Supplementary Information

Continuous flow biodiesel production from wet microalgae using a hybrid thin film microfluidic platform

Eko Kornelius Sitepu, Darryl B. Jones, Youhong Tang, Sophie C. Leterm, Kirsten Heimann, Wei Zhang *, Colin L. Raston*

1. Fluid dynamics within the T^{2}FD

The fluid dynamics within the T^{2}FD is highly complex. In the T^{2}FD device, the rotating blade sections of the rotor introduce Couette flow behaviour (a flow between a stationary and a moving plate) into the fluid. A Couette flow characteristically introduces shear stress within the fluid. In addition, the voids in the rotor create an air-fluid phase boundary which may introduce multi-phase flow characteristics. This phase boundary can further introduce gas into the fluid within the Couette flow regions. It is expected that the multi-phase flow can influence the films surface tension and evaporation of the film, with the Couette flow behaviour likely to dominate the fluid behaviour, which is discussed in further detail below.

In a Couette flow between two parallel plates (one stationary and one moving), the shear stress (τ) can be expressed through (eq. 1):

$$\tau = \gamma_{Av}\mu. \qquad \text{(eq. 1)}$$

where μ is the dynamic viscosity of the fluid and γ_{Av} the average shear rate. This average shear rate within the fluid relates to the moving plate's velocity, v, over the distance over which it acts, i.e the gap between the plates, d_s, (eq. 2)

$$\gamma_{Av} = \frac{v}{d_s}. \qquad \text{(eq. 2)}$$

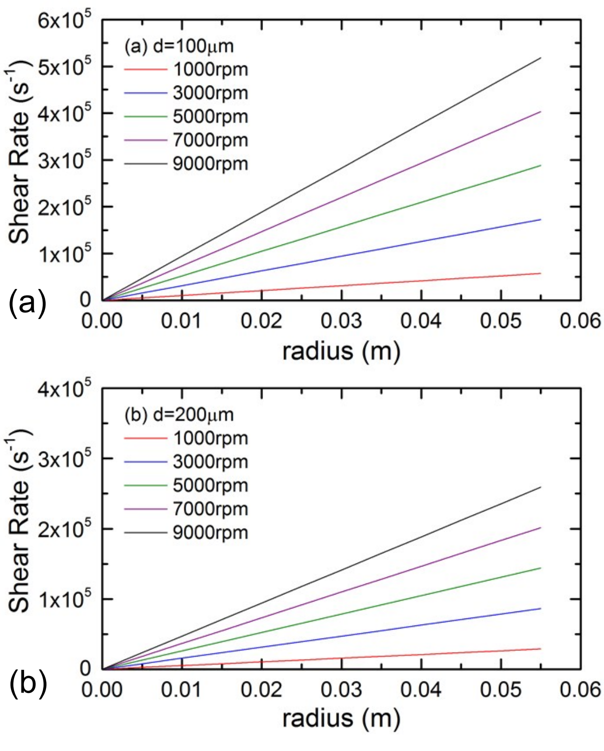
In the T[2]FD platform, the velocity of the conical surface, with respect to the stationary surface, increases as a function of its radial distance, r, from the axis of rotation at fixed angular speed, ω, (eq. 3)

$$v = r\omega. \qquad \text{(eq. 3)}$$

Therefore, it is expected that shearing rates (and shear stresses) within the T[2]FD increase linearly with the radial distance (eq. 4),

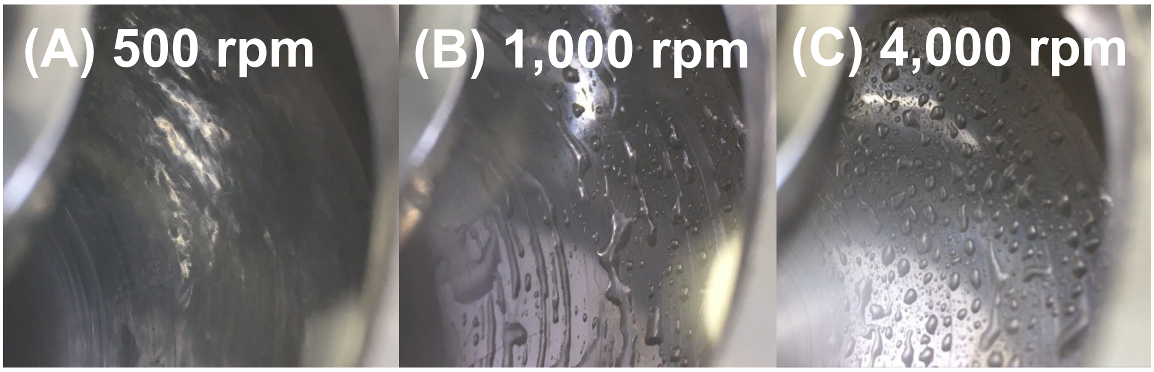
$$\gamma_{Av} = \frac{r\omega}{d_s}. \qquad \text{(eq. 4)}$$

where d_s is the gap between the rotating and stationary surfaces. The shearing rate based on this model for rotational speeds ranging from 1,000-9,000 rpm are shown in **SI Figure 1**, for gaps of (a) 100 μm and (b) 200 μm, as a function of the radial distance from the axis of rotation.



