular metal complexing fraction.

Optimisation of the extracellular metal complexing fraction is under further study in a pilot plant evaluation of continuous AMD loading regimes.

Integrated ASPAM system

Given the wide range of potential circumstances and applications required for the treatment of AMD or other heavy metal-containing wastewater streams, the ASPAM system would require operation in a number of possible formats. Figure 11 describes the integration of unit operations including the separate neutralisation and precipitation of metals in the incoming flow prior to its reaching the anaerobic compartment

of the facultative pond. This step provides for the contained recovery of the major fraction of metal precipitates, thus facilitating their disposal or downstream processing.

Where high levels of sulphide production are achieved it is anticipated that this component could be recovered by gas stripping and passed to the pre-treatment precipitation step 5 (Figure 11). Applications of sulphide gas stripping to SRB systems have been reported by Du Preez & Maree (1994). Carbon dioxide recovery in the stripping operation, and its recycle to HRAP 1, will be important in sustaining the high algal growth rates required. Preliminary work on this aspect of the process has been undertaken but is not reported here.

Recovery of sulphide and alkaline/metal complexing streams in the ASPAM process, which are then fed to a pre-treatment metal precipitation unit operation, offers the potential for the fine control of the selective precipitation of metal sulphide/carbonate/hydroxide complexes. This would enable the partial separation and refinement of incoming components suggesting the use of the process in the remediation of a range of metal waste streams in addition to AMD.

Conclusion

While the mechanisms underlying the role and use of SRB in AMD remediation have been the subject of considerable study, are quite well understood and have been applied with some success in wetland systems, further progress in process development of the active treatment approach are constrained by reactor size and cost, and the availability and cost of the carbon source, especially in the case of large water volume flows. The study reported here has attempted to demonstrate the utility of the waste stabilisation ponding process, providing an established reactor technology for the treatment of large water volumes, and the feasibility of linking co-disposal of organic waste streams with AMD treatment.

Tannery effluent provides a useful model for the evaluation of the co-disposal function. The application of anaerobic sulphide production in this medium, to the precipitation and removal of metal sulphides from mine and zinc refinery wastewaters, was demonstrated at both laboratory and pilot scale. Our development and operationalisation of a full-scale high rate ponding system receiving metal-contaminated tannery effluent enabled the demonstration of the anaerobic compart-

ment within a facultative pond as an effective sulphate reducing reactor for both COD reduction and the efficient removal of heavy metal contaminants. We also showed that the HRAP not only serves as a final metal polishing step but may contribute to primary treatment of AMD by both neutralisation and adsorption functions. The release of extracellular complexing compounds by microalgae and their role in metal binding was also shown. Algal biomass generated in the process provides a degree of independence from the supply of external carbon sources and its use in sulphide generation by SRB was demonstrated.

An integration of various components of the ponding approach to AMD treatment, which were investigated here, was proposed in the Algal Sulphate Reducing Ponding Process for Acidic and Metal Wastewater Treatment (ASPAM). The metal recovery and separation potential of the integrated system was noted. While the core unit operations of the proposed process, the facultative pond and the HRAP, have been demonstrated to full-scale, other components are currently under pilot-study evaluation together with the use of sewage as an alternative carbon source.

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References

- Andrews G (1989) An examination of the kinetics of coal pyrite decomposition. In: Scheiner G, Doyle FM & Katawa S (Eds) *Biotechnology in Minerals and Metals Processing*, (pp 87–94). Society of Mining Engineers, Littleton, USA
- APHA (1985) *Standard Methods for the examination of water and wastewater*. 16th edition, Washington.
- Barnes LJ, Janssen FJ, Scheeren PJH, Versteegh JH & Koch RO (1991) Simultaneous microbial removal of sulphate and heavy metals from waste water. First European Metals Conference, Bruxelles, Belgium, 15–20 Sept. 1991
- Barton LL (1995) *Sulphate Reducing Bacteria*. Plenum Press, New York.
- Barton LL & Tomei FA (1995) Characteristics and activities of sulphate-reducing bacteria. In: Barton LL (Ed) *Sulphate Reducing Bacteria* (pp 1–22). Plenum Press, New York.
- Bécharde G, Rajan S & Gould WD (1993) Characterization of a microbial process for the treatment of acidic drainage. In: Torma AE, Apel ML & Brierly CL (Eds) *Biohydrometallurgical Technologies* (pp 277–286). The Minerals, Metals & Materials Society

