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Algae for Defluoridation

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Potential Uses of Algae in Defluoridation

Introduction: *What are the potential uses of algae in defluoridation, and how can their sustainability be maximised?*

The objective of this report is to assess the potential of algal biomass as an alternative to reverse osmosis for the defluoridation of drinking water in the Andhra Pradesh region of India. Non-viable algae biomass has the ability to adsorb fluoride ions (biosorption), whilst viable algae have the ability to accumulate fluoride ions (bioaccumulation).

Both the economic and environmental sustainability of using non-viable algal biomass for biosorption could be maximised by utilising the waste from an algal biofuel plant. Although no such plant currently exists in India, the Indian company Reliance Industries Ltd. has provided the Australian producer of algal biodiesel, Algae.Tec Ltd., with an investment to build a pilot plant, most probably in the state of Gujarat. In addition, the global company Parabel (formerly PetroAlgae) is working in partnership with India's largest energy company, Indian Oil Corporation, to develop algal strains suitable for growth in Indian conditions with a view to producing renewable fuels on a large scale. Therefore, it is reasonable to predict that a reliable supply of waste algal biomass will be readily available in India in the near future.

Likewise, there is the possibility of linking bioaccumulation with algal biodiesel production. In the production of algal biodiesel, an initial period of nutrient-rich growth is often followed by exposure of the algae to stress conditions in order to maximise their lipid content. If the accumulation of fluoride ions could be incorporated into the stress phase of growth, then defluoridation could work complementarily in conjunction with biodiesel production.

In addition, there is the potential to grow algae in the flue pipes of power stations as these are such a rich source of carbon dioxide. In using this carbon dioxide for photosynthesis to fuel their rapid growth, the algae would effectively be performing carbon sequestration, which is one of the key climate change mitigation strategies. This is an active area of research, which promises to advance rapidly as energy companies become increasingly interested in algal biofuels.

Biosorption

Introduction: *What does the current literature suggest about the feasibility of biosorption?*

Three studies concerning the biosorption of fluoride onto algal biomass have formed the basis of our investigations. All three reports conclude that the algal biomass studied has the ability to adsorb fluoride and, in addition, one report emphasises that pre-treatment with Ca^{2+} 'converts microalgal biomass to an extraordinary tool for fluoride removal'.

1) First Report on Biosorption of Fluoride on the Microalga Spirulina Platensis: Batch Studies
   P. G Hiremath, P. Binnal
   Asian-American Journal of Chemistry Vol. 1, No. 1, January-June 2013, pp. 1-10
2) Biosorption of fluoride from aqueous phase onto algal *SpirogyraIO1* and evaluation of adsorption kinetics
   S. V. Mohan, S. V. Ramanaiah, B. Rajkumar, P. N. Sarma
   Bioresource Technology, Vol. 98, Is. 5, March 2007, pp. 1006-1011

3) Interactive biosorption by microalgal biomass as a tool for fluoride removal
M. Bhatnagar, A. Bhatnagar, S. Jha
Biotechnology Letters, No. 24, 2002, pp. 1079-1081

Comparison: *Of the algae studied, which is most suitable for use in biosorption?*

Overall, four different genera of algae are examined in the studies. Although the particular species is specified in most of the studies, this report aims to provide a slightly broader comparison of the genera. In doing so, it is possible to advise which genus is worth pursuing as a potential biosorbent whilst leaving a wide scope of species open to further research.

The main criteria for a suitable algal biosorbent for use in Andhra Pradesh are as follows:

- The algae should be non-toxic to obviate the need for costly, time-consuming and often unreliable processes to remove toxins following defluoridation of the water
- The algae should be easy to handle in culture, both for the purpose of further research and to minimise difficulties in large-scale growth
- The algae should occur naturally in India to minimise the energy costs of achieving suitable growth conditions, as well as transportation
- The non-viable algal biomass should have a high capacity for adsorption of fluoride
- The non-viable algal biomass should adsorb fluoride at a rate which allows the demand for drinking water to be met

