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Biodiesel production potential of an indigenous South African microalga, *Acutodesmus bajacalifornicus* ☆

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ABSTRACT

In this study, a South African indigenous microalga *Acutodesmus bajacalifornicus* was evaluated in different cultivation media. Eleven potential cultivation media were identified and tested on *A. bajacalifornicus*, a potential source for biodiesel production. *A. bajacalifornicus* had the highest growth rate in the JG medium (in-house formulation), with a competitive average specific growth rate of 0.47 d^{-1}. The highest biomass productivity was in the Hase medium, but with relatively low productivity of 53.1 $mg.L^{-1}.d^{-1}$.

Analysis of the biomass lipid content and profile of each cell culture, using fatty acid methyl ester (FAME) gas chromatography (GC) showed that the lipid content varied between cultivation media, with minimum lipid content of 20% (w/w) and an average close to 47% (w/w). Utilising biodiesel property predictive formulas, and the lipid profiles obtained in this study, it was possible to predict the properties of biodiesel that could be generated from *A. bajacalifornicus*. It was found that biomass from eight of the media adhered to South African summer grade biodiesel standards.

As such, *A. bajacalifornicus* is a potential candidate for microalgal biodiesel production in South Africa. However, the bioenergy yield rate would need to be improved to have a similar attractiveness to other studies.

Introduction

Biodiesel has widely been studied as a renewable energy source. However, one of the main challenges of traditional biodiesel is that it competes with local food and agriculture industries for feedstock and arable land. To overcome this, biodiesel derived from lipid-rich microalgal biomass feedstock has the potential to be used without competing with agriculture or food industries.

Microalgae are the focus of third-generation bio-energy research [1] due to their distinct advantages over other biomass sources such as corn, palm seed, sugar cane and sunflowers. This includes not competing with the food industry [2] for

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arable land [3], being able to be cultivated in dams or bioreactors, having its implementation made versatile [4], providing high oil yield, which can be converted to fatty acid methyl esters (biodiesel), and high carbohydrate yields, which can be converted to bioethanol [5,6]. Microalgae also have fast growth rates and can be harvested multiple times a year [5] to produce valuable co-products for the pharmaceutical or cosmetic industry, including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), laminarins, beta-carotene and astaxanthin [7,8]. Additionally, microalgae can utilise atmospheric carbon dioxide [5], be used for bio-remediating wastewater [9–12], integrated into other wastewater treatment [13], or cultivated in saltwater or low-quality fresh water.

While algal biodiesel technology has not matured as fast as was expected, mainly due to several processing difficulties (separating, oil extraction), part of the 'National Development Plan of South Africa 2030' is to exploit cleaner energy sources [14]. Since South Africa is home to many algal species, this could be one way of meeting this goal. Finding and characterising a suitable indigenous microalgal strain could act as a steppingstone to process development for that strain to contribute to this vision.

Indigenous microalgae have a few benefits: Indigenous microalgae are adapted to South African conditions. Usage of indigenous species eliminates the risk of accidental introduction of an invasive species to the environment. Usage of indigenous micro-algae reduces the intellectual property red-tape associated with usage of non-indigenous characterised microalgal strains. Usage of indigenous microalgae can benefit local communities in South Africa due to Bioprospecting compensation and increase of indigenous knowledge.

To help find such a suitable strain, the Microalgal Culture Collection of South Africa (MiCCSA) and the South African National Phycology Culture Collection (SANPCC) (www.sanpcc.org.za) are available. The MiCCSA is maintained on AF6 agarose medium in duplicate at the Council for Scientific and Industrial Research (CSIR), Pretoria, and the SANPCC is maintained in tris-acetate phosphate medium at the University of Witwatersrand, Johannesburg, South Africa. The culture collection consists of various South African indigenous microalgae isolated from eutrophic zones countrywide [15–17]. From this collection, a culture identified as an *Acutodesmus bajacalifornicus* (WCB 4.1 – MiCCSA numbering), has been shown in our previous studies to have a high growth rate and could be a potential biodiesel source. Species from the *Acutodesmus* family have previously been studied and shown to be able to accumulate high cellular lipids and carbohydrates [18].

