Use of Chemical and Biological Agents to Improve Water Quality of Effluent Discharge from Abattoirs*

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ABSTRACT : Intensive animal industries create large volumes of nutrient rich effluent which, if untreated, has the potential for substantial environmental degradationand to recover valuable nutrients that would otherwise be lost. Members of the family *Lemnaceae* are widely used in lagoon systems, to achieve inexpensive and efficient remediation of effluent. Only limited research has been conducted into their growth in highly eutrophic media and there has been little done to systematically distinguish between different types of media. This study examined the growth characteristics of duckweed in abattoir effluent and explored possible ways of ameliorating the inhibitory factors to growth on this medium. A series of pot trials was conducted to test the tolerance of duckweed to abattoir effluent partially remediated by a sojourn in anaerobic fermentation ponds, both in its unmodified form and after the addition of acid to manipulate pH, and the addition of bentonite. Unmodified abattoir effluent was highly toxic to duckweed, even at dilutions of 3:1. Duckweed remained viable and grew sub-optimally in simplified media with total ammonia nitrogen (TAN) concentrations of up to 100 mg/L. Duckweed grew vigorously in effluent diluted 1:4 v/v, containing 56 mg TAN/L when modified by addition of acid (to decrease pH to 7) and bentonite at 0.5%. The results of this study suggest that bentonite plays an important role in modifying the toxicity of abattoir effluent to duckweed. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 1 : 137-145)***

Key Words : Ammonium, Nitrogen, Meatworks, Abattoir Effluent, *Lemnaceae*, Bentonite, Duckweed, Phosphorus

INTRODUCTION

Intensive livestock producers and animal product processors, use large amounts of good quality, often potable water and produce equally large volumes of nutrient-rich effluent, unsuitable for release into either the environment, or municipal sewage without significant remediation. Technologies to achieve significant improvement in effluent quality, even to the point of making it suitable for re-use, already exist (Roux and Pretorius, 1997) but these are expensive to construct and to operate.

The most common form of treatment for abattoir effluent is discharge into anaerobic fermentation ponds, a process that, while significantly reducing nutrient content, also fundamentally alters the chemical makeup. An important distinction between abattoir effluent from anaerobic fermentation ponds and other classes of effluent, is that almost all the nitrogen present is derived from animal tissues and blood. Because this is broken down in the absence of oxygen, it is likely to be present in an unoxidised form, as ammonia. Even after treatment in anaerobic fermentation ponds, abattoir effluent contains nitrogen concentrations of 100 to 250 mg/L and dissolved phosphorus concentrations of 20 to 50 mg/L (Johns and Greenfield, 1992).

A proven method for producing high quality water from effluent is the use of natural or man-made, shallow lagoon systems that utilize aquatic plants to "capture" nutrients. Such systems have been employed for cleansing treated and untreated domestic sewage in the United States (Zirschky and Reed, 1988), the Middle East (Oron et al., 1985; Al-Nozaily, 2001) and the Indian subcontinent (Skillicorn *et al.*, 1993; van der Steen et al., 1998). An added advantage of this technology is that the plants can be harvested to provide a valuable source of animal food or compost. The growth and composition of duckweed (*Lemnaceae* spp.) grown on a wide range of eutrophic media, and the plant's potential as a quality animal feed have recently been reviewed (Goopy and Murray, 2003).

Investigations into adapting shallow lagoon systems to deal with the much higher levels of dissolved nutrients including nitrogen and phosphorus and other pollutants present in animal production systems, have met with mixed success (Oron et al., 1985; Whitehead et al., 1987, Al-Nozaily, 2001). Despite a large number of experiments on a limited number of species (mainly *Spirodella polyrhiza, S. punctata, Lemna gibba* and *L. minor*), the ability of these plants to grow in media with very high nutrient levels remains unclear. The variability in response of these plants is likely to be due to a combination of factors including large variations in the characteristics of growth media, substantial differences in elements of experimental methods

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Table 1. Characteristics of effluent released from abattoir anaerobic fermentation pond

COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	Total N (mg/L)	Total P (mg/L)	pH	Na (mg/L)	Cl (mg/L)
334	57	105	184	34.2	7.86	221	408

COD; Chemical oxygen demand. BOD_5; Biological oxygen demand (over 5 days). TSS; Total suspended solids.

between investigators, and variability within and between species. The variable performances observed may also arise because different molecular forms of the same element and differing proportions of these forms are present in the growth media used across these studies.