SI Figure 1: The calculated shear rate in the T[2]FD as a function of the radial distance from the axis of rotation for (a) d=100 μm, and (b) d=200 μm for rotational speeds between 1,000 and 9,000 rpm.

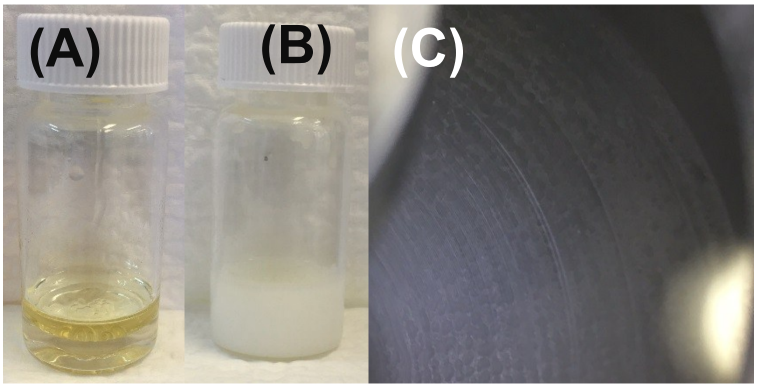
The behaviour of fluids under the influence of shear has been a subject of study over an extended period of time.[1, 2] Within the T^{2}FD, the high shear stresses experienced by the fluid leads to viscous fingering and phase deformation.[3] At the high shear rates present within the device, it is expected that the shear stresses will typically exceed the critical shear stresses required for single phase formation (homogenization).[1] We have therefore investigated the fluid behaviour within the T^{2}FD. The film was photographed at rotational speeds of 500, 1,000 and 4,000 rpm. At 500 rpm, the rotational motion of the rotor propels the liquid up the conical walls to produce a stable continuous film. At the higher rotational speeds (1,000 and 4,000 rpm), as the fluid is pumped into the device it is rapidly dispelled radially outward and up the conical walls. A supplementary video attachment recorded for emulsion experiments (discussed below) shows the fluid pulse moving rapidly out of the device. In this process, the large shear experienced by the fluid produces extensive viscous streaking and fingering, evidenced by the residual droplet film on the stationary conical surface. As the droplets are not in contact with the rotating surface they migrate around the conical surface owing to the physical forces they experience (gravity, surface tension, air flow/pressure). These droplets can recombine or get reabsorbed into a wave of fluid that is pumped into the device. When recombining, if they accumulate to a critical size they reinitiate contact with the rotating surface; leading to a viscous streaking of the droplet (as evident at 1,000 rpm, SI Figure 2B). This phenomenon makes the films characteristics particularly sensitive to the fluid volume available to the device (flow rate). We also note that the residual droplet formation is stabilized at higher flow volumes as it increases the available fluid volumes that are influenced by the viscous fingering and streaking, and reabsorption/recombination rates.



SI Figure 2: Photograph of the film created within the T^2FD when water flows into the device at 1 mL/min for rotational speeds of (A) 500, (B) 1,000 and (C) 4,000 rpm.