Several approaches can be used to improve the output of biomass, lipids and carbohydrates from microalgae production. These approaches include but is not limited to, medium optimisation, illumination optimisation, cultivation optimisation and harvesting optimisation. In the process of scaling up manufacturing it is generally a good approach to start with optimisations which can be tested at lab scale, which can improve results for later pilot-scale optimisation. In the above-mentioned optimisations, cultivation media optimisation is an excellent first step as it can be performed at lab scale and can provide insight into challenges which may arise in future optimisation.

To determine the type of medium which would be suitable for an organism, the biology of the organism must be considered. *Acutodesmus bajacalifornicus* (WCB 4.1) is a chlorophyte and has been isolated from a freshwater source. This indicates that freshwater cultivation media and media developed for chlorophytes and Acutodesmus and Scenedesmus strains should be good options to consider Lu et al. [19,20]). Seven media were identified, which can be used for the cultivation of *Scenedesmus* and *Tetradesmus,* from Andersen Algal Culturing Techniques [21]. The media identified were the AF6 medium, Bold Basal medium (BBM), C Medium, Chu #10 medium, DY-V Medium, Franquil medium and WC medium [21]. An additional three media were identified, the Zarrouk [22,19,20] media, which have been used for the cultivation of chlorophytes in open pond systems [19,20,22].

This research aimed to evaluate the performance of the *Acutodesmus bajacalifornicus* (WCB 4.1) strain in various cultivation media formulations and determine its potential as a source for producing bio-diesel.

Materials and methods

Strain selection and identification

Before starting laboratory work, the algal strain was identified. A monoseptic cell culture of WCB 4.1 was sent to Inqaba Biotech, an accredited genetic sequencing service provider, where a Fungal/Bacterial DNA Kit™ (Zymo Research) for DNA extraction was used. An 18S target region was amplified using primers 18S-AB1 and 18S-TW4, using OneTaq™ DNA polymerase (NEB) [23]. PCR products were extracted (Zymo Research, Zymoclean™ Gel DNA Recovery Kit) and sequenced in the forward and reverse directions on an ABI PRISM™ 3500xl Genetic Analyser. Sequencing data were analyzed using CLC Main Workbench 7 followed by a BLASTN search (NCBI). According to local alignment data, WCB 4.1 had a similarity to *Acutodesmus bajacalifornicus* strain ZA1-7 (GenBank Accession HQ246322.1) of 99%.

Cultivation media preparation

Eleven suitable media were chosen for this study (Table SM1). Seven of these (AF6 medium, Bold Basal medium (BBM), C Medium, Chu #10 medium, DY-V Medium, Franquil medium and WC medium) were from Andersen Algal Culturing Techniques [21], three more as described in the literature Zarrouk [22,19,20]), and a final in-house recipe (JG medium). The JG medium is a derivative of the Hase medium with a sodium and potassium molar ratio set to 4:1 and nitrate and phosphate molar ratio to 16:1 [24].

The media were all prepared, adjusted to pH 7 using 1 N HCl and 1 N NaOH and filter sterilised using 0.45 µm sterile filters.

Shake flask cultivation

Algal cultivation was performed at 150 mL scale in 250 mL Erlenmeyer flasks, initiated with a 10% inoculum with an optical density (OD 675) of 0.2 at a wavelength of 675 nm (200 µL, 96 well, BioTek PowerWave HT spectrometer). The cultures were incubated on a Sylvania GRO-LUX F18W/GRO-78 neon light illuminated orbital shaker, at a maximum light intensity of 1000 Lux, 120 rpm, with a 12 h day-night cycle at 25 °C and pH 7.5. Daily 1 mL samples were taken with TPP 2 mL sterile pipettes. Daily pH adjustments were performed aseptically using sterile 1 N HCl and 1 N NaOH.

From each sample, 200 µL were transferred in triplicate to independent wells in a 96 well plate. The OD 675 readings of the 96-well plate were taken on a BioTek PowerWave HT spectrometer. Natural logarithmic (OD 675) functions were used to determine the specific growth rates of WCB 4.1 in each medium. For all the natural logarithmic (OD) curves, a straight trend line could be seen between the time points of day 1 and 4 indicating exponential growth.