Information regarding the ability of duckweed to grow in the presence of high levels of nitrogen ([N] >20 mg/L) is conflicting (Oron et al., 1986; Whitehead et al., 1987; Leng 1999; Bergman et al., 2000; Al-Nozaily, 2001). The conflict in results may be because the form in which nitrogen is present is more important to plant metabolism than the overall concentration of the element. Interpretation of the results of some studies is hampered because the authors have not specifically reported all forms of nitrogen present.

Ammonia is recognized as being highly toxic to all higher forms of life, but when absorbed at a slow rate it is well tolerated by most organisms (Warren, 1962). Decreasing the proportion of free ammonia in solution may improve the survival of plants grown on eutrophic media. The relative concentrations of ammonia and ammonium ions in solution is governed by the dissociation constant (pK) of the molecule, and is determined by temperature, +type of solution, and above all, pH. The amount of total ammonia nitrogen (TAN) that is un-ionised in solution at pH 6 is about 0.05% and it increases in approximately one order of magnitude for every unit increase in pH (Warren, 1962).

If the toxic effects of TAN were only dependent on the proportion of the un-ionised form, it should be a relatively simple matter to control the toxic effects by adjusting pH. Available evidence, though not conclusive, suggests that this may not be the case. Caicedo et al. (2000) found a parabola-like effect of pH, with a maximum growth response at pH 7, on duckweed growth, indicating not only the anticipated growth inhibition at high pH/ammonia concentrations, but also poor growth where little (<0.05%) free ammonia was present. Increasing TAN concentrations in solution exacerbated the effect. Results varied across the four different media included in this study, and the regression analysis indicated that approximately half of the variation in plant growth was explained by ammonia concentration. There are therefore, other factors exerting a significant effect on plant growth in high nutrient media. Recently Britto et al. (2001) have determined that active transport of NH_4^+ out of the cell cytosol occurs in response to increased influx of ammonium ions at high ambient concentrations in susceptible barley (*Hordeum vulgare*) plants, leading to futile cycling of NH_4^- and ultimately metabolic exhaustion of the plant. This suggests a plausible mechanism for the poor growth of duckweed in the presence of high concentrations of ammonium ions (pH<6).

A promising candidate for reducing levels of ammonia in solution is bentonite. Montmorillonite type clays are hygroscopic and are recognized for their ability to absorb many times their own mass of water, at the same time adsorbing ammonia and other cations (Ashworth, 1978; Budavari, 1996; Ma and Uren, 1998). It appears that positively charged species are held by negatively charged exchange surfaces of the clay (Pratley, 1992). The suggested mechanism is by direct co-ordination of the NH_3 molecule to the surface and/or formation of the ammonium ion through reaction with water (Ashworth, 1978). Bentonite has been used successfully to reduce ammonia concentrations in aquaria (Booth, 1999) and in pig excrement (Venglovsky et al., 1998). These properties that bentonite exhibits may be valuable as an adjunct to the use of duckweed, acting to reduce ammonia and provide a high degree of buffering or a method for recovering dissolved nutrient from abattoir effluent.

At present, there are no reported attempts to adapt duckweed growing on lagoon systems, to deal with, and improve the quality of, water from red meat abattoirs. This paper examines the use of abattoir effluent as a growth medium for duckweed, with the aim of improving water quality of effluent by the use of duckweed to harvest the dissolved nutrient, especially nitrogen and phosphorus.

MATERIALS AND METHODS

Location and materials

All experiments were carried out at The University of Queensland's Gatton Campus, in South-East Queensland (27°55'S, 152°33'E) from July to October. The climate is subtropical with a summer dominant rainfall of 815.9 mm (Bureau of Meteorology, 2001). Effluent for this research was sourced from the final anaerobic treatment pond of a local abattoir, processing 400 to 600 animals (mostly cattle) per day. Effluent characteristics are regularly assessed by a National Association of Testing Authority (NATA) accredited laboratory and average values for the effluent discharge measured are shown in Table 1.