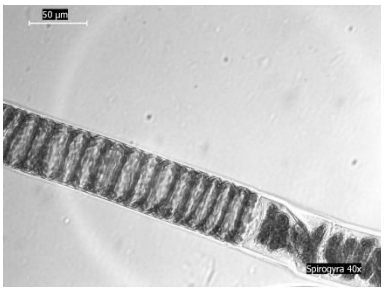
General Properties

The table below summarises the general properties of each genera of algae studied, thus allowing an initial comparison to be made based on the above criteria. (Note that the adsorptive properties of the algae are summarised in Figure 3)

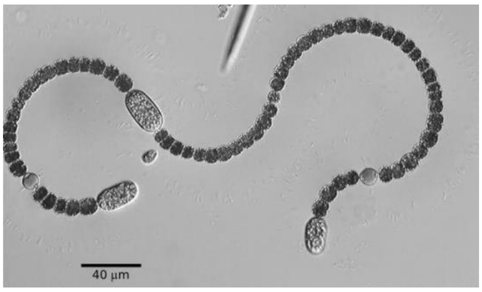
Genus	Phylum	Toxicity	Water type	Structure	Notes
Spirogyra	Chlorphyta	Non-toxic	Freshwater	Filamentous	• Common pond weed • Grows naturally in India
Anabaena	Cyanobacteria	Toxic	Freshwater	Filamentous	• Able to tolerate harsh conditions • Able to fix own nitrogen • Grows naturally in India
Chlorococcum	Chlorphyta	Non-toxic	Freshwater	Unicellular	• High lipid content • Grows naturally in India
Spirulina	Cyanobacteria	Potentially toxic*	Freshwater	Filamentous	• Sold as a health food • Grows naturally in India

Figure 1 General properties of the four genera of algae used in the biosorption studies

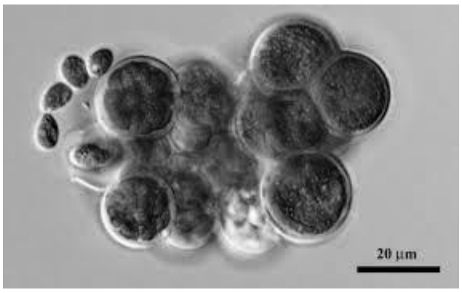
**Spirulina* is considered to be unique in that it is a type of cyanobacteria, yet can be consumed as a health food without having toxic effects. However, the toxicity of large doses of *Spirulina* is a heavily debated topic.



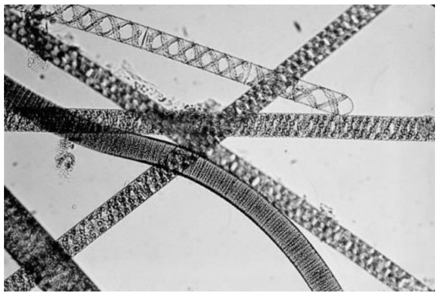
Spirogyra



Anabaena



Chlorococcum



Spirulina

Figure 2 Microscope images of the four genera of algae used in the biosorption studies

Examining the data in light of the criteria highlights several key points about the suitability of each genera for biosorption.

- *Spirogyra*, whilst being non-toxic, is particularly awkward to handle due to its filamentous structure. The fact that pond weed is considered so problematic suggests that large-scale growth of *Spirogyra* would be extremely efficient, yet difficult to carry out in a controlled fashion.
- *Anabaena* has the glaring disadvantage of being toxic. It produces especially harmful neurotoxins, the removal of which presents huge difficulties.
- *Chlorococcum* appears to fulfil the criteria based on its general properties and, in addition, its high lipid content makes it an important candidate for biodiesel production.
- *Spirulina* should not be dismissed based on its potential toxicity but, equally, further research would need to be conducted to provide verification. Although it is filamentous, the commercial growth of *Spirulina* for the health food industry confirms that it can be handled on a large scale. However, its monetary value as a health food far exceeds that as a biosorbent, so securing an affordable supply for the latter purpose could prove difficult.

This initial comparison – not taking into account the biosorption itself – yields *Chlorococcum* as a clear frontrunner.