An OD 675 to dry cell weight (DCW) correlation was performed to allow the conversion of OD values to DCW values. Culture to water ratios (v/v) of 16 mL dilutions between 100 and 0% (in 20% increments) were made. From each of the dilutions, 200 µL were transferred to 96-well microtiter plates in triplicate. OD 675 readings were performed using a BioTek PowerWave HT spectrometer with the aid of Gen5 (v1.06) software. Volumes of 5 mL from the dilutions were filtered in triplicate on an XX2702550 Millipore Model 1225 Sampling Manifold Vacuum Filtration Cell Harvester using pre-weighed 25 mm Macherey-Nagel GF2 glass fibre microfilters (~0.4 µm pore size), followed with a 10 mL distilled water wash. The filter papers were dried in a Mettler Toledo HS153 moisture analyser and weighed to determine the biomass DCW present on each filter.

The OD 675 values were converted to biomass ($g.L^{-1}$) concentrations using the OD/DCW correlation and plotted over time (DCW (biomass) = 0.2117 × (OD 675) – 0.0067). To calculate the average biomass productivity of WCB 4.1 in each medium over 17 days, linear trend lines were plotted for each curve. The slopes of these trend lines were indicative of the average specific biomass yield rate ($mg.L^{-1}.d^{-1}$) in each medium. On the 17th day, 16 mL samples were taken, using TPP 25 mL sterile pipettes.

Lipid content analysis

Samples for fatty acid methyl ester gas chromatography (FAME-GC) was prepared using a direct transesterification method [25]. The direct transesterification method is ideal for increasing microalgal biomass lipid extraction efficiency [25]. The GC analysis was performed using a Supelco Omega Wax 320 column (30 m × 0.32 mm × 0.25 µm film thickness, cat # 24152) on an Agilent Technologies 7890A GC system equipped with an Agilent Technologies 7693 Autosampler.

A FAME standard (Supelco 37 Component FAME Mix, 1 × 1 ml, varied concentration in dichloromethane Part Number: CRM47885) was run on the GC system to obtain the area under the peak and substance concentration standard curves for 37 FAMEs. A linear trend line was used to obtain straight line formulas for each correlation. These formulas were used to convert chromatogram peak areas to lipid concentration ($g.L^{-1}$) values. The areas under the peaks were used to determine the lipid contents of WCB 4.1 at day 17 cultivated in the eleven media. The sum of the lipid contents provided the total lipid content of WCB 4.1 in each medium.

Biodiesel properties

The properties of the potential biodiesel can be predicted using the generated FAME-GC lipid profiles. Cetane numbers (CN) can be calculated from various formulas Eqs. (1–3) from literature [26–28], while the CN of individual methyl esters can then calculated using the final equation given Eq. (4) [28].

$$CN = -107.71 + 31.126n - 2.042n^2 + 0.0499n^3 \quad (1)$$

$$CN = 109 - 9.292n + 0.354n^2 \quad (2)$$

$$CN = -21.157 + \left(7.965 - 1.785db + 0.235db^2\right)n - 0.099n^2 \quad (3)$$

Where:

CN = cetane number
n = the number of carbons in a fatty acid
db = the number of double bonds in a fatty acid

The cetane number for a specific fatty acid mixture can be calculated using Eq. (4) [28].

$$CN = 1.068 \sum (CN_i x_i) - 6.747 \quad (4)$$

Where:

CN_i = the CN for a specific methyl ester
x_i = the mass fraction of a specific methyl ester

To obtain the cold filter plug point (CFPP) Eq. (6) [39], the cloud point (CP) is required Eq. (5) [28,29]. The melting temperature (T_{mi}) and enthalpy of melting (ΔH_{mi}) values were sourced from [38] or predicted according to [40] (Table SM2).

$$\ln(x_i) = \frac{\Delta H_{mi}}{R}\left(\frac{1}{T_{mi}} - \frac{1}{CP}\right) \quad (5)$$

Where:

CP = the cloud point (°C)
R = the universal gas constant (8.31 $J.mol^{-1}.K^{-1}$)
T_{mi} = the melting temperature of individual methyl esters (°C)
ΔH_{mi} = the enthalpy of melting of individual methyl esters ($J.mol^{-1}$)
x_i = the mass fraction of individual methyl esters

$$CFPP = 1.0191 \times CP - 2.9 \quad (6)$$

Where:

CFPP = Cold filter plug point (°C)

According to the thermodynamic prediction of CP, the individual methyl esters are assumed to precipitate out independently [29]. This means the highest CP value for an individual methyl ester is the CP of the mixture.