For the first duckweed experiment (Experiment 3), two samples of duckweed from two sites were used. Plant samples were identified by botanists at the Queensland Herbarium. Samples of a mixed colony comprising *S. polyrhiza* and *Wolffia angusta* (Isolate 1) were gathered from a municipal sewage treatment plant on Queensland's Sunshine Coast. Samples of *L. aequinoctialis* (Isolate 2)

were taken from a municipal sewage treatment plant at Redcliffe, north of Brisbane. For the subsequent duckweed experiments, only Isolate 1 was used.

Experiment 1 : Effect of bentonite on the concentration of ammonia. A completely randomized design with six treatments: (0, 0.5, 1, 2, 4 and 8% bentonite), each with two replicates was utilized. Appropriate quantities of bentonite (Ebenite, Ipswich, Australia) for 2 litres of effluent were placed in rectangular plastic pots (27×13.5 cm). The pots were then placed on the concrete floor in a room where fresh undiluted effluent was added to each pot and the contents stirred to ensure thorough mixing. Samples were taken initially of the undiluted effluent, then of the undisturbed supernatant at days 1, 2, 3, 4, 7, 10 and 14 and samples processed immediately or frozen until analysis.

Experiment 2 : Effect of increasing levels of bentonite in treated abattoir effluent on the concentrations of Kjeldahl nitrogen, phosphorus, bacterial numbers, pH and turbidity. A completely randomized design featuring six treatments (0, 2, 4, 8, 12 and 16% w/v bentonite), each with two replicates, was utilized. Appropriate quantities of bentonite (Ebenite, Ipswich, Australia) for 2 L of effluent were placed in rectangular plastic pots (27×13.5 cm). The pots were then placed on the concrete floor in a room where fresh effluent was added to each pot and the contents stirred to ensure thorough mixing. Samples were taken initially of the undiluted effluent, then the undisturbed supernatant at days 1, 2, 3, 5, 7 and 9. Phosphorus, nitrogen, and ammonia concentrations were measured, along with pH, bacterial counts, and turbidity.

Experiment 3 : The growth of duckweed in different concentrations of treated abattoir effluent. A randomized block design with three blocks, five effluent dilutions (0, 25, 50, 75 and 100 percent in tap water), and two isolates was employed. Each pot was constructed from a plastic 200 L drum split longitudinally, with a surface area of 0.45 m^2, and was filled to 60 L with the effluent mixtures. The duckweed seeding rate was calculated at 500 g/m^2, thus each pot received 225 g (fresh weight). After introduction of the duckweed the pH of each pot was measured and a water sample taken for analysis. The pots were topped up weekly with tap water to maintain volume (approximately 10 L/week). Aside from empirical observations of the health status of the plants, the pots were left undisturbed until the end of the experiment. At day 28 the duckweed was harvested and weighed on site and pH of effluent in the pots was recorded.

Experiment 4 : Growth of duckweed in increasing concentrations of ammonia. A completely randomized design with three treatments (0, 50 and 100 mg/L NH_4^+), each at three levels of pH (5, 6 and 7), each with two replicates was used. Appropriate quantities of NH_4Cl for two litres were placed in rectangular plastic pots (27×13.5 cm). The pots were then taken to the greenhouse and placed on benches, where tap water was added to each pot and the contents stirred to ensure thorough mixing. Tap water was deemed to be approximately equal to pH 7 and to have 0 mg NH_4^+. Sulphuric acid (0.5 M) was added to the relevant pots, to lower the pH to approximately 5 and 6. Each pot was then seeded with 19 g fresh duckweed from Isolate 1 (approx. 500 g/m^2). After introduction of the duckweed the pH of each pot was measured and recorded and a water sample taken. Water samples were taken initially at one hour, then at days 1, 2, 4, 6, 9, 12 and 16. Phosphorus, Kjeldahl nitrogen and ammonia concentrations were measured, along with pH. A pot with only tap water in it was used to measure the evaporation rate, calculated by difference. Every fourth day the experimental units were recharged with tap water. At the conclusion of the experiment, all duckweed was harvested and processed.

Experiment 5 : Growth of duckweed in diluted abattoir effluent at neutral pH with or without bentonite added. A completely randomised design featuring a 25% v/v solution of abattoir effluent, with or without the addition of bentonite, each with two replicates, was utilized. Each pot, (surface area of 0.45 m^2) was filled to 48 L with the effluent mix allocated from the design. Where appropriate, bentonite was added to the pots by gently sprinkling over the surface at a rate equivalent to 0.5% w/v (240 g). Each pot was seeded with 225 g fresh duckweed from Isolate 1.