2. Mixing within the T^2FD

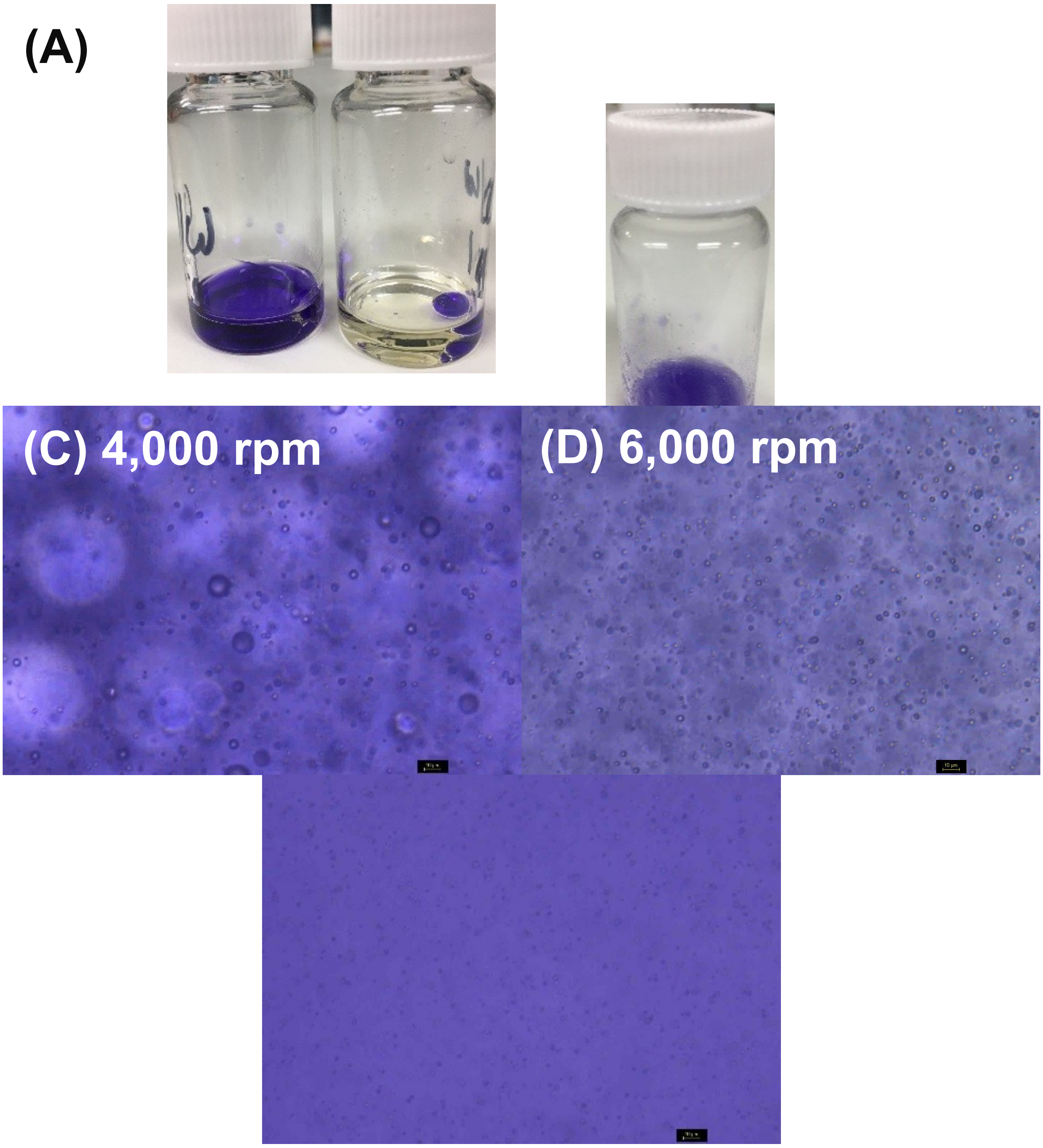
To investigate how the fluid dynamics influence mixing within the device, emulsification experiments were performed, using rice bran oil and water. The oil and water exists as a two phase system when prepared in a 1:1 volume ratio (**SI Figure 3A**). The oil and water were independently pumped at 1 mL/min through syringes into the T^2FD. A stable emulsion formed (**SI Figure 3B)** and the fluid within the device was a homogeneous emulsion (**SI Figure 3C)**. A supplementary video attachment of this emulsion process also demonstrates the rapid ejection of fluid that is pumped into the device. This video shows that there is a rapid wave of fluid moving outwards as fluid is pumped into the device.



SI Figure 3: (A) A photo of rice bran oil and water (1:1 ratio); (B) A photo of the emulsion formed in the T^2FD at 4,000 rpm. (C) A photo of the micro-drop emulsion seen within the T^2FD at 4,000 rpm.

To further assess mixing within the platform, we considered emulsification of soya bean oil and water. The water was stained with crystal violet to produce an optical contrast. Compared to the rice bran oil, it is more difficult to emulsify soya bean oil with water, making this mixture an ideal candidate to provide a more definitive test of the shear-induced mixing efficiency of the T^2FD device.

SI Figure 4A shows photos of oil in water and water in oil to illustrate the phase separation between the two components. In these experiments, water (flow rate 1.0 mL/min) and soya bean oil (0.1 mL/min) were delivered into the T^2FD at different rotational speeds. A photo of the emulsion generated at 2,000 rpm is shown in **SI Figure 4A**. Turbidity occurred in the water phase, but there is evidence of some phase separation. Soya bean oil-in-water emulsions are thermodynamically unstable, and are known to require an emulsifier to achieve a stable emulsion.[4] This phase separation may then occur as the liquid flows out of the device and is collected, so may not reflect the mixing within the device. Micrographs of the emulsions produced at 2,000-6,000 rpm (**SI Figure 4B**) reveal that there are oil droplets (yellow) contained within the water phase (purple). The size of these droplets characteristically decreased as the rotational speed of the T^2FD increased from 2,000 to 6,000 rpm. As a control experiment, soya bean oil in water was sonicated for 5 min (Pulse Swept Power: 70W), after which we did not observe any oil drops forming in the water phase. We believe that these emulsification experiments demonstrate a thorough shear-induced mixing within the T^2FD.