Adsorptive Properties

All three studies analyse the sorption kinetics underlying the removal of fluoride from the aqueous phase in order to assess the suitability of using algal biomass as a biosorbent. By fitting all the data to pseudo-first order and pseudo-second order models – as well as the intraparticle diffusion model in the case of study (2) – and calculating the correlation coefficient, the kinetics of the adsorption process for each genus of algae can be modelled.

In the studies the data was then fitted to two sorption isotherms – the Langmuir Isotherm and the Freundlich Isotherm – and the correlation coefficients were calculated once again to indicate which of these most accurately described the equilibrium relationship between the algal biomass (biosorbent) and the fluoride (adsorbate). These isotherms yield several constants which are crucial for making comparisons between the different genera of algae in terms of their suitability for biosorption.

Definitions of variable:

C_0 – initial fluoride concentration in solution
C_e – fluoride concentration in solution at equilibrium
q_e – mass of fluoride per unit mass of adsorbent at equilibrium

Langmuir isotherm:

$1/q_e = 1/bq_oC_e + 1/q_o$

b – equilibrium constant (volume of solution per unit mass of fluoride)
q_o – maximum monolayer sorption capacity (mass of fluoride per unit mass of biosorbent)

Hall separation factor (N.B. only calculated for data fitting the Langmuir isotherm):

$R_L = 1/(1+bC_o)$

$0<R_L<1$ – biosorption favourable
$R_L>1$ – biosorption unfavourable
$R_L=1$ – biosorption linear
$R_L=0$ – biosorption irreversible

Freundlich isotherm:

$\ln q_e = (1/n)\ln C_e + \ln K$

n - equilibrium constant (dimensionless)
K - maximum sorption capacity (mass of fluoride per unit mass of biosorbent)

After establishing which isotherm fits the data for each type of algal biomass most accurately, the most important comparison to make is between the maximum sorption capacity constants. These values serve as the best indication of how feasible it would be to reduce the fluoride concentration in water to a safe level for drinking, and approximately how much algal biomass that would require.

The table below summarises the results from the three studies. Unfortunately several of the correlation coefficients were not given, particularly for the Freundlich isotherm which tended to fit the data less closely than the Langmuir isotherm. Additionally, in study (3) the Hall separation factor was not calculated. The blank cells indicate which pieces of data are unavailable. Note that the emboldened correlation coefficient highlights which isotherm provided the better fit, and it is the maximum sorption capacity of this isotherm which is given.

Genus of algae	Langmuir isotherm correlation coefficient	Freundlich isotherm correlation coefficient	Hall separation factor (relating to Langmuir isotherm only)	Maximum sorption capacity (mg F^- g^{-1} biosorbent)	Volume of algal biomass required*
Spirogyra	**0.933**	0.709	0.253	1.272	7.5
Anabaena				0.99	9.6
Anabaena (Ca^{2+} treated)	**0.916**			7.0	1.4
Chlorococcum				0.3	31.7
Chlorococcum (Ca^{2+} treated)	**0.990**	0.951		4.5	2.1
Spirulina	0.9765	**0.9882**	0.08	2.87	3.3

Figure 3 Adsorptive properties of the four genera of algae used in the biosorption studies

*Based on a very rough calculation of reducing the fluoride concentration of 1 litre of water from a high concentration of 10 mg l^{-1} to the recommended level of 1.5 mg l^{-1}

It can be seen that Ca^{+2} treated *Anabaena* biomass has the highest maximum sorption capacity, yet, as previously mentioned, its toxicity makes it unsuitable for use as a biosorbent. The second highest maximum sorption capacity was observed with Ca^{+2} treated *Chlorococcum* biomass, which also emerged as the preferable genus based on its general properties. It is clear that treating the biomass with Ca^{2+} significantly enhances its adsorptive ability, and this is because doing so increases the number of positive sites available on the surface of the biomass to bind the negative fluoride ions. Unsurprisingly, the more Ca^{2+} adsorbed, the more the biosorption of fluoride is enhanced. This is supported by the fact that, in the Ca^{2+} treatment stage of the process, *Chlorococcum* adsorbed more Ca^{2+} than *Anabaena* (4.4 mg Ca^{2+} g^{-1} biomass as opposed to 2.8 mg Ca^{2+} g^{-1} biomass) and its maximum adsorption capacity saw a larger increase (15-fold as opposed to 7-fold).