After introduction of the duckweed, the pH of each pot was measured, and pH was lowered to pH 7 by the addition of 0.5 M sulphuric acid. The amount of acid required was the same across treatments. Fluid samples were taken after one hour, then after 3, 6, 8, 11 and 14 days. At each sampling time the pots were recharged with tap water to 48 L, pH was then measured, and adjusted to neutral by the addition of 0.5 M sulphuric acid. TAN concentrations were measured from collected samples. At the conclusion of the experiment, all duckweed was harvested and analysed for dry matter, phosphorus and Kjeldahl nitrogen.

Chemical analyses

Water samples to be analysed for total ammonia nitrogen were filtered through a 0.45 μm, syringe-driven filter unit (Millex, Millipore Corp., Bedford USA) and decanted into 10 mL sample tubes (Biolab, Australia) sealed and frozen at -20°C. Prior to analysis, samples were thawed at room temperature (approx. 20°C) then diluted with distilled water and processed according to the method of Wruck (2001).

Chloride concentration was determined using the silver nitrate based method (Mohr 1856, cited in: Storer, 1992). Sodium concentration was determined by the method of Hanson (1973). The Kjeldhal nitrogen content of water samples and plant was determined colormetrically using a

Table 2. Experiment 2: the effect of the addition of bentonite on average effluent clarity (absorbance), pH, bacterial count and $\log_{10}$ CFU

Treatment (% bentonite)	Absorbance	Log $_{10}$ CFU	pH
Control (0%)	-1.48^{a}	5.36^{ab}	7.98^{a}
2%	-1.89^{b}	5.23^{a}	8.02^{a}
4%	-1.98^{b}	5.17^{a}	8.09^{ab}
8%	-2.07^{b}	5.38^{ab}	8.17^{b}
12%	-1.89^{b}	5.60^{bc}	8.30^{c}
16%	-2.06^{b}	5.82^{c}	8.37^{c}
LSD (p =0.05)	0.202	0.316	0.0114

NB: Values in columns with different superscripts are statistically different.

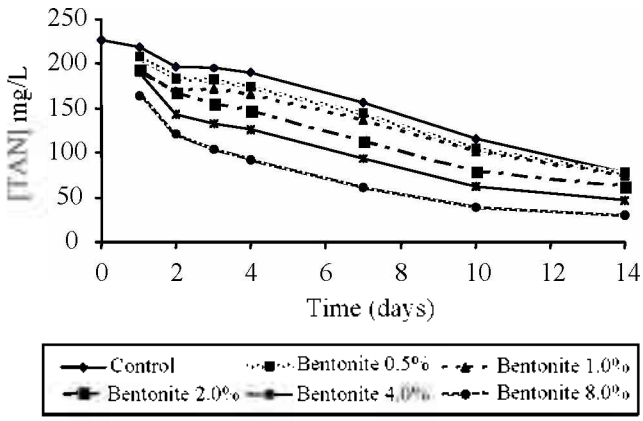

Figure 1. Change in total ammonia nitrogen (TAN) concentration over time in abattoir effluent with different quantities of bentonite.

modification of the Bertholt reaction (Storer, 1992). Phosphorus content was determined colormetrically at 420nm, after the method of O'Neill and Webb (1970). Absorbance was taken as a measure of relative turbidity, and was determined by spectrophotometry (Ultraspec) set at 600 nm.

Because of the high moisture content of duckweed, fresh samples of 15 to 20 g were dried at 100°C to determine DM. Samples used to determine the DM content were subsequently ashed at 550°C. Organic Matter (OM) content was calculated by difference.

Bacterial count : 0.5 mL samples of effluent were serially diluted in ten fold increments to a final dilution of 10^{-5} of the original sample. A standard plate count agar (Brindson, 1995) was used as a total count medium. All samples were cultured in duplicate for 48 h incubation at 37°C and bacterial colonies on each plate (CFU) were enumerated using a stereo dissection microscope at 15X power.