Results and Discussion

Cultivation

Microalgal growth data over 17 days were obtained using all eleven media in the form of OD readings. An OD/DCW correlation curve was used to convert OD values to biomass values. Plotting the biomass over time (Fig. 1) indicated that WCB 4.1 had the fastest growth in the JG and Hase media.

WCB 4.1 had the highest growth rate in the JG medium followed by the Hase and Lu media with growth rates of 0.47-, 0.42- and 0.31 d^{-1} respectively (Fig. SM1). The growth rate of WCB 4.1 in the JG medium was 14.8% higher than in the Hase medium and 33.7% higher than in the Lu medium. WCB 4.1 had the lowest growth rate in the BBM and Zarrouk media with growth rates of 0.12- and 0.14 d^{-1} respectively. The growth rates of WCB 4.1 in the AF6, C-medium, Chu # 10, DYV, Franquil and WC media ranged from 0.18- up to 0.26 d^{-1}. According to the growth rates, the JG and Hase media would be good media for cultivating WCB 4.1 for optimal biomass generation.

The determined biomass yield rates for WCB 4.1 in eleven different media is displayed in Fig. SM2. The Hase and JG media had the highest biomass yield rates at 53.1- and 51.1 $mg.L^{-1}.d^{-1}$ respectively. The Franquil and Zarrouk media showed the lowest biomass yield rate at 7.8 $mg.L^{-1}.d^{-1}$ each.

The JG medium provided the fastest growth rate at 0.47 d^{-1} indicating faster growth rates than microalgal growth rates sourced from other sources such as 0.34 d^{-1} for *Neochloris oleabundans*, 0.38 d^{-1} for *Chlorella vulgaris* and 0.41 d^{-1} for *Nannochloropsis sp*. [30–32] Fig. 2. This demonstrates the potential of WCB 4.1 to compete with other industrially significant microalgal strains. There are some microalgal growth rates such as 0.87 d^{-1} for *Chaetoceros sp*., 0.97 d^{-1} for *Isochrysis sp.* and 1.11 d^{-1} *Scenedesmus sp.* [33,34]) which showed higher growth rates than WCB 4.1 in JG medium. Although this indicates that microalgae are outcompeting WCB 4.1, it also indicates possible room for improvement of WCB 4.1 growth rates.

Microalgal biomass yield rates determined by other authors range from 120 $mg.L^{-1}.d^{-1}$ up to 783 $mg.L^{-1}.d^{-1}$ Fig. 2. Compared to these biomass yield rates, the biomass yield rate of WCB 4.1 is quite low.

It is important to consider both the growth rate and the biomass yield rate. The growth rate is measured during the exponential growth phase of the organism and is indicative of the growth potential if exponential growth can be maintained. The biomass yield rate is indicative of the rate at which biomass is generated within a certain time frame. The biomass yield rate can vary depending on the time point at which the yield rate is calculated. The growth rate and the biomass yield rate can shed light on the strong and weak points of a cultivation.

The *Neochloris oleabundans, Dunaliella* sp. and *Botryococcus braunii* growth rates (0.47, 0.34 and 0.30 d^{-1}) and biomass yield rates (227, 231 and 271 mg/L/d) in Fig. 2 are from the work of Choi and Lee [30]. This is interesting given that the top growth rate of WCB 4.1, as 0.47 d^{-1} in JG medium, are superior to that of *Neochloris oleabundans, Dunaliella* sp. and *Botryococcus braunii*, while the biomass yield rate of WCB 4.1 is inferior to the same cultures [30]. This indicates that the low biomass yield rate is not due to the low growth rate. This means that the biomass yield rate can be improved using measures to keep the culture in exponential growth for a longer duration. The biomass yield rate can thus be improved by improving the production process through measures such as feeding, continuous or semi-continuous cultivation, improved illumination, and optimising harvesting times.