Whilst Ca^{2+} treatment could play an important role in making algal biosorption feasible, it is, nevertheless, an extra step in the process which introduces extra costs and complications. Therefore, it is worth identifying the untreated biomass with the highest maximum sorption capacity, which – by a considerable margin – is *Spirulina*. It is also worth mentioning that the Freundlich isotherm for this genus – which is omitted from the table, but only had a slightly smaller correlation coefficient than the Langmuir isotherm – yielded an incredibly high maximum sorption capacity of 34.82 mg F^- g^{-1} adsorbent. The study did not dwell on this anomaly, but it does raise the question of whether *Spirulina*'s supposed miraculous health benefits may be mirrored in its ability to perform defluoridation.

The two Hall separation factors calculated both fall within the range $0<R_L<1$, thus confirming that biosorption is favourable in the case of both *Spirogyra* biomass and *Spirulina* biomass. The Hall separation factor of the latter falls much closer to $R_L=0$, at which the biosorption process is irreversible. This highlights the issue of reversibility, which is only briefly touched upon in the studies. Reversibility of the biosorption process is a favourable quality, since it would minimise costs by allowing the same biomass to be used repeatedly. Alternatively, once the fluoride had desorbed, the biomass could be used as algal meal for livestock. This would cut waste out of the process completely, and would ultimately be providing the villagers with a source of food and income in addition to clean water.

Biosorption Conditions

So far, the final criterion relating to the rate of adsorption has not been addressed. The biosorption in each of the studies was carried out under different conditions, but there was general agreement that equilibrium was reached after a contact time of 120 minutes. The exception to this is study (1), in which the equilibrium of the *Sprulina* biomass adsorbent and the fluoride was reached after just 20 minutes. Even the longer contact time of 120 minutes should easily allow supply to meet demand, assuming that the systems used in the studies could be scaled up to process the necessary volumes of water.

The approach to the conditions under which the biosorption was carried out varied massively between the studies, as did the information provided regarding these conditions. Studies (1) and (2) carried out multiple experiments to rigorously establish optimum conditions, whereas the conditions were not expanded upon in study (3). The information provided regarding the conditions for biosorption is summarised below.

Algae genera	Initial fluoride concentration / mg l^{-1}	pH of feed solution	Adsorbent dosage / g l^{-1}	Contact time / min
Spirogyra	20	2		120
Anabaena	10			120
Anabaena (Ca^{2+} treated)	10			120
Chlorococcum	15			120
Chlorococcum (Ca^{2+} treated)	15			120
Spirulina	500	2	4	20

Figure 4 Conditions under which the biosorption studies were carried out

Although it is difficult to draw many conclusions from this data, a few observations can be made. With the exception of the study on *Spirulina*, the initial fluoride concentration was within the range 10.00-20.00 mg l^{-1}, which lies only slightly above the fluoride concentration occurring in groundwater in the Andhra Pradesh region.

The extremely high initial fluoride concentration used in the study on *Spirulina* does not reflect natural levels of fluoride. This may be the reason for the remarkably high maximum adsorption capacity yielded by the Freundlich isotherm for *Spirulina* biomass, although that would not explain why the maximum adsorption capacity yielded by the Langmuir isotherm was in the same order as those for the other genera of algae. This illuminates the fact that it is invalid to make such direct comparisons between the constants in Figure 3 due to the variation in initial fluoride concentrations.

A low pH is favourable because, like the Ca^{2+} treatment, it increases the number of positive binding sites on the surface of the algal biomass. The pH of groundwater in Andhra Pradesh tends to be slightly alkaline, in the range 6-8.5, so would need to be acidified in order to recreate the conditions used in the studies. However, a pH was not stated for the study in which Ca^{2+} treatment was used, and it is possible that this treatment would render acidification unnecessary.