Statistical analysis

All statistical analyses were carried out using the Minitab statistical package (release 13). Data were initially analysed by ANOVA using a general linear model. A logarithmic transformation of data was carried out for regression analysis in experiment one and for bacterial counts in experiment two. Significant differences revealed through the general linear model were further examined using Fisher's Least Significant Difference. Multiple regression analysis was used to model the disappearance of ammonia over time in experiment 1.

RESULTS

Experiment 1

Over 65% of the TAN initially present disappeared from the control during the length of the 14 day experiment. The addition of bentonite to the abattoir effluent, rapidly decreased the amount of TAN in solution (Figure 1) and TAN disappeared at approximately four times the rate of the control group at the 8% bentonite treatment level for the first two days. Total removal from the fluid (86.7%) and reduction of TAN in the first 24 h (27.8%) were higher ($p<0.05$) for the highest bentonite treatment (8%) than for the control. Overall, the change of TAN concentration was proportional to the quantity of bentonite added, and time in contact (Figure 1).

Models were developed using multiple regression analysis to describe the relationship between TAN concentration, bentonite level and time. The model described by the equation below best fits the relationship between the three variables and explains 95.3% of the variation.

$$\text{Log}_e(\text{Concentration of TAN}) = 5.33\ (0.019) - 5.92\times10^{-2}\ (3.0\times10^{-3}) \times \text{Time (days)} - 1.25\times10^{-2}\ (1.246\times10^{-2}) \times \text{Bentonite (\%)} \times \text{Time (interaction)}$$

(Numbers in brackets = S.E.)

Experiment 2

The highly hygroscopic nature of bentonite was evident in the 12 and 16% treatments, where it swelled to take 80 to 90% of the volume of the effluent, making sampling difficult. Our observations suggested that the addition of bentonite had a positive effect on water clarity, and turbidity was significantly reduced by addition of bentonite ($p<0.01$) and over time ($p<0.001$). The treatment with no added bentonite had a higher absorbance than the treatments with bentonite added which did not differ in absorbance ($p<0.01$).

Table 3. Experiment 3: chemical composition of two isolates of duckweed on a dry matter basis (% mean values±SE) measured at the start and end of the experiment and changes in final values expressed as a percentage of initial values

	DM	Ash	Crude protein	Phosphorus
Isolate 1 (initial)	3.9 (0.18)	17.0 (0.47)	30.2 (0.44)	1.7 (0.001)
Isolate 2 (initial	4.3 (0.08)	20.7 (1.86)	28.9 (0.22)	1.7 (0.07)
Isolate 1 (final)	7.7 (0.28)	10.3 (0.28)	11.2 (0.3)	0.6 (0.02)
Isolate 2 (final)	8.1 (2.07)	12.1 (0.93)	11.8 (0.69)	0.6 (0.08)
Isolate 1 (%)	293.6	179.0	108.5	93.9
Isolate 2 (%)	171.8	100.8	70.0	54.3

Table 4. Experiment 4: mean yields of duckweed (g fresh weight) after 16 d growth on media different initial concentrations of ammonia and different initial pH

TAN [mg/L]	pH		
	5	6	7
0	42.2^{fe}	39.9^{e}	43.5^{f}
50	35.3^{cd}	33.8^{bc}	35.4^{cd}
100	26.3^{a}	31.7^{b}	36.2^{d}
	$LSD_{(p=0.05)} = 2.42.$		

Measures with the same superscripts are not significantly different from each other.

(Table 2)

Increasing levels of bentonite increased the number of Colony Forming Units (cfu), although the increase was not linear or regular (Table 2). The effluent became more alkaline at higher levels ($p<0.001$) of bentonite addition and pH also increased over time ($p<0.001$), but the change was not of great magnitude (7.7 to 8.5) (Table 2). Increased amounts of bentonite in effluent did not significantly affect ($p>0.05$) the concentration of phosphorus in the effluent. There was an effect of time ($p<0.001$), with an increase in phosphorus concentrations at all levels, but this change was attributable to the last day only.

Experiment 3

Initial values for pH varied little across treatments, (7.58 to 7.70). Most of the N in effluent samples was present as ammonia, and the phosphorus content was high (~35 mg/L). All measured values were within the expected range based on prior analyses of the effluent, and the composition of the two isolates was similar to each other (Table 3), and within reported range for duckweed (Leng, 1999).