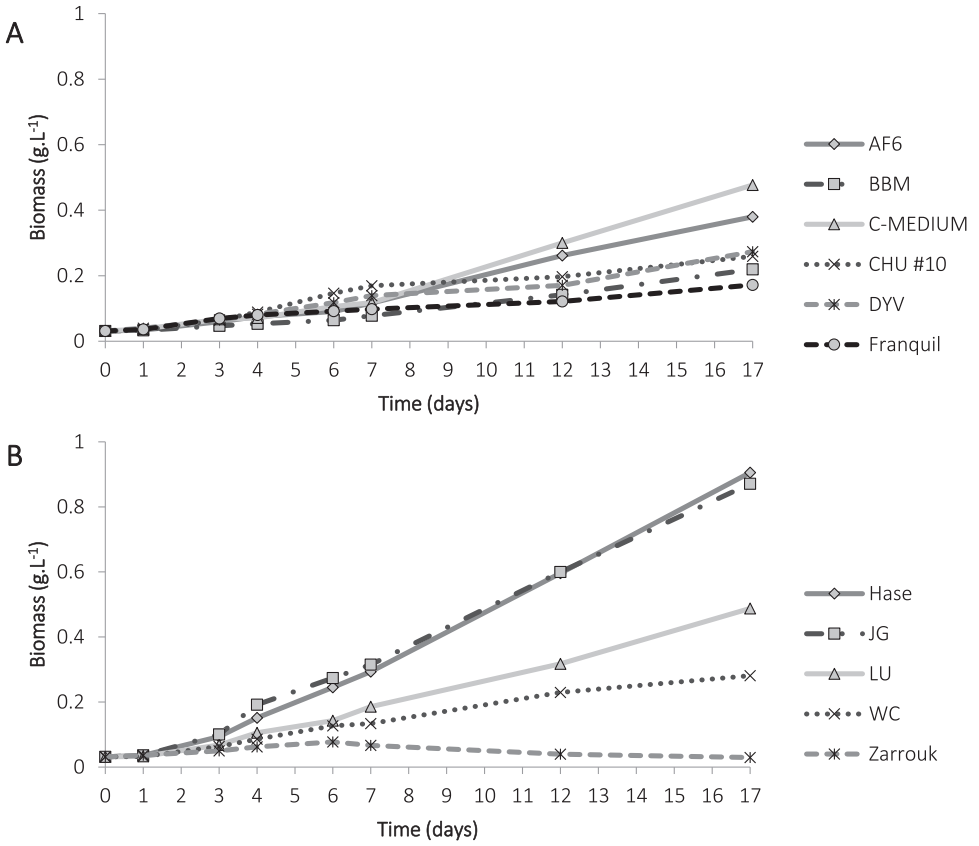

Fig. 1. Biomass over time for the cultivation of WCB 4.1 in six different media (A) and five different media (B) over 17 days.

