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Effect of nitrogen source and acclimatization on specific growth rates of microalgae determined by a high throughput *in vivo* microplate autofluorescence method

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Abstract

Specific growth rates (SGR) of fresh water algal strains (*Chlorella vulgaris*, *Auxenochlorella protothecoides*, and *Chlorella sorokiniana*) and the marine strain *Nannochloropsis oculata* on various nitrogen sources (ammonium carbonate, ammonium chloride, sodium nitrate, and urea) could be determined by *in vivo* chlorophyll-a autofluorescence. These preferences could be determined before large pH changes occurred in the media, with no significant difference ($P > 0.05$) between buffered and non-buffered media. In all algal species, acclimatization was observed with no significant difference ($P > 0.05$) between SGRs of second and third cultivations. ANOVA of SGRs in the acclimatized second and third cultivation revealed preferences for nitrogen sources among most of the algae; *C. vulgaris* preferred sodium nitrate over other nitrogen sources, *A. protothecoides* adapted to urea after no growth in the first cultivation, and the SGRs of *N. oculata* showed an aversion for sodium nitrate over other nitrogen sources ($P < 0.05$).

Aims

- Developing a microplate screening technique for high throughput screenings of microalgae.
- to determine the preference of nitrogen source for algal growth for four industrially important microalgae
- Determine the acclimatized SGR of each algae species to each nitrogen source.
- Determine the SGR before large changes in pH occur.

