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Enhancing Low-Carbon Wastewaters with Flue Gas for the Optimal Cultivation of *Desmodesmus multivariabilis*

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Phycovalorization of wastewater into algal biomass and extracellular polysaccharides (EPS) is an attractive alternative for producing raw materials for biofuels. Industrial flue gas has also been demonstrated as a potential substrate for growing microalgae. Combining wastewater (WW) and flue gas in a single process would provide an attractive process for valorisation of both waste-streams. This study investigated enhancing the potential of low carbon wastewaters with flue gas and mapping the interaction between the organic carbon present in the wastewater and the carbon dioxide introduced with the flue gas, with the aim of achieving optimal biomass, fatty acid methyl esters (FAME) and EPS yields.
Desmodesmus multivariabilis, isolated from a local eutrophic reservoir in South Africa was used in the study. Wastewater media from a local wastewater treatment works and synthesized flue gas (13 % CO_2) were used as feed. Experiments were performed in triplicate. The ratio of organic: inorganic carbon was manipulated by varying the CO_2 concentration. In the control experiment, BBM media with no organic carbon was used instead of wastewater. CO_2 uptake was calculated by means of performing a carbon balance.
The experimental results indicated a point of optimal efficiency where all production parameters (Biomass Production, EPS Production, Nitrogen Uptake) were at maximum value, and the input ratio was at minimum value (Input ratio = mg TOC_0/mg CO_2 uptake). EPS production only occurred at low input ratios and organic substrate utilization conversely only occurred at high input ratios, indicating a switch between autotrophic and mixotrophic metabolism. The results evidenced an association between higher productivity and autotrophic metabolism.

1. Introduction

Microalgal cultures are well known for being able to grow mixotrophically, and it has been demonstrated that CO_2 addition to microalgal cultures cultivated in wastewater substrate has the potential to stimulate COD (chemical oxygen demand) uptake, with some of the authors finding optimal growth at 4 % CO_2 when supplemented with air (Cabanelas et al., 2013). Successful integration of the integrated microalgal biorefinery with wastewater and combustion gas treatment will represent a significant step in the direction of making the technology both economically feasible and competitive in the long term (Zhu et al., 2014). Flue gases from industrial processes generally have a CO_2 concentration that varies between 3 and 25 % and can be diluted with air to provide a suitable source of inorganic carbon (Thomas et al., 2016) for the supplementation. Lipid accumulation in microalgae has generally been shown to increase under conditions of nutrient depletion; therefore, there is significant research potential to determine the optimal growth conditions for maximising the commercial productivity and yield of the most promising strains (Griffiths et al., 2012, 2014). The main aim of the research was to investigate the enhancement of low-carbon domestic wastewater treatment with the addition of flue gas (using simulated flue gas with a composition typical of that of cleaned coal flue gas) and explore the biomass yield and the fatty acid methyl ester (FAME) distribution and yield. The experiments examined the impact on wastewater quality, FAME yield, growth rate and biomass yield when the cultivation of microalgae in low-carbon domestic wastewater was supplemented with gaseous carbon dioxide in the form of simulated flue gas. The concentration of flue gas and the subsequent ratio of organic to inorganic carbon was varied by diluting the flue gas with air to obtain experiments with CO_2 concentrations at 5 %, 2.5 % and 0 %

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respectively. In the control experiment, the wastewater was substituted with 3N-BBM+V (Bold's Basal Medium with threefold nitrogen and vitamins).

2. Methods and Materials

2.1 Algal culture preparation

The microalgal culture was derived from cultures isolated from the Hartbeespoort Dam reservoir and identified as *Desmodesmus multivariabilis* using 18s rRNA and ITS sequencing (Lubbe & Brink, 2019). Algal starter cultures were cultivated in a Triple Nitrogen, Bold Basal Medium with Vitamins (3N-BBM+V) to a cell density of 200 - 450 mg/l before inoculation (Roestorff & Chirwa, 2018).

2.2 Wastewater media

WW media was collected from the feed of an activated sludge WW treatment works on a private housing estate and it underwent primary sedimentation and flow reduction (by means of a septic tank and French drain system). The media was autoclaved at 121 °C and 2 bar for 20 min and stored at 4 °C to slow the rate of bio-degradation. Total Carbon (TC) and Total Organic Carbon (TOC) analysis (Shimadzu TOC-Vwp), Total Nitrogen (TN) analysis (Spectroquant 14763/14537 kit with Spectroquant Nova 60), and PO_4^{3-} analysis (Hanna Instruments HI 93717) were performed to characterize the effluent prior to usage.