- Boshoff G, Duncan J & Rose PD (1996) An algal-bacterial integrated ponding system for the treatment of mine drainage waters. *J. Appl. Phycology* 8 (4–5): 442
- Brady D, Rose PD & Duncan JR (1994) The use of hollow fibre cross-flow microfiltration in bioaccumulation and continuous removal of heavy metals from solutions by *Saccharomyces cerevisiae*. *Biotech. Bioeng.* 44: 1362–1366
- Braun M & Stolp H (1985) Degradation of methanol by a sulphate-reducing bacterium. *Arch. Microbiol.* 58: 786–793
- Buisman CJN, Post R, Ijspeert P, Geraats G & Lettinga G (1989) Biotechnological process for sulphide removal with sulphur reclamation. *Acta Biotechnol.* 9: 255–267
- Butlin KR, Selwyn SC & Wakerley DS (1956) Sulphide production from sulphate-enriched sewage sludges. *J. Appl. Bacteriol.* 19: 3–15
- Burgess SG & Wood LB (1961) Pilot plant studies in production of sulphur from sulphate enriched sewage sludge. *J. Sci. Food Agric.* 12: 326–341
- Chian ESK & DeWalle FB (1983) Removal of heavy metals from a fatty acid wastewater with a complete mixed anaerobic filter. *Proceedings 38th Industrial Waste Conference*, Purdue University, West Lafayette, IN, pp 920–927
- Conradie PJA & Grutz PWE (1973) The treatment of acid mine waste in a mixture with sewage sludge in an anaerobic digester. Report to the Chamber of Mines (File No. w6/534/3). National Institute for Water Research, Pretoria.
- Crist RH, Oberholse K, Schwartz D, Marzoff J, Ryder D & Crist DR (1988) Interaction of metals and protons with algae. *ES & T Environmental Science and Technology* 22: 755–760
- De Pauw N & Salomoni C (1991) The use of microalgae in wastewater treatment: achievements and constraints. In: Madoni P (Ed) *Biological Approach to Sewage Treatment Processes: Current Status and Perspectives*, pp 329–352. Perugia, Italy.
- DeWalle FB, Chian ESK and Brush J (1979) Heavy metal removal with completely mixed anaerobic filter. *J. Water Poll. Control Fed.* 51: 22–36
- Du Preez LA & Maree JP (1994) Pilot-scale biological sulphate and nitrate removal utilizing producer gas as energy source. *Wat. Sci. Tech.* 30: 275–285
- Du Preez LA, Odendaal JP, Maree JP & Ponsonby M (1992) Biological removal of sulphate from industrial effluents using producer gas as an energy source. *Environ. Technol.* 13: 875–882
- Filip DS, Peters T, Adams VD & Middlebrooks EJ (1979) Residual heavy metal removal by an algae-intermittent sand filtration system *Wat. Res.* 13: 305–313
- Gadd GM & White C (1993) Microbial treatment of metal pollution – a working biotechnology? *TIBTECH* 11: 353–359
- Gale NL & Wixson BG (1979) Control of heavy metals in lead industry effluents by algae and other aquatic vegetation. In: *Conf. Management and Control of Heavy Metals in the Environment*, London, 1979 (pp 580–583)
- Greene B & Bedell GW (1990) Algal Gelsor immobilized algae for metal recovery. In: Akatsuka I (Ed) *Introduction to Applied Phycology*, (pp 109–136). Academic Publishing, The Hague.
- Grobicki A & Stuckey DC (1992) Hydrodynamic characteristics of the anaerobic baffled reactor. *Wat. Res.* 23(3): 371–378
- Herrera LJ Hernández P, Ruiz & Gantenbein S (1991) *Desulfovibrio desulfuricans* growth kinetics. *Environ. Toxicol. Water Qual.* 6: 225–238
- Jennett JC, Hassett JM & Smith JE (1979) Control of heavy metals in the environment using algae. *Heavy metals in the environment. Intl. Conf. London* (pp 210–217)
- Johnson DB (1995) Acidophilic microbial communities: candidates for bioremediation of acidic mine effluents. *Int. Biodet. & Biodeg.* 1995: 41–58
- Kuenen JG & Robertsen LA (1992) The use of natural bacterial populations for the treatment of sulphur containing wastewater. *Biodegradation* 3: 239–254
- Lawty R, Ashworthy DB & Mara DD (1995) Waste stabilisation pond decommissioning: a painful but necessary decision. *Wat. Sci. Tech.* 31: 1–8.
- Lichtenhaler HK (1987) Chlorophylls and Carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* 148: 350–371
- Mann H & Fyfe WS (1988) Biogeochemical cycling of the elements in some freshwater algae from gold and uranium mine districts. *Biorecovery* 1: 3–26
- Mara DD, Pearson HW & Silva SA (1996) Waste stabilisation ponds: technology and applications. *Wat. Sci. Tech.* 33: 1–262
- Mara DD & Do Monte M (1987) Waste Stabilization Ponds. *Wat. Sci. Tech.* 19: 1–401
- Maree JP, Gerber A & Hill E (1987) An integrated process for biological treatment of sulphate-containing industrial effluents. *Journal WPCF* 59: 1069–1074
- Maree JP & Hill (1989) Biological removal of sulphate from industrial effluents and concomitant production of sulphur. *Wat. Sci. Tech.* 21: 265–276
- Odom JM & Singleton R (1993) *The Sulphate Reducing Bacteria: Contemporary Perspectives* (p 289). Springer-Verlag, New York
- Oleszkiewicz JA & Hilton BL (1986) Anaerobic treatment of high sulphate wastes. *Canadian Journal of Civil Engineering* 13: 423–428
- Oswald WJ (1988) Micro-algae and waste-water treatment. In: Borowitzka MA & Borowitzka LJ (Eds) *Micro-algal Biotechnology*, (pp 305–328). Cambridge University Press, Cambridge, UK
- Oswald WJ (1991) Waste treatment by Pond Systems, Engineering aspects. *Proc. IAWPRC Conference on Appropriate Waste Management Technologies*, Perth, Australia
- Oswald WJ (1995) Ponds in the Twenty-first Century. *Wat. Sci. Tech.* 31: 108
- Pescod MB (1996) The role and limitations of anaerobic pond systems. *Wat. Sci. Tech.* 33: 11–22
- Peters RW, Ku Y & Bhattacharyya D (1985) Evaluation of recent treatment techniques for removal of heavy metals from industrial wastewaters. *AIChE Symposium Series* 81: 165–203
- Pipes WO (1960) Sludge digestion by sulphate reducing bacteria. *Proc. 15th Ind. Waste Conf.*, Purdue University, Lafayette, Indiana
- Postgate JR (1984) *The sulphate-reducing bacteria*. Cambridge University Press, Cambridge
- Pronk JT & Johnson DB (1992) Oxidation and reduction of iron by acidophilic bacteria. *Geomicrobiology Journal* 10: 153–171
- Riviera AL (1983) Heavy metal removal in a packed-bed, anaerobic upflow (ANFLOW) bioreactor. *J. Water Poll. Control Fed.* 61: 1576–1587
- Robb GA (1994) Environmental consequences of coal mine closure. *The Geographical Journal* 106: 33–40
- Robinson JDF & Robb GA (1995) Methods for the control and treatment of acid mine drainage. *Coal International* July 1995: 152–156
- Rose PD, Maart BA, Dunn KM, Rowsell RA and Britz P (1996) High rate algal oxidation ponding for the treatment of tannery effluents. *Wat. Sci. Tech.* 33: 219–227
- Scheeren PJH, Koch RO & Buisman CJN (1993) Geohydrological containment system and microbial water treatment plan