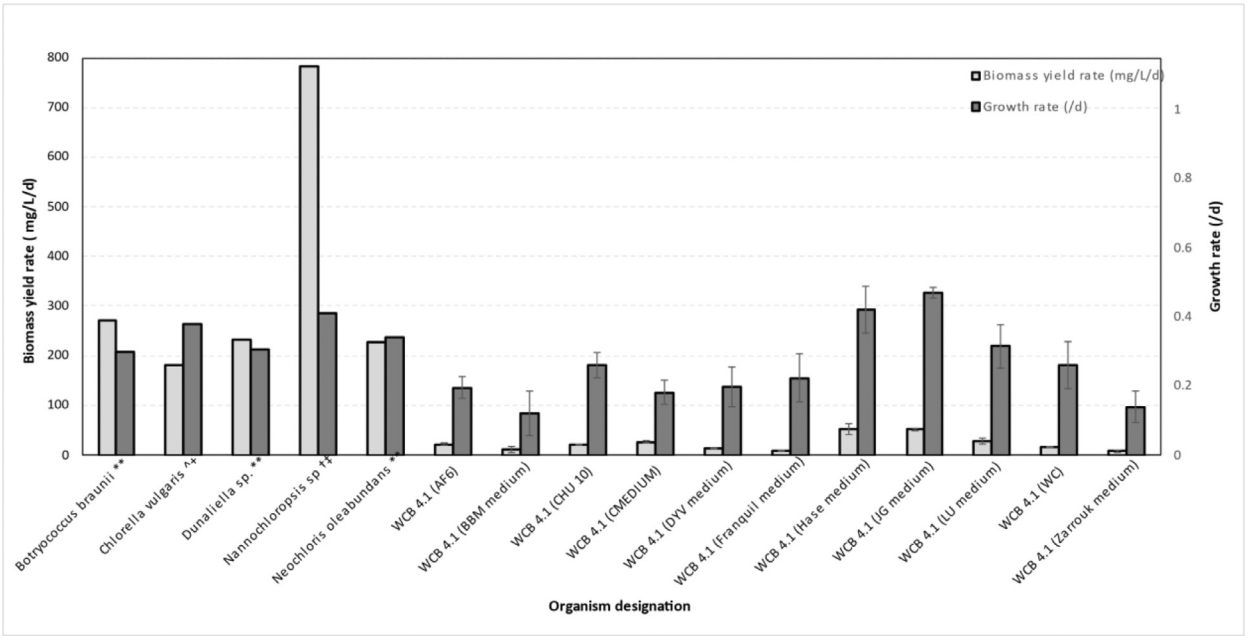

Fig. 2. Comparison between biomass yield and microalgal growth rates from literature [31]$^{+}$,[30]*,[32]‡,[36]† and [37]$^{\wedge}$) *vs.* the current study.

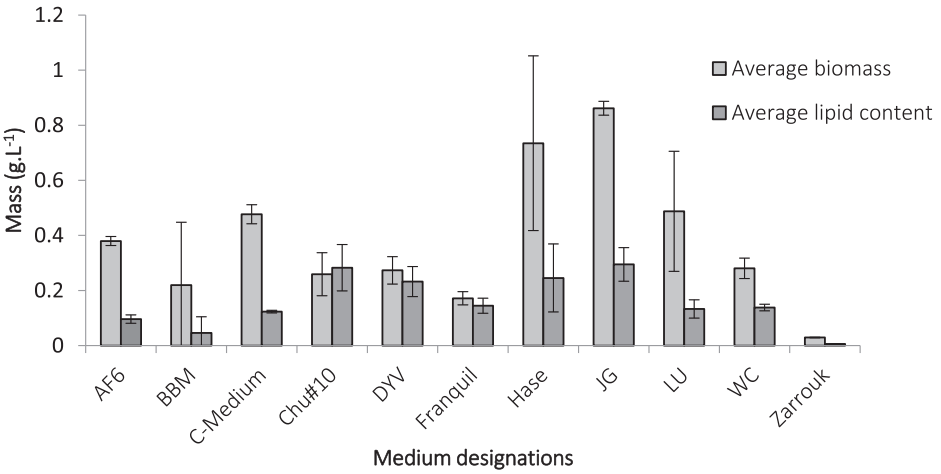

Fig. 3. Medium specific lipid and biomass yield of WCB 4.1 after 17 days of cultivation.

Lipid content analysis

The lipid contents of WCB 4.1 from all eleven media can be seen in Fig. 3. The JG, Chu#10, Hase and DYV media provided the highest lipid yields in descending order.

The JG and Hase media are by far the highest biomass yielding media. The Chu#10 and DYV are not within the top five biomass producing media. This raises the question as to how such low biomass yielding media can be yielding such high lipid contents. This can be related to which life stages WCB 4.1 is in each medium at day 17 and the cellular lipid percentage. Considering lipid yield in relation to biomass yield, showed that WCB 4.1 had a very high cellular lipid content (> 80% w/w) in the Chu#10, DYV and Franquil media Fig. 3. In most of the other media, WCB 4.1 has cellular lipid contents percentages ranging between 20- and 50%. The high degree of lipid content variation corresponds to data reported by [44], wherein variations as drastic as 16.7 – 71% lipid content was described for a single strain. It was noted that the lipid content of Chu#10 found here was higher than the average biomass value, possibly a statistical issue around the error.

[41,42] demonstrated that nitrogen limitation can lead to lipid content increase in *Acutodesmus dimorphus*, close family to *Acutodesmus bajacalifornicus*. The high lipid content in Chu#10, DYV and Franquil can be accounted for due to nutrient stress. The most important nutrient stress resulting in increased lipid stores is nitrogen stress. Considering the 11 medium compositions, the Chu#10, DYV and Franquil media have the lowest nitrogen contents. Having the lowest nitrogen contents will result in the earliest nitrogen depletion in these media. The WC medium has the fourth-highest cellular lipid percentage after the Chu#10, DYV and Franquil media. WC medium also has the fourth-lowest nitrogen content at 1 mM. WCB 4.1 in the WC medium started to show lipid accumulation due to nitrogen depletion. The AF6 medium with a nitrogen content of 1.64 mM however did not show signs of increased lipid accumulation. Most healthy cultures in media, with nitrogen concentrations of 1.64 mM and higher, had higher growth rates compared to nitrogen-limited media such as the Chu#10, DYV, Franquil and WC media.