At introduction and for the next four days, the plants in all pots appeared healthy. Thereafter, all treatments except the control (0% effluent), became etiolated, and it was estimated that over 80% of individual plants lysed. By the end of the experiment all of the plants in the undiluted effluent and the 75 and 50% concentrations had died. In the 25% concentration treatment, all plants of Isolate 2 were dead, but 46% of the original mass of Isolate 1 was harvested. Only Isolate 1, grown on tap water, showed any net increase on a fresh weight basis (110.7 g).

Therefore, measurements at the conclusion of the experiment were limited to the 25% effluent concentration (Isolate 1 only) and the controls (Table 3). In the control pH was almost 1 unit higher, sodium, chloride and phosphorus concentrations increased, and nitrogen was detectable at levels of less than 1 mg/L. In the 25% effluent treatment group, pH increased by over 2 units above the initial value, sodium and chloride increased by 20 and 12% respectively, nitrogen almost disappeared from the solution and phosphorus decreased by over 50%.

Owing to the large amount of floating dead material, it was considered impossible to get an accurate assay of the composition of the material growing in the 25% solution, so only the material growing in tap water was collected for analysis. The composition of the two isolates at the conclusion of the experiment were similar to one another, but different from their initial compositions (Table 3), DM content doubled (from 4 to 8%); and ash content almost halved, while both CP and phosphorus content fell by two-thirds (30 to 11% and 1.7 to 0.5% respectively). The increase in DM% produced a very different profile of plant growth when characteristics were compared on a DM basis (Table 3) with a marked increase in dry matter production for both isolates, with Isolate one higher than Isolate 2.

Experiment 4

Duckweed grew in the presence of TAN up to 100 mg/L and pH in the range 3.3-7.6. Biomass increased for all treatments over the duration of the experiment. The greatest increase ($p<0.05$) in fresh biomass of duckweed was for the control group (tap water). The increase in fresh biomass differed between treatments, and there was significant interaction between TAN and pH (all $p<0.001$) (Table 4). DM concentration of duckweed did not differ ($p>0.05$) between treatments, and when the plant material was assessed on a dry matter basis, most interaction effects disappeared. Thus, further analyses performed to assess the effects of ammonia on duckweed, have been described separately.

DM production decreased ($p<0.001$) with increasing concentrations of TAN, but both CP concentration and CP production of duckweed increased significantly, both when compared to the control and between treatments, but production of CP was highest for the 50 mg/L treatment ($p< 0.05$; Table 5). CP content of duckweed for both treatment groups (24.0 and 25.4 %) was similar to that of the donor

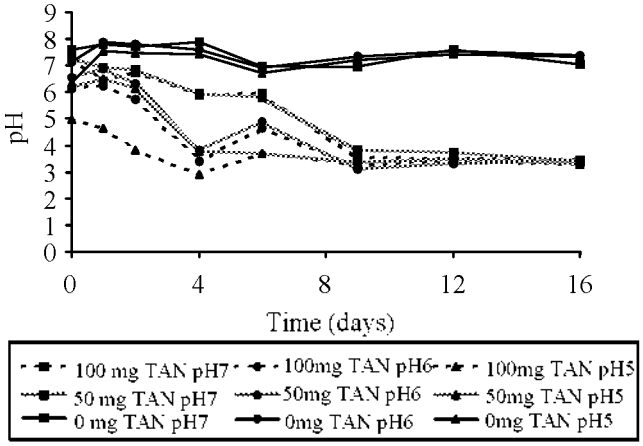

Figure 2. Change in pH over time in pots growing duckweed, as influenced by initial pH and TAN concentration.

material (26.1%), but the difference between the two treatments was significant ($p<0.05$). Phosphorus concentration in the plant material also rose with increased TAN levels ($p<0.05$), but this did not translate into a significant increase in phosphorus being taken up from medium, due to the lower DM production (Table 5).

In contrast to the first experiment, pH levels did not all rise. All experimental units were close to their target pH (5, 6 or 7) at the commencement of the experiment, however the groups with no ammonium chloride added (tap water) rose quickly to pH 7-7.6, irrespective of the degree of initial acidification and remained within pH range of 7.2-7.9 for the duration of the experiment (Figure 2). In contrast, the pH of all treatments with added ammonium chloride fell, so that by the end of the experiment all were in the range of 3.3-3.6, with the low pH treatments reaching these levels earlier.

