# Bio prospecting for High Lipid-producing Indigenous Algal

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## INTRODUCTION AND BACKGROUND

The earth's surface is 71% covered by water. Microalgae comprise a vast group of photosynthetic, heterotrophic organisms which have a good potential for cultivation as renewable feedstock for biodiesel production due to their rapid growth rates and are more productive than plants and microalgae. Microalgae are genetically diverse organisms that exist as unicells, colonies and extended filaments; they are distributed ubiquitously throughout the biosphere growing under the widest possible variety of conditions (Sheehan *et al.*, 1998).

The scope of this project covers the screening and characterisation of selected indigenous algal strains and screening them for lipid production potential. The algae were isolated from salt- and freshwater bodies, along the Western Cape coastline of South Africa. Isolates were purified into individual isolates and screened for lipid-production. Isolate B7.4, displayed a promising lipid producing capability and fast growth rate. Key nutrients such as nitrate and silicate concentration present in the medium were identified as target handles in an attempt to boost lipid producing capability of the isolate, without, impacting negatively on the inherent growth. This optimization could prove highly beneficial when the technology is applied commercially.

## MATERIAL AND METHOD

**Sampling and Isolation**

The temporal and spatial collection strategy was adopted to cater for any succession that can occur at the sampling site (Anandraj *et al.*, 2008; Bernal *et al.*, 2008). Once samples reach the laboratory, cultures are revived using protocols outlined in Figure 1. Thereafter, preliminary screening is undertaken on samples collected in order to ascertain whether the isolates demonstrated the ability to produce relevant lipid intermediates.