Preparation Methods

Aside from slight variations, the same basic procedure was followed in each study to prepare the algal biomass. The steps of this procedure are as follows:

1. Washing with de-ionised water
2. Drying, either by long-term exposure to sunlight or short-term exposure to an artificial heat source (60-80ºC)
3. Crushing to a powder

Note

- In study (2) the algal biomass was also treated with HCl
- In study (3) some of the algal biomass was also treated with $CaCl_2$ for comparison with the untreated algal biomass

Although the three basic steps could be carried out with relative ease on-site in the villages of Andhra Pradesh, this may be unnecessary as the by-product from algal biodiesel plants already tends to be available in a washed, dried and crushed form. This is the aforementioned product, algal meal, which can be fed to livestock as a rich source of protein.

The cost of algal meal depends largely on the quantity required and how refined the product is, but the company SEE ALGAE Technology suggests a guide price of approximately £140 per tonne. An extremely crude calculation based on an average village population of 800 with each villager consuming 5 litres of water per day suggests that the cost of the adsorbent itself could be as little as £1.20 per day. Moreover, this calculation ignores the possibility that the biomass could be used repeatedly if the adsorption process is sufficiently reversible.

Conclusion: *Which of the genera of algae, if any, is worth pursuing as a potential biosorbent?*

In conclusion, of the genera studied, *Chlorococcum* seems to be the most suitable for biosorption, but only when treated with Ca^{2+}. This treatment appears to increase the cost and complexity of the process, but crucially, *Chlorococcum* – with its high lipid content – is the only genus likely to be used in biodiesel production. Consequently, unlike the other algae which would probably need to be grown specifically for the purpose of biosorption, it is possible that *Chlorococcum* could be obtained cheaply as a by-product of biodiesel production. Clearly, saving the start-up, energy and labour costs of purpose-growing the algae would more than compensate for the cost of the Ca^{2+} treatment.

Further Research: *What needs to happen next?*

- The species of *Chlorococcum* studied was *Chlorococcum Humicola*, but there are over 30 species in total. Further research could investigate the adsorption of some of the other commonly occurring species, both with and without Ca^{2+} treatment.
- The optimum conditions for biosorption onto *Chlorococcum* biomass need to be firmly established by investigating a range of initial fluoride concentrations, pH values, temperatures and adsorbent dosages.
- The reversibility of biosorption onto *Chlorococcum* biomass should be studied with respect to variables such as pH and temperature. If desorption could be easily induced by altering conditions, it would then need to be established how many times the same algal biomass could be re-used before becoming ineffective as a biosorbent.
- The methods of treating algal biomass with Ca^{2+} need to be investigated further with a view to minimising the cost and complexity of this additional step in the preparation of the adsorbent.
- Further research could be done into the biosorption onto *Spirulina* biomass at lower initial concentrations of fluoride in order to establish whether the Langmuir isotherm yields a similarly high maximum adsorption capacity. If so, the toxicity of *Spirulina* would then need to be conclusively assessed.
- The development of the planned algae biodiesel pilot plant in India must be followed closely, and communication with the companies involved may be worthwhile to ascertain what by-products may become available once the plant is operational.
- The scalability of the biosorption process needs to be looked into. In particular, the maximum volume of water which could be processed in a single vessel at one time needs to be established.

Bioaccumulation

Introduction: *What does the current literature suggest about the feasibility of bioaccumulation?*

There was just one readily accessible study that provided relevant, specific information concerning the bioaccumulation of fluoride by viable algae. In this study, four different species of algae were investigated, but showed little variation in their ability to accumulate fluoride. Therefore, this section of the report will focus less on a comparison between different types of algae, and more on the overall feasibility of bioaccumulation as a method of defluoridation, particularly in light of the biosorption findings.

> Fluoride and aluminium tolerance in planktonic microalgae
> G. Ali
> *Fluoride Vol. 37, No. 2, 2004, pp. 88–95*

Assessment: *How feasible is bioaccumulation as a method of defluoridation, and how does it compare to biosorption?*

General Properties

The table below summarises the properties of the four genera of algae used in the study.