Experiment 5

The pH of the solution in all units continued to rise after the initial correction, until Day 11 (Table 6). Initially, duckweed in both treatments appeared healthy, although they did not proliferate rapidly. The pH of solution in both treatments was also similar ($p>0.05$) and required equal quantities of acid to neutralize the effluent. However, by day 8, the plants in the acid-only treatment looked unwell, and algae appeared to be beginning to grow. From that point, the plants in the acid only treatment deteriorated, until by Day 14 all had died.

In contrast, duckweed in the treatment with bentonite added appea red to grow vigorously and by day 14, covered the surface of the water, with individual plants 4 to 6 deep. Because of the death of the plant material in the acid-only treatment and the subsequent contamination of the medium, it was considered inappropriate to include data from that treatment group and further analysis was confined to differences between initial and final values of plant and effluent for the treatment in which bentonite was added. Fresh weight of duckweed and DM, CP and phosphorus production, all increased significantly ($p<0.01$) in the diluted effluent with bentonite added (Table 7). Of all changes the greatest difference was in CP production, which increased three-fold ($p<0.003$) a combination both of increasing DM and CP content.

Initial values for nitrogen and phosphorus in the diluted effluent were similar to those of experiment 3 and similar to values expected from the effluent (52 mg N/L; 9.9 mg P/L). By Day 14, these values had dropped to 4.6 and 6.8 mg/L respectively.

DISCUSSION

The results of experiment 3 demonstrated clearly that unmodified abattoir effluent was very toxic to the species of duckweed examined in these experiments. The composition of abattoir effluent is notably dissimilar to other types of highly eutrophic effluent that has been used to grow duckweed (Oron et al., 1985; Whitehead et al., 1987; Vermaat and Haniff, 1998). The most immediately obvious difference between abattoir effluent discharged from anaerobic fermentation ponds and media described in the

Table 5. Experiment 4: effects of increasing concentrations of total ammonia nitrogen (TAN) on selected duckweed production characteristics

TAN, mg/L	Percentage increase in fresh wt.	DM production (g)	% DM	P (mg/g DM)	% Crude protein
0	109.6	2.82^{c}	6.74^{a}	7.23^{c}	8.97^{c}
50	74.3	2.28^{b}	6.55^{a}	9.97^{b}	24.04^{b}
100	52.8	2.00^{a}	6.55^{a}	11.32^{a}	25.35^{a}
$LSD_{(p=0.05)}$	NM	0.202	0.587	0.79	0.508

Table 6. Experiment 5: change in pH and amount of acid required for neutralizing abattoir effluent diluted 1:3 with tap water and with or without added bentonite at 0.5% (w/v)

Day	0	3	6	8	11	14
Acid only treatment	7.80	7.80	7.08	7.39	7.17	**
Acid+bentonite treatment	7.8	7.8	6.98	7.34	6.81	5.92
Amount acid added	45 mL	30 mL	0	10 mL	0	0

** not measured.

Table 7. Experiment 5: change in selected characteristics of duckweed grown for 14 days on dilute abattoir effluent to which bentonite was added (SE given in brackets.)

	Fresh mass (g)	DM (g)	DM (%)	P (%)	P (g)	Crude protein, %	Crude protein, (g)
Initial	225 (00.0)	10.9 (0.14)	4.9 (0.06)	1.9 (0.13)	0.2 (0.02)	40.3 (0.31)	4.4 (0.02)
Final	541 (28.5)	24.3 (1.54)	4.5 (0.05)	1.8 (0.02)	0.4 (0.03)	55.8 (0.57)	13.6 (0.72)
Change ±	+315.9	+13.4	-0.4	-0.13	+0.2	+15.6	+9.2
p value	0.004	0.007	0.024	N.S.	0.013	0.001	0.003

literature was that ammonia was the predominant nitrogen source in the abattoir effluent, comprising over 95% of total N. This feature, combined with documented sensitivity of duckweed to ammonia (Wang, 1991; Caiciedo et al., 2000), suggested that ammonia toxicity was a likely reason for the death of these plants. On the other hand, the proposition that plant death in even the most dilute effluent was attributable to ammonia was not supported by the literature.