A trade-off in WCB 4.1 cultures can be seen between a fast growth rate and high cellular lipid content due to nitrogen abundance or limitation respectively. This corresponds with results from Cobos *et al*., (2017), where an *Acutodesmus obliquus* displayed a similar trade-off where high nitrogen content allowed fast growth rates, where nitrogen limitation led to slower growth rates but higher cellular lipid percentages. One of the reasons for this might be "*the temporal and metabolic overlap between lipid accumulation and programmed cell death due to nitrogen starvation*" [35]. This displays why the balance of nitrogen is so important in microalgal culture.

Biodiesel production potential

During FAME-GC the lipid contents are measured in the form of fatty acid methyl esters which is also referred to as biodiesel. The FAME-GC results are thus indicative of the biodiesel yield after 17 days. The yield after 17 days can be used to provide specific biodiesel productivity of WCB 4.1in each medium. The determined biodiesel yield and yield rate of WCB 4.1 in each medium can be seen in Table SM3.

[43] demonstrated microalgal biodiesel yield rates ranging from 22.6- up to 204.9 $mg.L^{-1}.d^{-1}$ (Table 1). Comparing this data with the maximum yield rate of WCB 4.1 at 17.4 $mg.L^{-1}.d^{-1}$ shows that the biodiesel yield rate of WCB 4.1 is low in comparison with other sources. Low lipid yield rates can be caused due to low biomass yield rates or low cellular lipid contents. WCB 4.1 cultivated in JG medium had a growth rate of 0.47 d^{-1}, which is higher than nine of the growth rates determined by [43]. WCB 4.1 cultivated in JG medium had a cellular lipid content of ~34% at harvest, which is higher than 10 of the cellular lipid contents determined by [43]. If WCB 4.1 has a growth rate and cellular lipid percentage competitive

Table 1
Specie specific lipid yield rates with accompanying growth rates, biomass yield rates and cellular lipid contents as determined by [43].

Species	Growth rate (d^{-1})	Biomass yield rate ($mg.L^{-1}.d^{-1}$)	Lipid yield rate ($mg.L^{-1}.d^{-1}$)	Cellular lipid content (%)
Ankistrodesmus falcatus	0.57	340	56.07	16.49 ± 0.44
Ankistrodesmus fusiformis	0.39	240	49.58	20.66 ± 2.07
Kirchneriella lunaris	0.25	140	24.22	17.30 ± 1.12
Chlamydomonas sp.	0.3	240	36.17	15.07 ± 0.95
Chlamydocapsa bacillus	0.75	320	43.26	13.52 ± 0.65
Coelastrum microporum	0.13	110	22.61	20.55 ± 0.99
Desmodesmus brasiliensis	0.28	130	23.39	17.99 ± 0.42
Scenedesmus obliquus	0.21	160	26.77	16.73 ± 1.37
Pseudokirchneriella subcapitata	0.27	80	22.74	28.43 ± 5.40
Chlorella vulgaris	0.53	730	204.91	28.07 ± 4.31
Botryococcus braunii	0.14	250	112.43	44.97 ± 4.00
Botryococcus terribilis	0.13	200	98.00	49.00 ± 1.48
WCB 4.1 (JG medium)	0.47	51.1	17.35	34.29 ± 6.53

with that of the strains mentioned by [43], the main cause for low lipid yield rate is due to low biomass yield rate. The low biomass yield rate is also not due to a low growth rate of WCB 4.1. This indicates that improvement in process design can play a huge role in improving biomass and lipid yield rates. Process optimisations, which might greatly increase yield rates, would include optimising the harvest time and keeping the culture in exponential growth for longer. Due to yield rates being a function of time, reducing the harvesting time can often lead to an increase in yield rate. Keeping the culture growing exponentially for longer would allow the biomass to increase more rapidly and instantly increase biomass yield rates. Fed-batch and continuous cultivation can be used to keep a culture in an exponential phase for longer.

The competitive growth rates and the cellular lipid contents of WCB 4.1 demonstrate its potential to be an industrially competitive isolate.