SI Figure 4: (A) Left to right: photos of oil in water, water in oil, T²FD 2,000rpm emulsion. (B-D) Micrographs of soya bean oil in water emulsions produced in the T²FD at different rotational speeds 2,000-6,000 rpm. Scale bar 10 μm.

3. Microalgae Cultivation

Microalgae *Chloroparva pannonica* (FC40) biomass was obtained from SARDI and was grown using F_2SI media which consists of 75.0 g L^{-1} $CO(NH_2)_2$, 5.0 g L^{-1} NaH_2PO_4, 2.6 g L^{-1} $FeCl_3.6H_2O$, 8.7 g L^{-1} Na-EDTA, 40 mg L^{-1} $CuSO_4.5H_2O$, 25.2 mg L^{-1} $Na_2MoO_4.2H_2O$, 88 mg L^{-1} $ZnSO_4.7H_2O$, 40 mg L^{-1} CoCl2.$6H_2O$, 1.44 mg L^{-1} $MnCl_2.4H_2O$. The pH was adjusted to 4.5 by adding 1 M NaOH prior to autoclaving. The vitamin content per 100 mL of MilliQ water was 10 mg vitamin B_{12}, and 10 mg biotin. A 11 L photo-bioreactor with two cool white fluorescent lamps operating for 12 hours illumination was used in this cultivation. The CO_2 was augmented only during illumination. The cells were collected after 7 days of cultivation and harvested using centrifugation (6000 $\times$ *g*, 10 min), then freeze-dried, ground to 250 μm and stored in a cold room until used. Methanol, sodium hydroxide, sulfuric acid and hexane were purchased from Sigma Aldrich and were of analytical grade (AR).

4. FT-IR and ^{1}H-NMR studies

The production of biodiesel was confirmed using a FT-IR spectrophotometer (Perkin Elmer FT-IR 400) equipped with an attenuated total reflectance (ATR) probe. The biodiesel spectra were observed in the range of 4000 – 400 cm^{-1}. Following the published method, a 600 MHz Bruker spectrometer ^{1}H-NMR with typical condition of 64 scans and 1 second D1 delay was used to measure the conversion yield based on the integration value of the specific chemical shift of methoxy protons (OMe) and α-methylene protons (α-CH_2), using eq. 5 below [5-8].

$$C = \frac{2A_{Me}}{3A_{CH_2}} \times 100 \qquad \text{(eq. 5)}$$

where C = percentage conversion of triglyceride (TG) to fatty acid methyl ester, A_{Me} = integration value of the methoxy protons of methyl ester and A_{CH_2} = integration value of α-methylene protons.

Table 1. The appearance of specific peaks of triglyceride and biodiesel in FT-IR spectra.[9]

Absorption (cm^{-1})	Functional Group	Appearance	
		Triglyceride	Biodiesel
1445	CH_3 asymmetric	-	√
1238 - 1248	O-H deformation	√	√
1200	O-CH_3 stretching	-	√
1170	C-O-C symmetric stretching; C-C stretching	√	√
1100	O-CH_2-C asymmetric, -CH_2-OH	√	-