Genus	**Phylum**	**Toxicity**	**Water type**	**Structure**	**Notes**
Microcystis	Cyanobacteria	Toxic	Freshwater	Unicellular	• Colonial • Grows naturally in India
Nitzschia	Heterokontophyta	Toxic	Marine/ Freshwater	Unicellular	• Tolerates high salinities • Grows naturally in India
Scenedesmus	Chlorophyta	Non-toxic	Freshwater	Coenobic	• Colonial • Grows naturally in India
Anabaena	Cyanobacteria	Toxic	Freshwater	Filamentous	• Able to tolerate harsh conditions • Able to fix own nitrogen • Grows naturally in India

Figure 5 General properties of the four genera of algae used in the bioaccumulation studies

Since a detailed comparison is not necessary, the one main point which should be noted is that *Scenedesmus* is the only non-toxic genera, so, realistically, is the only alga suitable for use in the defluoridation of water. Thus, the bioaccumulation data for *Scenedesmus* – despite not being radically different to that for the other genera – should be given special consideration.

Accumulative Properties

The table below shows the fluoride accumulation over 10-14 days in mg fluoride kg^{-1} dry algal biomass, with an initial fluoride concentration of 4 mg l^{-1}.

	Microcystis	*Nitzschia*	*Scenedesmus*	*Anabaena*
pH 7.3	2.25	2.60	2.30	2.75
pH 6.0	2.50	2.20	2.30	2.60
pH 4.5	2.60	1.20	2.50	2.00

Figure 6 Fluoride accumulation in the four genera of algae used in the bioaccumulation studies

N.B. Approximate values as read from graphs

Aside from one exception (*Nitzschia*, pH 4.5), all of the results lie in the narrow range 2.00 - 2.75 mg kg^{-1}, with *Scenedesmus* falling fairly centrally within this band. There is no consistent trend relating the pH to the accumulation of fluoride; the response to changes in pH varies between genera, with *Microcystis* and *Scenedesmus* accumulating more fluoride at lower pH values, and *Nitzschia* and *Anabaena* accumulating more fluoride at higher pH values. This does not make pH an irrelevant factor, but rather one which would have to be investigated within the context of a particular genus, or even a particular species.

In contrasting this data with the maximum adsorption coefficients in Figure 3, it is crucial to note the difference in units; whilst the maximum adsorption coefficients are quoted in mg fluoride g^{-1} biosorbent, the above figures are quoted in mg fluoride kg^{-1} dry algal biomass. Therefore, the capacity of non-viable algae to adsorb algae over a period of two hours appears to be approximately three orders of magnitude higher than the capacity of viable algae to accumulate fluoride over a period of two weeks. This reveals the inefficiency of biosorption, and deems it unfeasible for performing defluoridation on a regular-supply basis. Nevertheless, it does not rule the process out completely, since it could be implemented in long-term storage tanks in which the water has a fluoride concentration slightly above the recommended levels. Considering the Indian climate, whereby a wet season in the high-sun months is followed by a longer dry season in the low-sun months, there is certainly a need for long-term water storage. However, over a long period of time it may be difficult to control the growth of algae, especially a colonial genus such as *Scenedesmus*.

Alternative Applications

It is another aspect of the study, however, which raises an intriguing possibility, and takes the concept of bioaccumulation in a slightly different direction. As indicated by the name of the study, aluminium accumulation was also investigated. This was done independently, as well as in combination with fluoride. The table below shows the accumulation of fluoride and aluminium over 10-14 days in mg fluoride/aluminium kg^{-1} dry algal biomass, with an initial fluoride and aluminium concentration of 4 mg l^{-1}.

	Microcystis		*Nitzschia*		*Scenedesmus*		*Anabaena*	
	Fluoride	Aluminium	Fluoride	Aluminium	Fluoride	Aluminium	Fluoride	Aluminium
pH 7.3	2.50	0.75	2.10	2.00	1.40	0.50	1.90	0.40
pH 6.0	2.50	1.20	2.50	2.80	1.80	0.50	2.00	0.40
pH 4.5	3.20	2.20	2.80	3.40	3.00	0.10	2.50	1.40

Figure 7 Fluoride and aluminium accumulation in the four genera of algae used in the bioaccumulation studies