2.3 Experimental setup

Algal cultures were all cultivated in 500 ml culture flasks as shown in Figure 1. Flasks were equipped with specially designed airlocks equipped with a filter which allowed the throughput of air but reduced contact with airborne contaminants. Flasks were mounted on Velp Am4 magnetic stirrers with stirrer setting at 6.
Cultivation was performed at 25-28 °C at the required lighting conditions (Osram L 36W/77 Floura x 2) (Roestorff & Chirwa, 2018). The light/dark cycle was adjusted to replicate peak summer lighting conditions in Pretoria, South Africa (14 h light/10 h dark).

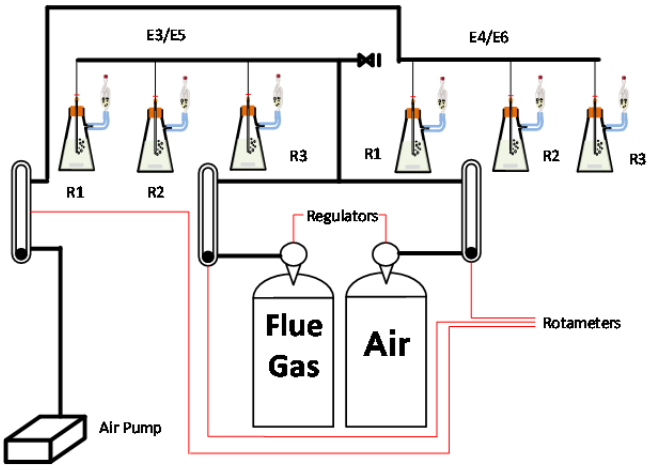

Figure 1 : Setup of the gas supply of Experiments 3–6

The ~13 % CO_2 synthetic flue gas was diluted to different values for comparison as shown in Table 1.

Table 1: Description of the CO_2 enhanced experiments

Experiment	Repeats	% CO_2 by volume	Media
Exp3	3	5	WW
Exp4[1]	3	0	BBM
Exp5	3	2.5	WW
Exp6[1]	3	0	WW

[1]In Experiments 4 and 6 unenriched atmospheric air was supplied to the reactor containing 410 ppm CO_2

2.4 Sampling and analysis

Sampling of the media was conducted on a daily basis in in 10 ml centrifuge tubes. Biomass was separated by centrifuging at 6500 rpm for 10 min (Hettich Universal 320R Centrifuge). The biomass was dried at 50 °C for 24 h before being weighed (Mettler-Toledo XS205 Dual Range). Samples were filtered using Whatman No. 1 filter paper and diluted to a ratio of 1:30 with de-ionized, distilled water prior to TOC analysis. The experiments were conducted until the growth rate decreased and cultures exhibited characteristics of maintenance growth, i.e. metabolically active yet non-reproducing.
At the termination of each experiment, the growth and mixed liquor were harvested, and the cellular material separated by means of centrifuging at 6500 RPM for 10 min in 50 ml tubes. The cell-free media was analyzed for TOC, TN, pH and PO_4^{3-} concentration. Transesterification was performed on the algal mass according to the method of Indarti et al., (2005) and a measure of the FAME constituents was obtained via GC-MS (Perkin-Elmer Clarus 600), using the analysis method described by Li (2012). Table 2 refers to the equations implemented for analysis.

Table 2: Metrics and equations for the CO_2 experiments

Description	Parameter	Unit	Equation/Definition	
Carbon dioxide uptake efficiency		mg/mg	$\frac{\text{Total mass of } CO_2 \text{ uptake by cells}}{\text{Total mass of } CO_2 \text{ fed to reactor}}$	(1)
Effective input ratio		mg/mg	$\frac{TOC_0}{\text{Total mass of } CO_2 \text{ uptake by cells}}$	(2)
N utilised	$\%N$	%	$\%N = \frac{N_f - N_0}{N_0} \cdot 100$	(3)
P utilised	$\%P$	%	$\%P = \frac{P_f - P_0}{P_0} \cdot 100$	(4)
Parameters	TOC_0	mg/l	Initial concentrations of organics in the media	
	N_f, N_0		Final and initial total nitrogen concentrations in the media, respectively	
	P_f, P_0		Final and initial total phosphate concentrations in the media, respectively	