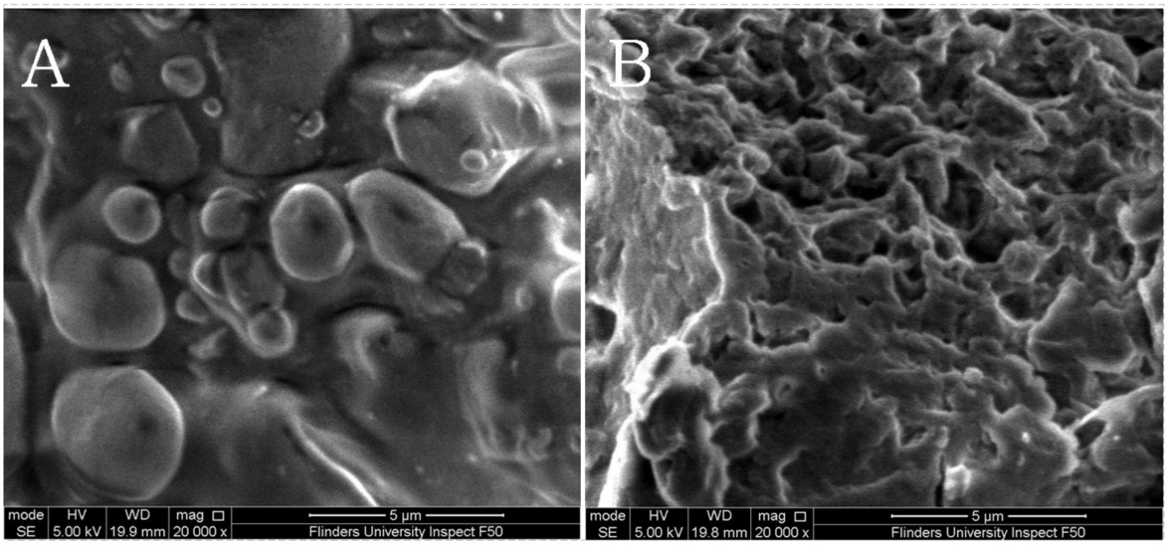
6. Lipid quantification

The Folch method [10] was used to quantify lipid content in the microalga *Chloroparva pannonica*. The dry biomass (0.05 g) was loaded into a conical polypropylene tube and stirred in a vortex mixer with a mixture of 1.4 mL 0.9% saline and 2 mL methanol. After standing for 5 min, 4 mL of chloroform was added to the mixture and shaken. The homogenate phase formed after 5 min standing was separated through centrifugation at $3000 \times g$ for 10 min. The chloroform layer containing the extracted lipid was removed and the solvent evaporated under a nitrogen stream. The mass of lipid obtained was determined gravimetrically.

The *Chloroparva pannonica* lipid content was determined to be 23.4%. Oleic acid ($C_{18:1}$) was the major constituent present in *Chloroparva pannonica*, with 30.2% of the total FAME content, followed by Linoleic acid ($C_{18:2}$) and α-Linolenic acid ($C_{18:3}$) and Palmitic acid ($C_{16:0}$) with 24.7, 21.9 and 16.7%, respectively (Table 2). The total saturated fatty acid content was 18.2%, of which palmitic acid ($C_{16:0}$) is the major compound. This profile was similar to other reported data.[11]

7. Scanning Electron Microscopy

SEM images of the fresh harvested microalgae biomass and solid residues after DT processing with sulfuric acid or sodium hydroxide as the catalyst were investigated using a FEI F50 Inspect FE-SEM (SI Figure 5). The dried sample was mounted on carbon tape and sputter-coated with 2 nm platinum, then examined at an accelerating voltage of 5 kV at a working distance of 19.9 mm. The images were captured at 20,000x magnification for a dwell time of 100 ms.



SI Figure 5 SEM images of microalgae *Chloroparva pannonica* biomass (A) before (reproduced from Sitepu *et al* [8]) and (B) after T^2FD processing.

Table 2: Quantified ^{1}H-NMR spectra for C18:3, C18:1 and C16:0 of T^{2}FD-derived FAME samples of wet biomass of *Chloroparva pannonica* operated in continuous extraction mode. n.d. – not detected; b.d.l. – below detection limit.