Biodiesel Quality prediction

Using the FAME-GC lipid profiles generated for WCB 4.1 for each medium, the biodiesel properties were predicted using Eqs. (1–4). In Table SM4 the predicted CN, CP and CFPP values for biodiesel produced from each medium can be seen. For biodiesel to adhere to South African biodiesel standards, the CN should be 51 or higher. The predicted biodiesel CN generated from WCB 4.1 biomass cultivated in BBM (CN = 60.7), C-medium (CN = 54.5), DYV medium (CN = 54.5), Franquil medium(CN = 54.4), Hase medium (CN = 51.1), JG medium (CN = 53.5), Lu medium (CN = 51.6), WC medium (CN = 53.5) and Zarrouk medium (CN = 73.8) adheres to South African biodiesel standards. The predicted biodiesel CN generated from WCB 4.1 biomass cultivated in AF6 medium (CN = 48.6) and Chu # 10 medium (CN = 49.6) does not adhere to South African biodiesel standards. For the CFPP of biodiesel to adhere to South African summer grade biodiesel standards, the CFPP should be lower than 3 °C. WCB 4.1 cultivated in all eleven media (CFPP of 0.4 °C – 2.5 °C) except for the Zarrouk medium (CFPP = 5.4 °C) is predicted to generate biodiesel with CFPP values in adherence to South African summer grade standards. Biodiesel predicted to be generated from WCB 4.1 biomass in the Zarrouk medium have been predicted to have a CFPP of 5.4 °C, which is too high to adhere to South African biodiesel standards. The predictions for the top-performing media, the Hase and JG media, provided excellent fuel properties with CN values of 51.1 and 53.5 respectively and CFPP values of 1.3 °C and 1.9 °C respectively.

The predicted biodiesel properties for WCB 4.1 mainly adhere to South African biodiesel standards. This further demonstrates the feasibility of using WCB 4.1 for biodiesel production.

WCB 4.1 shows great potential for use in biodiesel production with bioethanol as a secondary product. The productivity of both biodiesel and bioethanol would however need to be greatly increased to compete with productivities described by other sources.

Several options could be considered in future research, which may improve productivity. Media could be further optimised to find the ideal nitrogen and phosphor medium contents for the cultivation of WCB 4.1. This would require cultivating WCB 4.1 in varying conditions of nitrogen or phosphor with all other medium components kept constant. Fed-batch cultivation could be investigated, including fed-batch process development for the cultivation of WCB 4.1, process optimisation and yield rate analysis of biomass, carbohydrates, and lipids. Continuous cultivation could be investigated in a similar way to fed-batch cultivation, exploring continuous cultivation of WCB 4.1 and looking at continuous cultivation process development, process optimisation and yield rate analysis of biomass, carbohydrates, and lipids. Due to yield rate being a factor of time, harvesting time plays a huge role in increasing the yield rate of cultivation. The ideal harvesting time can increase the yield of the batch, fed-batch and continuous cultivations and should be investigated. Carbon dioxide plays a huge role and its inclusion in the feed can increase the growth rate of microalgal cultures. This would open a new avenue for further process optimisation. Different carbon dioxide feeding regimes could be explored and different carbon sources such as gaseous carbon dioxide and carbonate salts explored. The optimisation of lighting not only includes finding the ideal lighting intensity and regime but would also require identifying different reactor designs to optimise light utilisation and improving areal yield.

Conclusion

Acutodesmus bajacalifornicus (WCB 4.1) was cultivated on eleven different cultivation media. The highest growth rate was in the in-house JG medium, with a competitive growth rate of 0.47 d^{-1}. The highest biomass productivity was in the Hase medium, with a productivity of 53.1 $mg.L^{-1}.d^{-1}$, which is meagre to other literature biomass productivities such as 160 $mg.L^{-1}.d^{-1}$ as described by [43] in *Scenedesmus obliquus*.

While WCB 4.1 showed potential for use as a bioenergy source, biomass productivity alone does not provide the full picture, and lipid productivity should also be taken into account. In WCB 4.1 it was seen that Chu#10, DYV, Hase and JG media resulted in the highest lipid productivities. Furthermore, it was possible to predict the biodiesel properties of biodiesel generated from WCB 4.1 biomass, with the WCB 4.1 biomass from the BBM, C-medium, DYV, Franquil, Hase, JG, Lu and WC media adhering to South African summer grade biodiesel standards. In closing, WCB 4.1 showed potential to become a commercially competitive strain for bioenergy production, provided further process development and improvements could be made on an industrial scale.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2021.e00952.

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