3. Results and Discussions

3.1 Algal growth & Nutrient uptake

Experiment 3 showed the most rapid growth and also reached the stationary phase in the shortest time. The measured cell density upon termination in Experiment 5, which reached the stationary phase after around five days, was nearly identical to that in Experiment 3, indicating an equivalent yield of cells at a slower rate of growth (see Figure 2).

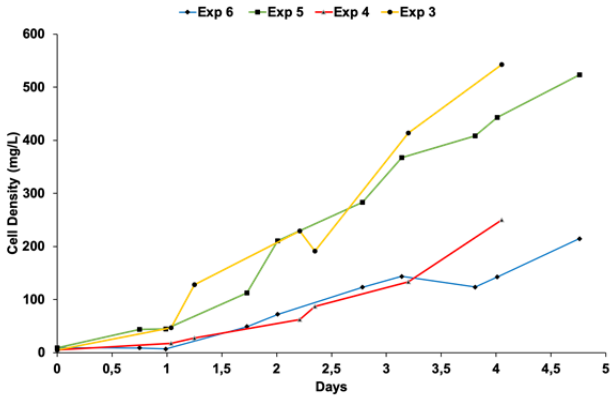

Figure 2: Cell density growth curve for Experiments 3 – 6

Table 3: Biomass yield and final nutrient analysis for Experiments 3 – 6. The values reported indicate the mean ± the standard deviation of the triplicate runs.

Exp	Total N (mg/l)	% N Utilized	Total PO_4^{3-} (mg/l)	% PO_4^{3-} Utilized	pH Final	Total Biomass Harvested	Biomass Harvested (mg Biomass)	Specific Biomass Yield (mg/mg)
Exp3	0.5 ± 0.3	99 ± 0.5	0.88 ± 0.98	92 ± 8.9	6.9 ± 0.05	353 ± 45.3	341 ± 42.9	89.5 ± 1.58
Exp4	143 ± 6.08	5.2 ± 4.1	>32.9	-	7.0 ± 0.09	24.7 ± 7.31	18.6 ± 11.2	32.5 ± 10.8
Exp5	4.2 ± 0.55	89 ± 1.4	4.7 ± 2.4	62 ± 19	6.5 ± 0.08	274 ± 28.4	243 ± 29.1	42.2 ± 6.50
Exp6	4.5 ± 0.62	88 ± 2.1	0.033 ± 0.058	100 ± 0.48	10.4 ± 0.229	128 ± 5.29	114 ± 4.73	16.6 ± 1.52

In Experiments 3 and 5 (WW and CO_2 at 5 % and 2.5 % respectively), the high rate of CO_2 replenishment effectively stabilised the water at a pH of 6.5 – 6.9, which is in the optimal range for algal growth. In Experiment 6, which showed the most significant disparity in pH, the carbonate equilibrium shifted as a result of the mass transfer of CO_2 into the solution being the rate-limiting step. This was not because of the aeration rate being a limiting factor, but was a result of the low concentration of CO_2 inherently present in atmospheric air (Lubbe & Brink, 2019), This led to some of the dissolved inorganic carbon present in the water being utilized before equilibrium was re-established, increasing the pH (seeTable 3).

The metabolic processes that relate to the release of organics appear to be independent of cellular growth in the growth phase the release of organics appeared to be mainly a mechanism occurring during the latency phase of the growth of the organism. There may be competing metabolic mechanisms at play, where the experiments with high carbon input ratios did not use only the EPS that was produced, but also the organics present in the wastewater as a substrate. This indicates a higher prevalence of mixotrophic metabolism, where both organic and inorganic substrates were preferred. The specific growth rate during this metabolism is lower, possibly indicating that the organism is unable to derive the same equivalent of energy from this metabolic pathway.

The nitrogen utilization for all the wastewater experiments, with the exception of was greater than 88 %. The phosphate utilization was directly proportional to a decrease in FAME yield, showing a high correlation coefficient. As the maximum FAME yield occurred at the point where mixotrophic metabolism was dominant, and as phosphorous uptake is related to the production of ATP (Cabanelas et al., 2013), this lends further credit to the idea that this is a lower energy pathway.