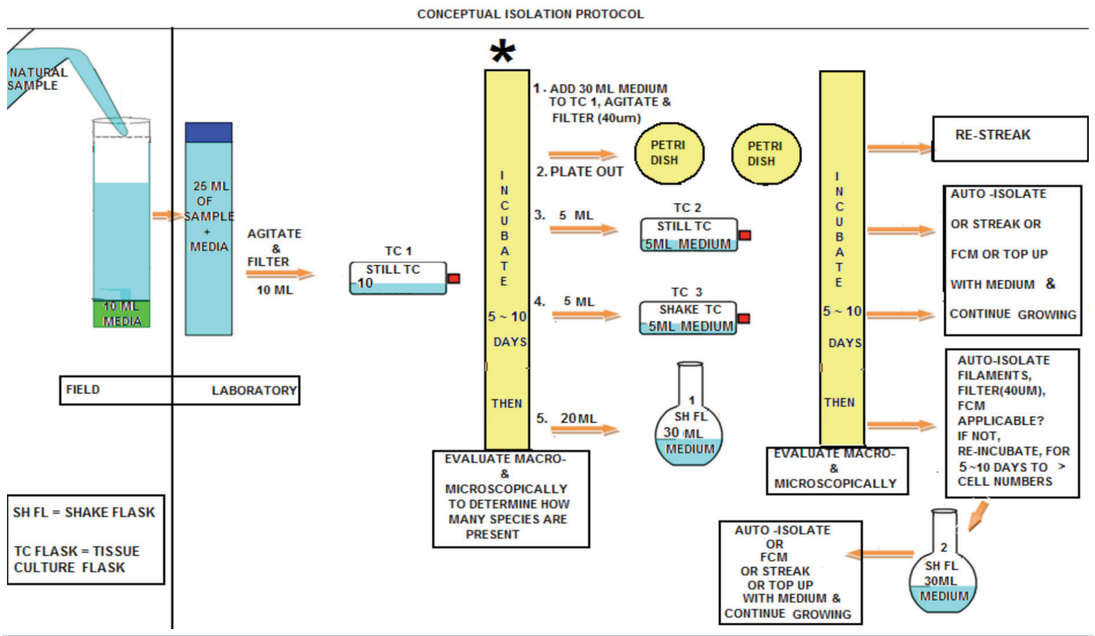


**Figure 1:** Outline of the isolation protocol.

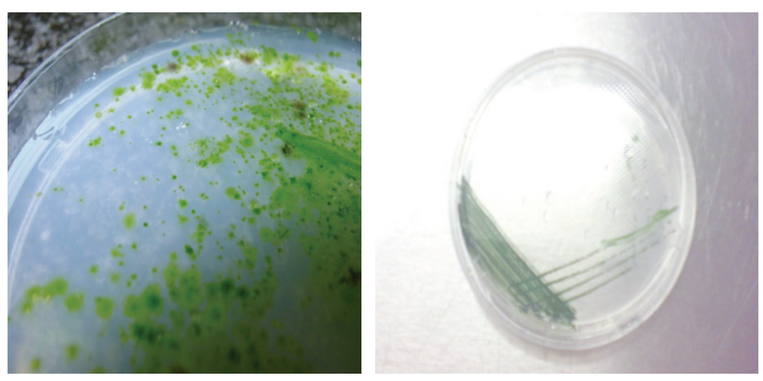

**Figure 2:** (a) Spread plate of sample WCC 8 (b) 3rd streak plate of isolate WCC 8.1 obtained from sample 8.

***Growth Rate and Doubling Time using Micro titre plate***

Mono-algal colonies from streak plates were transferred into 15 ml test tubes containing 10ml of medium using a sterile inoculation loop. These test tubes were incubated at ambient temperature at on a Heidolph Vibramax 100 (Schwabach, Germany) test tube shaker at 50 rpm and under constant illumination from Sylvania® Growlux (L58W/77) lamps.

***Fluorescence Spectrophotometer***

The algal cell suspensions were taken from a 50 ml BD Falcon tube (BD Biosciences, San Jose, California, USA) used for sampling and 250ul was aspirated using a pipette into a micro-titre plate. 250µl was dispensed per well in triplicate or more into a 96 well black micro titre plate (Greiner Bio One, Germany).

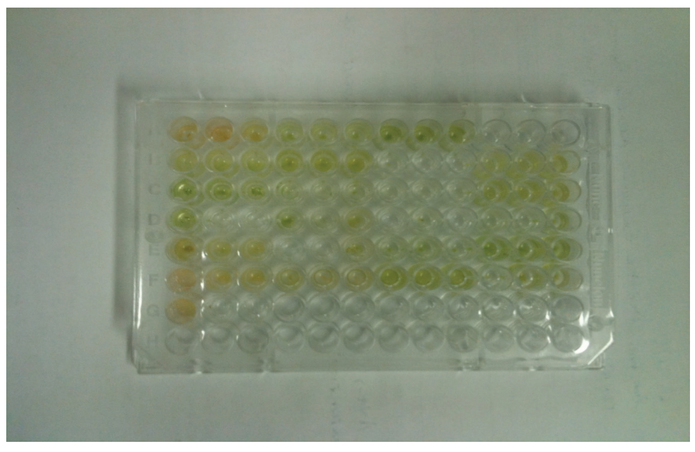

**Figure 3:** Transparent Micro titter plate for growth rate and doubling time.

## RESULTS AND DISCUSSION

The growth of B 7.4 differed greatly over the range of nitrogen concentrations tested in this study. Growth was absent in the 0.5x medium for the first 4 hours of cultivation (Figure 6), thereafter an increase in cell concentration was observed. The highest cell concentration ($4.20 \times 10^6$ cells.ml$^{-1}$) was achieved when B7.4 was grown in medium containing double the original nitrate concentration (Figure 6). The 1.0x and 3.0x medium produced maximal cell concentrations of $4.1 \times 10^6$ cells.ml$^{-1}$ and $4.0 \times 10^6$ cells.ml$^{-1}$ after 7 and 6 hours of cultivation respectively (Figure 6).