						Polyunsaturated Fatty Acid		Monounsaturated Fatty Acid		Saturated Fatty Acid			ΣPUFA	ΣMUFA	ΣSFA	$\Sigma_{C18:3,C18:1,C16:0}$
						$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{24:1}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$				
GC	**Dry biomass non T^{2}FD**					21.9	24.7	30.2	0.7	0.4	16.7	0.7	48.6	33.1	18.2	
NMR	**Wet biomass turbo thin film device using base catalyst**															
	Ratio Biomass : MeOH (wt./v)	**Flow Rate (mL/min)**	**Rotation Speed (rpm)**	**Catalyst Conc. (wt.%/v)**	**Water Content (wt%)**											
	1:12	2	6,000	0	67.6	20.48 ± 0.9	b.d.l	7.77 ± 1.79	n.d.	n.d.	10.7 ± 0.18	n.d	n.d.	7.77 ± 1.79	n.d.	38.95
	1:12	2	6,000	0.5	67.6	22.03 ± 3.09	b.d.l	4.05 ± 4.61	n.d.	n.d.	10.66 ± 0.07	n.d	n.d.	4.05 ± 4.61	n.d.	36.74
	1:12	2	6,000	1	67.6	12.87 ± 2.89	b.d.l	17.55 ± 3.5	n.d.	n.d.	10.08 ± 0.23	n.d	n.d.	17.55 ± 3.5	n.d.	40.50
	1:12	2	6,000	3	67.6	25.06 ± 19.74	b.d.l	8.11 ± 20.14	n.d.	n.d.	11.57 ± 1.98	n.d	n.d.	8.11 ± 20.14	n.d.	44.74
	1:12	2	6,000	5	67.6	7.33 ± 0.92	b.d.l	25.5 ± 8.98	n.d.	n.d.	9.47 ± 0.86	n.d	n.d.	25.5 ± 8.98	n.d.	42.30
	1:12	2	6,000	7	67.6	11.71 ± 1.06	b.d.l	17.67 ± 0.88	n.d.	n.d.	10.03 ± 0.04	n.d	n.d.	17.67 ± 0.88	n.d.	39.41
	1:12	2	6,000	9	67.6	6.84 ± 1.21	b.d.l	25.79 ± 2.45	n.d.	n.d.	9.78 ± 0.12	n.d	n.d.	25.79 ± 2.45	n.d.	42.41
	1:12	2	6,000	12	67.6	n.d	b.d.l	n.d	n.d.	n.d.	n.d	n.d	n.d.	n.d	n.d.	NA
	1:12	1	6,000	1	67.6	12.96 ± 1.58	b.d.l	14.13 ± 2.99	n.d.	n.d.	10.02 ± 0.2	n.d	n.d.	14.13 ± 2.99	n.d.	37.11
	1:12	2	6,000	1	67.6	12.87 ± 2.89	b.d.l	17.55 ± 3.5	n.d.	n.d.	10.08 ± 0.23	n.d	n.d.	17.55 ± 3.5	n.d.	40.50
	1:12	3	6,000	1	67.6	15.28 ± 1.2	b.d.l	13.64 ± 1.7	n.d.	n.d.	10.3 ± 0.08	n.d	n.d.	13.64 ± 1.7	n.d.	39.22
	1:12	4	6,000	1	67.6	17.21 ± 1.73	b.d.l	11.37 ± 1.35	n.d.	n.d.	10.35 ± 0.06	n.d	n.d.	11.37 ± 1.35	n.d.	38.93
	1:12	5	6,000	1	67.6	14.21 ± 0.59	b.d.l	14.95 ± 0.78	n.d.	n.d.	10.26 ± 0.11	n.d	n.d.	14.95 ± 0.78	n.d.	39.42
	1:12	3	2,000	1	67.6	11.68 ± 0.72	b.d.l	20.91 ± 0.6	n.d.	n.d.	10.53 ± 0.04	n.d	n.d.	20.91 ± 0.6	n.d.	43.12
	1:12	3	2,500	1	67.6	11.41 ± 0.7	b.d.l	22.09 ± 0.7	n.d.	n.d.	10.57 ± 0.08	n.d	n.d.	22.09 ± 0.7	n.d.	44.07
	1:12	3	3,000	1	67.6	10.87 ± 0.54	b.d.l	22.96 ± 0.67	n.d.	n.d.	10.55 ± 0.05	n.d	n.d.	22.96 ± 0.67	n.d.	44.38
	1:12	3	3,500	1	67.6	10.28 ± 0.73	b.d.l	22.97 ± 0.53	n.d.	n.d.	10.48 ± 0.04	n.d	n.d.	22.97 ± 0.53	n.d.	43.73