3.2 Carbon dioxide Supply and Uptake

From Figure 3, the mass of organics released initially in experiment 3 was much higher than for the other experiments. The initial mass of organics present in the WW was approximately 43.5 mg, and by the end of the lag phase, the organics had increased by over 534 %. Thereafter, the mass of organics slowly decreased over the course of the experiment, presumably as the extracellular organics initially formed were utilized in cellular growth. TOC loading comparison for the experiments with 0 % and 2.5 % CO2 and WW the initial increases in organics were much lower, being 69 % and 33 % respectively.

In Experiment 5, there was a similar decrease, but between day 3 and day 4 there had been a sudden increase in the concentration of organics. This corresponded to a possible cell lysis event, as the density of cells in the experiment decreased between those days (see Figure 2). Possibly triggered by this stress factor (Lubbe & Brink, 2019) showed that the alga secreted organics in response to stress), the alga started producing extracellular organics again between days 4 and 5. The cause of the possible cell lysis event is not known.

As the initial loading of organics was by design 0 mg/l, this possibly relates to the switching of metabolism of the algae as displayed in Lubbe & Brink (2019), where the algae, under conditions of stress, would release a large concentration of organics, possibly to manipulate growing conditions. The rate of organics released increased markedly after the third day.

As air enriched with CO_2 was continually supplied in Experiments 3 and 5 and the rate of uptake was limited only by the rate of utilization of the organism and thus the CO_2 and media were not in equilibrium. A conjecture can then be made that the effective concentration of CO_2 encountered by the organism was the concentration in the headspace and was thus not limited by Henry's law. This, however, was not true of Experiments 4 and 6. In Experiment 6, which was not buffered with mono- and dipotassium phosphate like Experiment 4, a pH shift could clearly be seen.

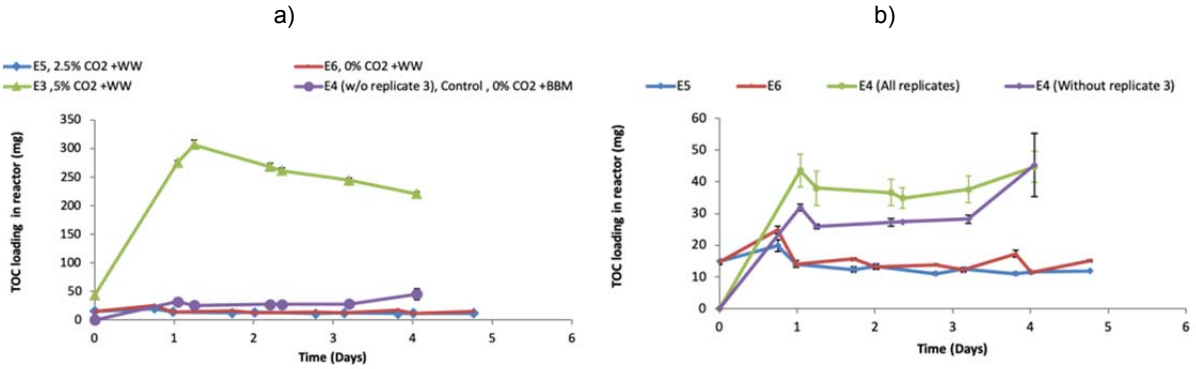

Figure 3: TOC loading comparison of a) Experiments 3 – 6 and b) Experiments 4 – 6. Experiment 4 with and without replicate 3 are shown to demonstrate the significant deviation caused by experiment 4 replicate 3.

3.3 FAME production & Analysis

From Figure 4, it can be seen that the experiment with 2.5 % CO_2 (Experiment 5) had the lowest variety of components, and the control (Experiment 4) had the highest.