There is substantial evidence that duckweed will grow in the presence of high concentrations of ammonia nitrogen. When those experiments that have used predominantly non-ammonia nitrogen are excluded, there are studies that clearly describe duckweed growing in TAN concentrations of at least 100 mg/L (Oron et al., 1985; Whitehead et al., 1987; Bergmann et al., 2000a; Cheng et al., 2002). This was approximately twice the level of N that proved fatalto duckweed in the third experiment. Thus it was necessary to modify the approach, firstly to determine whether ammonia was actually responsible for the death of the plants, and secondly to develop an approach that might be used to modify the chemical qualities of effluent that made it toxic to duckweed.

Experiment 1 and 2 demonstrated that bentonite significantly reduced the concentration of TAN in solution and thus may be a useful agent for reducing very high TAN concentrations in effluent from anaerobic fermentation ponds. Additionally, these experiments demonstrated the spontaneous disappearance of TAN from solution. Most likely the loss is from volatilisation of ammonia and this provides another potential modality for nutrient stripping of effluent. The results of the experiments suggest that volatilisation makes a quantitatively larger contribution to TAN disappearance from solution than bentonite over longer periods.

In contrast to Experiment 3, duckweed grew in the presence of 100 mg TAN/L, but at a lower rate than the lower TAN concentrations in the simplified media of Experiment 4. This strongly suggests that TAN *per se* was not the cause of death of the duckweed. However, the unanticipated shift in pH caused the TAN to be present predominantly as ammonium ions in the treatment groups un-ionized ammonia, which can diffuse across cell membranes in an unregulated manner (Ford and Clarkson, 1999) may still have been a candidate for the toxic effect observed in experiment 3. Because of the possible deficiency of other nutrients in the growth media, it seemed unwise to attempt to draw conclusions about duckweed growth and productivity from this trial.

Experiment 5 demonstrated that, when considered with the previous experimental outcomes, TAN is not the sole, and is unlikely to be the principal, toxic agent, at least in diluted abattoir effluent. The reason for the positive effect on the growth of duckweed, of the addition of bentonite to effluent, is unclear. However, it seems plausible to suggest that it may be related to the decrease in suspended particulate matter in the effluent. As well as providing a promising way forward to allow the cultivation of duckweed on abattoir effluent, this suggests a method to elucidate the identity of such a toxic agent. Close comparison of untreated effluent, with that to which bentonite has been added, may provide the answer. This was beyond the scope of this study. The results of experiment 5 also indicated that duckweed will grow profusely on diluted abattoir effluent to which bentonite has been added. Duckweed will also efficiently accrete both nitrogen and phosphorus into its tissue mass when grown in this medium, although the limit of its tolerance to TAN in effluent was not determined.

CONCLUSION

The results of these experiments suggest that duckweed can be grown on abattoir effluent, and produce a plant material that is high in both CP and phosphorous, suggesting that it may provide a feedstuff with high nutritional value. There are however challenges that must be met before this can take place. Firstly, it seems that some constituent (other than ammonia) of unmodified effluent is highly toxic to duckweed. Bentonite appears to be able to ameliorate this effect, but further development will be hampered unless the toxic agent can be isolated, and removed or neutralized. The usefulness of bentonite as an agent for removing ammonia reduction from solution depends in part on whether it holds the molecules tightly, saturating the clay, and effectively removing the ammonia from the system, or whether it functions as a buffer, giving up its captured particles when concentrations fall, thus allowing harvesting of the ammonia and effectively renewing the clay's properties

A second important issue is that of crop management. Any system, mechanical or natural is constrained by its throughput capacity. The rate at which the plants take-up

nutrient appears positively influenced by the nutrient concentration in the media. but uptake is not infinite and once the plant has reached its potential for nutrient removal. the only way for more nutrient to exit the system is for more plants to grow. and for that there must be sufficient surface area. Harvesting rate will be a major determinant of the extent to which nutrients can be stripped from effluent.

The need to dilute effluent before the successful introduction of plants is not a drawback to implementation because low rate systems require time to operate and in effect new effluent will be diluted by partly remediated material already *in situ*.

Handling and food safety are both concerns that will also need to be addressed before this technology can be acknowledged as viable by industry but these concerns are worth addressing in view of the large quantities of nutrients, especially nitrogen and phosphorus are lost from abattoirs under current practices.

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