					Polyunsaturated Fatty Acid		Monounsaturated Fatty Acid		Saturated Fatty Acid			ΣPUFA	ΣMUFA	ΣSFA	$\Sigma_{C18:3,C18:1,C16:0}$
					$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{24:1}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$				
1:12	3	4,000	1	67.6	10.4 ± 0.5	b.d.l	23.1 ± 0.81	n.d.	n.d.	10.48 ± 0.06	n.d	n.d.	23.1 ± 0.81	n.d.	43.98
1:12	3	4,500	1	67.6	10.56 ± 0.55	b.d.l	23.03 ± 1.1	n.d.	n.d.	10.5 ± 0.05	n.d	n.d.	23.03 ± 1.1	n.d.	44.09
1:12	3	5,000	1	67.6	11.49 ± 0.4	b.d.l	21.51 ± 1.01	n.d.	n.d.	10.47 ± 0.05	n.d	n.d.	21.51 ± 1.01	n.d.	43.47
1:12	3	5,500	1	67.6	11.35 ± 1.18	b.d.l	21.81 ± 1.56	n.d.	n.d.	10.5 ± 0.06	n.d	n.d.	21.81 ± 1.56	n.d.	43.66
1:12	3	6,000	1	67.6	15.28 ± 1.2	b.d.l	13.64 ± 1.7	n.d.	n.d.	10.3 ± 0.08	n.d	n.d.	13.64 ± 1.7	n.d.	39.22
1:12	3	6,500	1	67.6	14.99 ± 1.67	b.d.l	15.43 ± 1.99	n.d.	n.d.	10.3 ± 0.14	n.d	n.d.	15.43 ± 1.99	n.d.	40.72
1:12	3	7,000	1	67.6	15.22 ± 0.6	b.d.l	14.11 ± 0.76	n.d.	n.d.	10.25 ± 0.02	n.d	n.d.	14.11 ± 0.76	n.d.	39.58
1:12	3	2,000	0.5	67.6	15.13 ± 0.46	b.d.l	15.79 ± 0.54	n.d.	n.d.	10.55 ± 0.02	n.d	n.d.	15.79 ± 0.54	n.d.	41.47
1:12	3	3,000	0.5	67.6	16 ± 1.37	b.d.l	13.66 ± 1.89	n.d.	n.d.	10.5 ± 0.07	n.d	n.d.	13.66 ± 1.89	n.d.	40.16
1:12	3	4,000	0.5	67.6	17.45 ± 0.72	b.d.l	11.22 ± 0.83	n.d.	n.d.	10.51 ± 0.06	n.d	n.d.	11.22 ± 0.83	n.d.	39.18
1:12	3	5,000	0.5	67.6	17.56 ± 1.66	b.d.l	10.69 ± 2.01	n.d.	n.d.	10.49 ± 0.08	n.d	n.d.	10.69 ± 2.01	n.d.	38.74
1:12	3	2,000	0	67.6	23.63 ± 1.17	b.d.l	13.2 ± 0.89	n.d.	n.d.	10.98 ± 0.06	n.d	n.d.	13.2 ± 0.89	n.d.	47.81
1:12	3	3,000	0	67.6	24.03 ± 0.19	b.d.l	14.56 ± 1.55	n.d.	n.d.	11.14 ± 0.17	n.d	n.d.	14.56 ± 1.55	n.d.	49.73
1:12	3	4,000	0	67.6	23.83 ± 1.43	b.d.l	14.75 ± 1.01	n.d.	n.d.	11.27 ± 0.16	n.d	n.d.	14.75 ± 1.01	n.d.	49.85
1:12	3	5,000	0	67.6	23.28 ± 1.55	b.d.l	13.39 ± 1.17	n.d.	n.d.	11.32 ± 0.2	n.d	n.d.	13.39 ± 1.17	n.d.	47.99
1:6	3	4,000	1	67.6	14.19 ± 0.65	b.d.l	15.89 ± 1.19	n.d.	n.d.	10.22 ± 0.08	n.d	n.d.	15.89 ± 1.19	n.d.	40.30
1:9	3	4,000	1	67.6	14.11 ± 0.55	b.d.l	16.22 ± 0.66	n.d.	n.d.	10.24 ± 0.06	n.d	n.d.	16.22 ± 0.66	n.d.	40.57
1:15	3	4,000	1	67.6	13.66 ± 1.53	b.d.l	16.67 ± 1.25	n.d.	n.d.	10.39 ± 0.07	n.d	n.d.	16.67 ± 1.25	n.d.	40.72
1:12	3	4,000	1	67.6	10.4 ± 0.5	b.d.l	23.1 ± 0.81	n.d.	n.d.	10.48 ± 0.06	n.d	n.d.	23.1 ± 0.81	n.d.	43.98
1:18	3	4,000	1	67.6	12.32 ± 0.95	b.d.l	19.09 ± 2.58	n.d.	n.d.	10.35 ± 0.08	n.d	n.d.	19.09 ± 2.58	n.d.	41.76
1:12	3	4,000	1	67.6	10.4 ± 0.5	b.d.l	23.1 ± 0.81	n.d.	n.d.	10.48 ± 0.06	n.d	n.d.	23.1 ± 0.81	n.d.	43.98
1:12	3	4,000	1	75	15.32 ± 1.08	b.d.l	14.93 ± 1.14	n.d.	n.d.	10.6 ± 0.1	n.d	n.d.	14.93 ± 1.14	n.d.	40.85
1:12	3	4,000	1	80	15.76 ± 0.61	b.d.l	14.24 ± 0.68	n.d.	n.d.	10.51 ± 0.04	n.d	n.d.	14.24 ± 0.68	n.d.	40.51
1:12	3	4,000	1	85	16.66 ± 0.55	b.d.l	12.67 ± 0.62	n.d.	n.d.	10.53 ± 0.04	n.d	n.d.	12.67 ± 0.62	n.d.	39.86
1:12	3	4,000	1	90	16.05 ± 0.55	b.d.l	13.03 ± 0.76	n.d.	n.d.	10.31 ± 0.05	n.d	n.d.	13.03 ± 0.76	n.d.	39.39

Table 3: FA to FAME conversion efficiencies of the DT of wet biomass of *Chloroparva pannonica* in T^{2}FD operated in continuous extraction mode.