- for metal-contaminated groundwater at Budelco. International Symposium – World Zinc, '93 Proceedings (pp 373–384)
- Silver M (1989) Control of acid mine drainage including coal pile and ash pond seepage. In: Hammer DA (Ed) *Constructed Wetlands for Wastewater Treatment*, (pp 753–760). Lewis Publishers, Inc., Chelsea, Michigan
- Szewzyk U & Pfennig N (1987) Complete oxidation of catechol by the strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. *Arch. Microbiol.* 147: 163–168
- Ueki K, Kotaka K, Itoh K & Ueki A (1988) Potential availability of anaerobic treatment with digester slurry of animal waste for the reclamation of acid mine water containing sulphate and heavy metals. *J. Ferment. Technol.* 66: 43–50
- Umita T, Nenov V, Omura T, Aizawa J & Onuma M (1988) Biological ferrous-iron oxidation with fluidized bed reactor. *Water Pollution Control in Asia*. pp 479–485
- van Hille R & Duncan J (1996) Bioremediation of heavy metal polluted acidic mine effluent by *Spirulina* sp. *J. Appl. Phycology* 8(4–5): 461
- van Hille RP, Boshoff GA, Rose PD & Duncan JR (1997) A continuous process for the biological treatment of heavy metal contaminated acid mine water. RSA Provisional Patent no. 97/7616
- Van Houten RT, Hulshoff Pol LW & Lettinga G (1994) Biological sulphate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. *Biotechnol. Bioeng.* 44: 586–594
- Van Zyl HC (1996) Environmental systems in Amcoal. *Mining Environment management*, March issue (pp 18–21)
- Widdel F & Hansen TA (1992) The dissimilatory sulphate- and sulphur-reducing bacteria. In: Ballows A, Trüper HG, Dworkin M, Harder W & Schleifer K-H (Eds) *The Prokaryotes*, Vol I (pp 583–624). Springer-Verlag, Berlin
- Wieder, RK (1993) Ion input/output budgets for five wetlands constructed for acid coal mine drainage treatment. *Water, Air, and Soil Pollution* 71: 231–270
- Wilde EW & Benemann JR (1993) Bioremoval of heavy metals by the use of microalgae. *Biotech. Adv.* 11: 781–812
- Wildeman T, Brodie GA & Gusek JF (1991) Draft Hand Book for Constructed Wetlands Receiving Acid Mine Drainage. US Environmental Protection Agency, Ohio 45268
- Younger PL, Curtis TP, Jarvis A & Pennell R (1997) Effective passive treatment of aluminium-rich acidic colliery spoil drainage using a compost wetland at Quaking Houses, County Durham. *J. Chartered Inst. Water and Environment Mgt.* 11: 200–208
- Zarouk C (1966) Contribution a l'Etude d'une Cyanophyce. Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthese de *Spirulina maxima*, Thesis, University of Paris, France