N.B. approximate values as read from graphs

The trend relating accumulation to pH is much more consistent in this set of data; aside from one exception (*Scenedesmus*, Alumunium, pH 4.5) the accumulation of both fluoride and aluminium is higher at lower pH values. In addition, comparison with Figure 6 reveals that the fluoride accumulation is generally higher in combination with aluminium. Moreover, coagulation with aluminium is a method of defluoridation sometimes used as an inexpensive alternative to adsorption onto activated alumina. The problem with this method is the residual aluminium resulting from the soluble fluoride-aluminium complexes remaining in solution. Biosorption may overcome this problem to some extent by accumulating these fluoride-aluminium complexes, thus removing them from solution.

One complex - AlF_4^- - is of particular interest due to its ability to act as an analogue of PO_4^{3-}. Ordinarily, PO_4^{3-} binds to the enzyme ATPase, which plays a role in the uptake of NO_3^- and further PO_4^{3-}. Both of these are essential nutrients for growth, so when AlF_4^- competes for binding sites on ATPase and inhibits the uptake of NO_3^- and further PO_4^{3-}, the algae are effectively being subjected to nutrient starvation. Both nitrate starvation and phosphate starvation – but in particular the former – are techniques used in the stress phase of algae growth during biodiesel production, as the lipid content of the cells is maximised as a result.

The study did not correlate fluoride and aluminium accumulation with lipid content, but instead did so with the chlorophyll a content. Although this is not stated explicitly in the study, a general examination of the literature would suggest that high chlorophyll a content goes hand in hand with high protein content, whilst low chlorophyll a content goes hand in hand with high lipid content. In the study, all four genera of algae showed a reduction in the chlorophyll a content following accumulation of fluoride and aluminium. The table below gives a qualitative indication of the extent to which the chlorophyll a content was reduced.

	Microcystis	*Nitzschia*	*Scenedesmus*	*Anabaena*
pH 7.3	Slight	Slight	Negligible	Negligible
pH 6.0	Significant	Significant	Significant	Significant
pH 4.5	Very significant	Slight	Significant	Significant

Figure 8 Chlorophyll a reduction in the four genera of algae used in the bioaccumulation studies

Assuming that the inversely proportional relationship between chlorophyll a content and lipid content broadly holds true, the table may also be loosely interpreted as an indication of the extent to which lipid content would increase following fluoride and aluminium accumulation.

Conclusion: *How, if at all, can bioaccumulation be applied to defluoridation?*

In conclusion, bioaccumulation seems to be unsuitable for defluoridation alone, but could potentially work well in conjunction with biofuel production. To summarise this potential complementarity between defluoridation and biodiesel production, aluminium could be introduced into water with a high fluoride concentration as a coagulant, which would lead to the formation of fluoride-aluminium complexes. If combined in the correct proportions and under suitable conditions, the complex AlF_4^- would form, which would subsequently accumulate in the algal cells. In doing so, the complex would inhibit ATPase enzymes, leading to nitrate and phosphate starvation. This would lead to an increase in lipid content, and therefore a maximised yield of biofuel.

One main advantage of this multi-stage process is that the time-scale of algae growth for biofuel production lends itself well to bioaccumulation, although the water undergoing the defluoridation would, again, need to be water in storage.

There are numerous potential flaws in the combination of defluoridation of stored water and biodiesel production, but one in particular which should be given immediate consideration is the strong possibility that the lipids extracted from the algae could have a significant aluminium content. This would need to be rectified before converting the lipids into biofuels, since the aluminium oxides which would otherwise be produced during combustion are pollutants which carry certain health hazards. Effectively removing aluminium from the lipids could prove costly and energy-intensive.

Further research: *What needs to happen next?*

- It needs to be established whether a decrease in chlorophyll a content following accumulation of fluoride and aluminium does indeed correlate with an increase in lipid content, which could be done by carrying out a longer-term version of the study referred to above.
- It needs to be determined whether or not the aluminium accumulated in the algal cells resides in the lipids and, if it is found to do so, the methods of removing this aluminium must be investigated.
- Further research must be done into defluoridation using coagulation by aluminium, particularly in terms of the optimum conditions for this process, the residual aluminium, and how selectively the AlF_4^- ion can be produced.