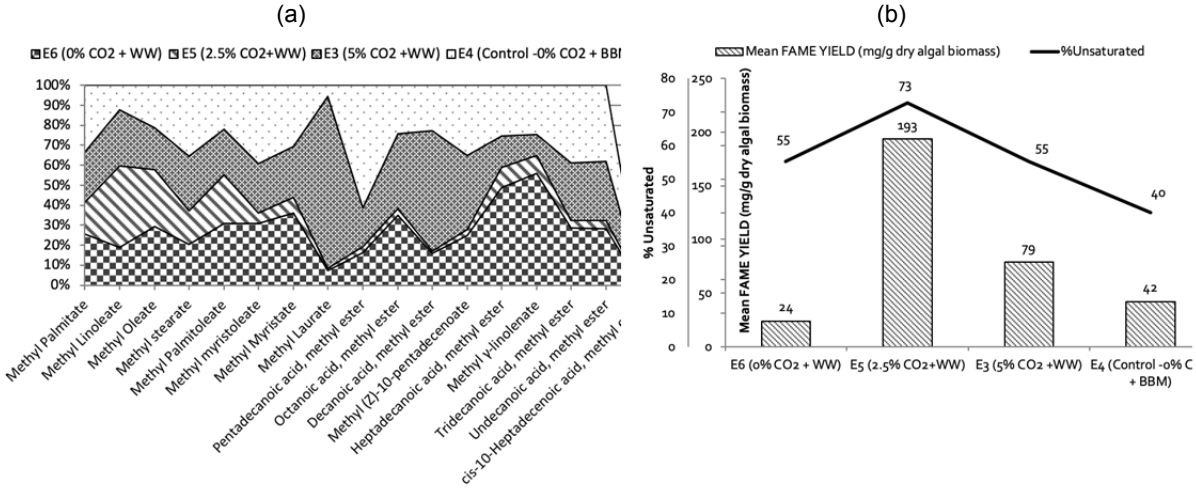

Figure 4: a) Overall FAME yields and b) Component distribution for the experiments

The high selectivity, combined with the high yield is an extremely favorable characteristic for commercial production as it ensures an easier separation of the different FAME components. According to Hoekman et al. (2012), the degree of unsaturation of an algal biofuel is positively correlated with the volumetric higher heating value (HHV) of the biodiesel, therefore the total biodiesel energy yield of the algae at 2.5 % CO_2 in wastewater will be much higher than the other combinations of % CO_2/WW if the biomass harvested is considered. However, the much higher degree of unsaturation may also present a problem with oxidative stability (Hoekman et al., 2012) as the biodiesel contains a high percentage of methyl linoleate, which is polyunsaturated. However, this can be offset through separation, blending and stabilizing with oxidizers (Hoekman et al., 2012).

3.4 The optimal point of operation

For the combination of wastewater and simulated flue gas for the production of FAMEs by *D. multivariabilis*, a 0.47 mg/mg input ratio at 2.5 % CO_2 was determined as the optimal point. It resulted in the highest FAME yield, the highest FAME selectivity, the highest FAME production, the highest proportion of unsaturated FAMEs, the highest uptake of CO_2 and Nitrogen. At this point, it also showed the greatest inclination towards mixotrophic metabolism and the least inclination towards autotrophic metabolism

4. Conclusion

It was determined that wastewater and flue gas phycovalorization could be successfully integrated into a single treatment process with *D. multivariabilis*. The optimal point of operation point was demonstrated to be near 0.47 mg TOC_0 to CO_2 utilized.

At the optimal point, FAME yield, selectivity and production are at their maximum value for any CO_2/WW combination. It was determined that a mixotrophic metabolism was dominant at this point, which meant that the organism utilized the lowest proportion of carbon dioxide in proportion to the quantity fed. The experiments that showed a preference for mixotrophic growth also had higher effective input ratios and utilized the organics present in the wastewater as substrate. The results also indicated that during mixotrophic metabolism, D. *multivariabilis* followed a lower-energy metabolic pathway as the overall specific growth rates observed were comparatively lower than in the predominantly autotrophic pathways.

With regard to growth and organics yield, it was demonstrated that *D. multivariabilis* followed a characteristic growth pattern whereby organics were released at the end of the latency phase, possibly in preparation for exponential growth.

When the valorization of the nutrients in the wastewater is considered, the percentage of nitrogen reduction was high for all the experiments with wastewater and gas, being greater than 88 %, but the percentage of phosphorous reduction showed a strong correlation with decreasing FAME yield, indicating that the organism took up less phosphorous (which is used in the production of ATP) from the wastewater as FAME production increased. This is a further indication of a decrease in metabolic activity associated with FAME production.

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