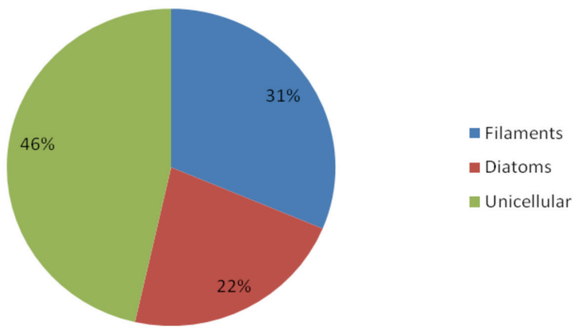


**Figure 4:** Represents three different types of algae present in the sample set C.

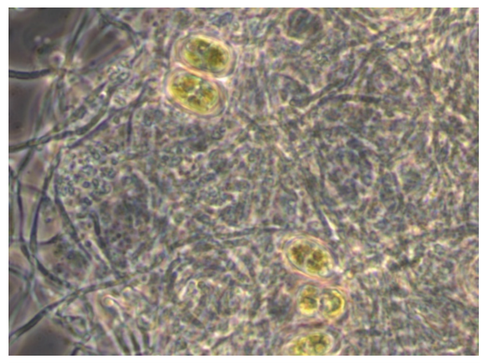

**Figure 5:** Micrographs of isolates C8.1. The unicellular algae form part of a biofilm.

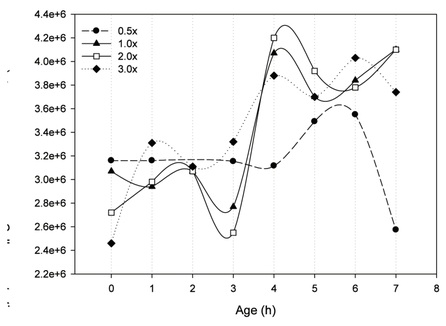


**Figure 6:** Cell concentration profiles of B 7.4 in 2 L bioreactors at ambient temperature and across a nitrate concentration range of 0.5x (●), 1.0x (▲), 2.0x (□) and 3.0x (◆).

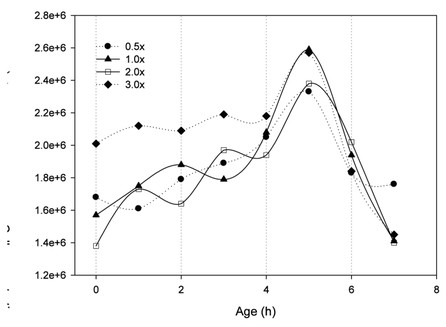


**Figure 8:** Cell concentration profiles of B 7.4 in 2 L bioreactors at ambient temperature and across a silicate concentration range of 0.5x (●), 1.0x (▲), 2.0x (□) and 3.0x (◆).

Nitrate concentration, up to a maximum of 176 mg.l$^{-1}$ i.e. double the original medium concentration. Once the nitrate concentration increased to triple that of the original concentration, a decrease in cell concentration was observed (Figure 7 a). A maximum growth rate of 0.113 h$^{-1}$ was achieved when B7.4 was grown in the standard ASW medium (Figure 7b). When isolate B7.4 was cultivated in medium containing triple the nitrate content, a large decrease in growth rate was observed. B7.4 demonstrated the weakest cell productivity ($3.0 \times 10^6$ cells.ml$^{-1}$) when it was cultivated in a medium containing half the original nitrate content (Figure 7c). The reduced growth at the highest nitrate concentration is probably directly attributed to the decrease in specific nitrate uptake rate associated with higher nitrate concentrations. Kudela and Dugdale (2000) observed that there was large variability in specific nitrate uptake rates in algae which could be directly correlated to nitrate concentrations.

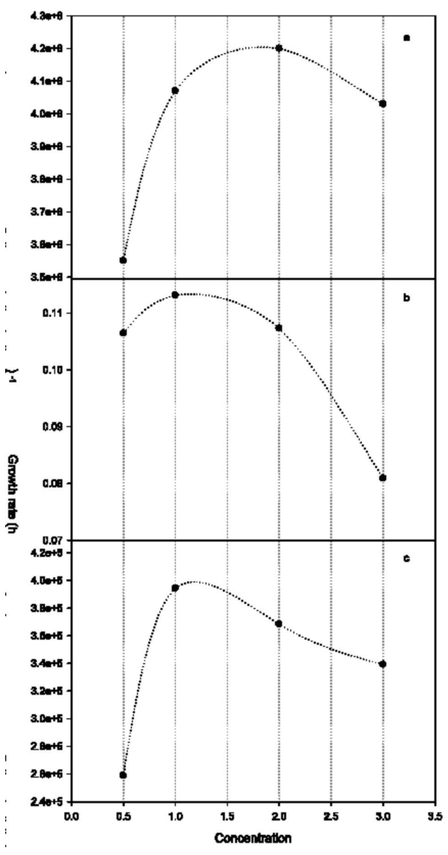


**Figure 7:** Cell concentration (a), growth rate (b) and cell productivity (c) of B 7.4 after growth in 2 L bioreactors over a nitrate range of 70 - 420mg.l$^{-1}$.

## CONCLUSIONS

The application of the conceptual protocol that was used in the above statistics in the isolation of different strains was effective, easy and quick and it can be applied to the bio prospecting of algae for biodiesel production with greater success compared to the application of only standard and traditional isolation techniques. This study makes a valid contribution to the field of biodiesel production as it demonstrates a medium to high throughput isolation and purification protocol for potential biodiesel algal isolates.

## ACKNOWLEDGMENTS

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