Methods

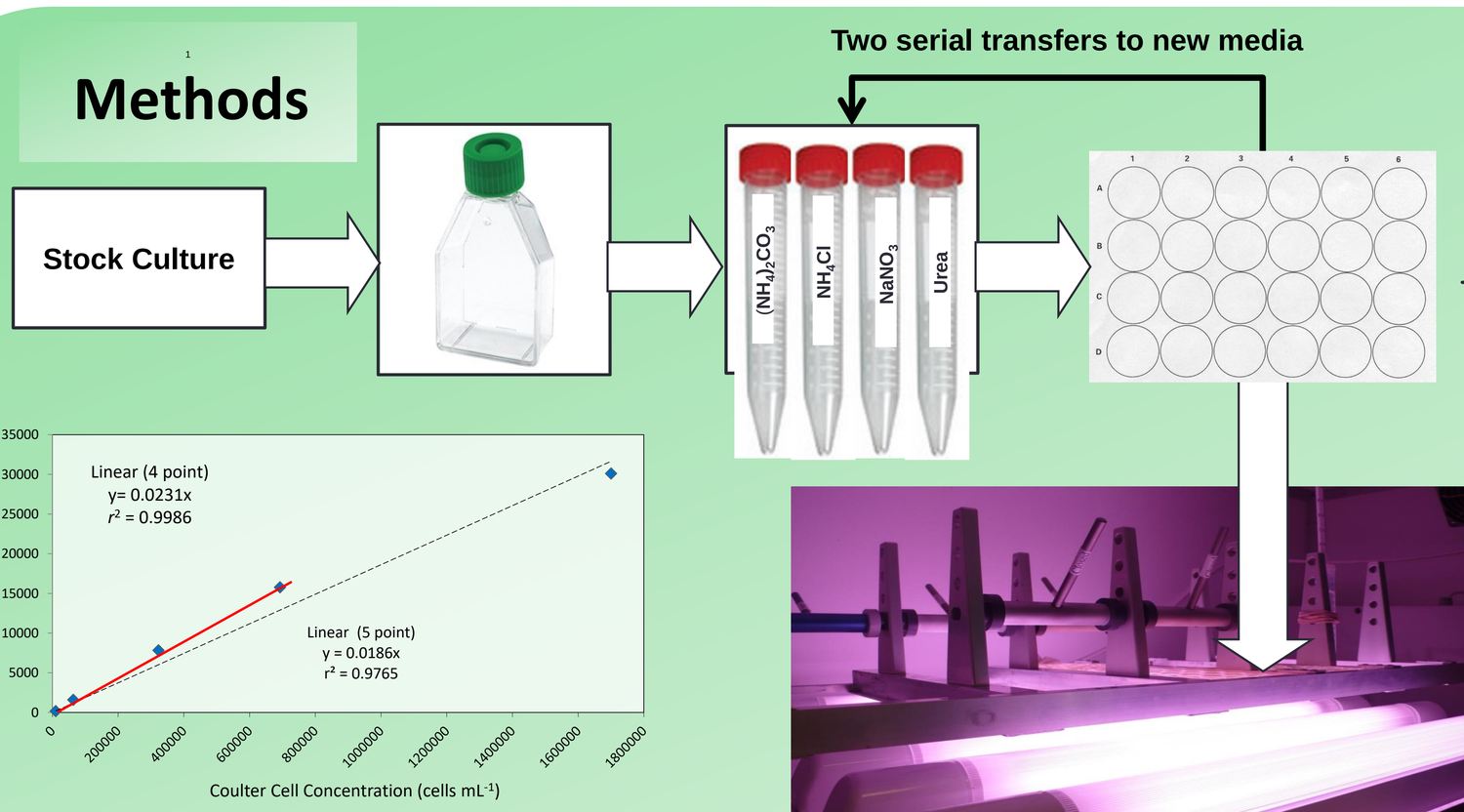

Fig. 2 Five point linear (large dashed line), and four point linear (solid red line) representations of fluorescence vs. coulter cell concentration calibration curve of *Nannochloropsis oculata*. Direct correlation to cell concentration <15,000 RFU.

Results

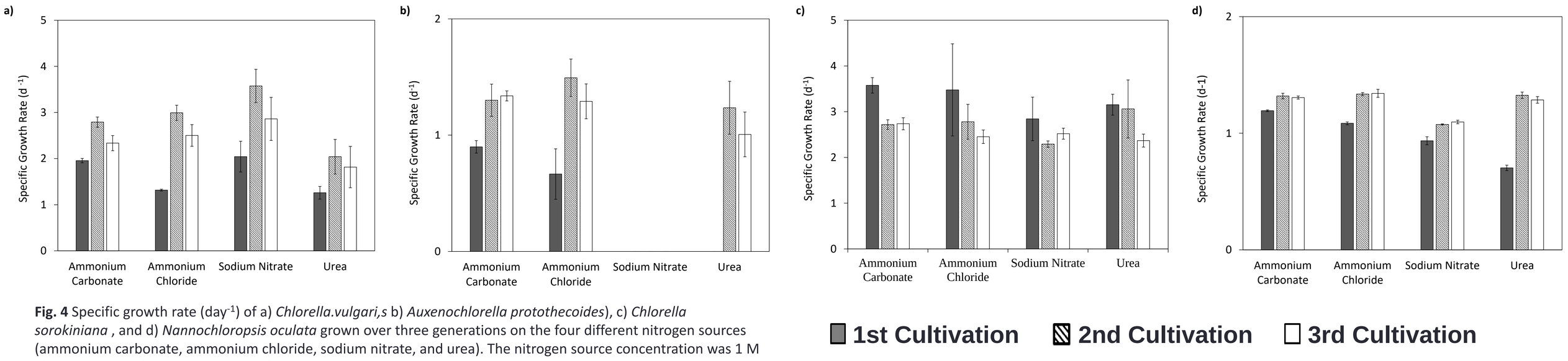

Fig. 4 Specific growth rate (day^{-1}) of a) *Chlorella.vulgari,s* b) *Auxenochlorella protothecoides*), c) *Chlorella sorokiniana* , and d) *Nannochloropsis oculata* grown over three generations on the four different nitrogen sources (ammonium carbonate, ammonium chloride, sodium nitrate, and urea). The nitrogen source concentration was 1 M nitrogen. Note different scale of y-axis.

Conclusions

- *In vivo* microplate batch cultures can be used to compare specific growth rates of microalgae containing chlorophyll-a.
- This method can be used to determine the specific growth rate before significant pH changes in the media occur, making the method useful for comparison of various modes of nitrogen assimilation.
- It has been demonstrated that, at certain conditions, sodium nitrate is the most preferred nitrogen source and urea is the least favored nitrogen source for *Chlorella vulgaris* .
- *Auxenochlorella protothecoides* demonstrated an acclimatization to urea, with no growth occurring in nitrate media in autotrophic conditions
- *Nannochloropsis oculata* showed a clear aversion for sodium nitrate as a nitrogen source.

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