Overall conclusion: *What can be drawn from this report?*

Overall, there appears to be a much stronger case for the defluoridation of water using biosorption as opposed to bioaccumulation, since it is a much more efficient process. Of the genera of algae studied so far, *Chlorococcum* appears to be the most promising as an adsorbent, both in terms of performance and economic viability.

Bioaccumulation could only feasibly be used for the defluoridation of water in storage due to the time-scale of the process. To make this more worthwhile, it could play an important role in maximising the lipid content of algae in biodiesel production.

Both biosorption and bioaccumulation require extensive further research; in the case of the former, more specific data needs to be amassed, whereas in the case of the latter the underlying principles must be thoroughly investigated.

Future of the project*: How could this project be taken forward?*

The biodiesel pilot plant to be built in India presents a particularly exciting opportunity for the development of this project: it provides a focus for research, and could one day play a crucial role in enabling algae defluoridation technology to be implemented in the field. Below is an outline of how this project could progress alongside the pilot plant.

1) Ascertain the planned location for the pilot plant and what species is to be used in biodiesel production
2) Assuming the species is non-toxic, investigate its adsorptive properties:
    - Systematically establish the optimum conditions for adsorption
        - pH
        - Temperature
        - Adsorbent dosage
        - Initial fluoride concentration
        - Contact time
    - Under these optimum conditions, determine the maximum adsorption capacity of this species by fitting adsorption data to Langmuir and Freundlich isotherms
    - Assess the reversibility of adsorption
        - Identify desorption trends in adsorption data
        - Investigate desorption over a wider range of conditions
    - Repeat above investigations with Ca^{2+} treatment
3) Investigate the bioaccumulative properties of the species under the conditions used for growth in the biodiesel plant
    - Measure the rate of bioaccumulation of fluoride and aluminium over the full growth period of the algae
    - Determine the effects of bioaccumulation of fluoride and aluminium upon lipid content by assessing the composition of algae samples at regular intervals
4) Investigate aluminium coagulation
    - Systematically establish the optimum conditions for forming the AlF_4^- complex
    - Establish the best combination of reactants for forming the AlF_4^- complex
    - Measure the aluminium content in the lipids extracted from the algae after the growth period
    - Measure the aluminium content in the biofuel produced by the transesterification process
    - Assess the residual biomass for suitability as algal meal

Facilitating this plan:

- Use of suitable laboratory space
    - Laboratory space at the University of Bristol
    - Laboratory space at a nearby university with a specialist biofuel research team (e.g. University of Bath)
- Support from academics
    - Academics at the University of Bristol
    - Academics at a nearby universities with specialist biofuel research teams (e.g. University of Bath)
    - Academics at universities nearby to the pilot plant location
    - Academics conducting research on behalf of Algae.Tec for the pilot plant
- Funding
    - Grant from an NGO
    - Investment from Algae.Tec

Glossary of terms

Viable – Living
Non-viable – Not living
Adsorption – The binding of atoms, ions or molecules of a gas, liquid or solution to a solid surface
Biosorption – Adsorption onto biomass
Bioaccumulation – The accumulation of a substance within an organism
Genus – A rank in the taxonomical classification hierarchy, falling above species and below family
Phylum – A rank in the taxonomical classification hierarchy, falling above class and below kingdom
Filamentous – A chainlike arrangement of cells
Unicellular – Single-celled
Coenobic – A colony containing a definite number and arrangement of unicellular organisms
Lipids – Fat molecules, broadly defined as hydrophobic
Nitrogen fixation – The conversion of atmospheric nitrogen into ammonium for biosynthesis
Complex ion – A metal ion surrounded by molecules or other ions
AlF_4^- - Tetrafluoroaluminate, a negatively charged complex ion comprising one aluminium atom and four fluoride atoms
PO_4^{3-} - Phosphate, a negatively charged ion comprising one phosphorous atom and four oxygen atoms
NO_3^- - Nitrate, a negatively charged ion comprising one nitrogen atom and three oxygen atoms