Ratio Biomass : MeOH (wt./v)	Flow Rate (mL/min)	Rotation Speed (rpm)	% Catalyst Concentration (wt./v)	Water Content (wt%)	Fatty Acid to FAME Conversion Efficiency (%)
Effect of catalyst concentration					
1:12	2	6,000	0	67.6	7.57 ± 1.34
1:12	2	6,000	0.5	67.6	84.04 ± 4.35
1:12	2	6,000	1	67.6	91.03 ± 1.28
1:12	2	6,000	3	67.6	93.44 ± 2.12
1:12	2	6,000	5	67.6	90.2 ± 5.07
1:12	2	6,000	7	67.6	94.46 ± 3.31
1:12	2	6,000	9	67.6	90.39 ± 2.86
1:12	2	6,000	12	67.6	52.9 ± 17.01
Effect of flow rate					
1:12	1	6,000	1	67.6	73.99 ± 8.53
1:12	2	6,000	1	67.6	91.03 ± 1.28
1:12	3	6,000	1	67.6	94.01 ± 1.19
1:12	4	6,000	1	67.6	89.79 ± 1.96
1:12	5	6,000	1	67.6	89.75 ± 3.47
Effect of rotation speed					
1:12	3	2,000	1	67.6	91.49 ± 3.04
1:12	3	2,500	1	67.6	95.27 ± 1.65
1:12	3	3,000	1	67.6	92.49 ± 2.27
1:12	3	3,500	1	67.6	94.01 ± 2.32
1:12	3	4,000	1	67.6	96.56 ± 0.66
1:12	3	4,500	1	67.6	95.8 ± 1.54
1:12	3	5,000	1	67.6	89.43 ± 2.89
1:12	3	5,500	1	67.6	87.44 ± 3.51

Ratio Biomass : MeOH (wt./v)	Flow Rate (mL/min)	Rotation Speed (rpm)	% Catalyst Concentration (wt./v)	Water Content (wt%)	Fatty Acid to FAME Conversion Efficiency (%)
1:12	3	6,000	1	67.6	94.01 ± 1.19
1:12	3	6,500	1	67.6	87.57 ± 0.55
1:12	3	7,000	1	67.6	89.49 ± 3.37
1:12	3	2,000	0.5	67.6	87.26 ± 3.42
1:12	3	3,000	0.5	67.6	88.21 ± 1.92
1:12	3	4,000	0.5	67.6	88.19 ± 2.63
1:12	3	5,000	0.5	67.6	87.28 ± 2.1
1:12	3	2,000	0	67.6	14.39 ± 3.85
1:12	3	3,000	0	67.6	9.22 ± 2.58
1:12	3	4,000	0	67.6	8.34 ± 2.97
1:12	3	5,000	0	67.6	5.54 ± 1.57
Effect of ratio biomass to methanol					
1:6	3	4,000	1	67.6	90.75 ± 4.84
1:9	3	4,000	1	67.6	91.57 ± 2.64
1:12	3	4,000	1	67.6	96.56 ± 0.66
1:15	3	4,000	1	67.6	93.5 ± 3.37
1:18	3	4,000	1	67.6	90.48 ± 6.28
Effect of water content					
1:12	3	4,000	1	67.6	96.56 ± 0.66
1:12	3	4,000	1	75	94.46 ± 0.98
1:12	3	4,000	1	80	90.28 ± 0.6
1:12	3	4,000	1	85	90.66 ± 1.79
1:12	3	4,000	1	90	91.58 ± 0.8

Table 4: Tukey test for C18:3, C18:1 and C16:0 of T^2FD-derived FAME samples of wet biomass of *Chloroparva pannonica* operated in continuous extraction mode. Significance Values below $\alpha<0.05$ are colour coded in red.

Cell No.	Tukey HSD test; variable C18:3 (CC-T2FD microalgae biodiesel_FA extraction efficiency(5)) Approximate Probabilities for Post Hoc Tests Error: Between MS = 2.5554, df = 8.0000				
	Cat. conc. [wt/v %]	{1}	{2}	{3}	{4}
1	0		0.001908	0.000247	0.000232
2	1	0.001908		0.012181	0.001169
3	5	0.000247	0.012181		0.231906
4	12	0.000232	0.001169	0.231906	

Cell No.	Tukey HSD test; variable C18:1 (2) (CC-T2FD microalgae biodiesel_FA extraction efficiency(5)) Approximate Probabilities for Post Hoc Tests Error: Between MS = 32.050, df = 6.0000			
	Cat conc. [wt/v %]	{1}	{2}	{3}
1	0		0.166868	0.020237
2	1	0.166868		0.273135
3	5	0.020237	0.273135	

Cell No.	Tukey HSD test; variable C16:0 (CC-T2FD microalgae biodiesel_FA extraction efficiency(5)) Approximate Probabilities for Post Hoc Tests Error: Between MS = .26648, df = 8.0000				
	Cat conc. [wt/v %]	{1}	{2}	{3}	{4}
1	0		0.505179	0.076266	0.000454
2	1	0.505179		0.505331	0.001310
3	5	0.076266	0.505331		0.006314
4	12	0.000454	0.001310	0.006314	

Cell No.	Tukey HSD test; variable C18:3 (Ratio-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = .84607, df = 10.000					
	Ratio	{1}	{2}	{3}	{4}	{5}
1	1:18		0.432721	0.010072	0.972441	0.951982
2	1:15	0.432721		0.152932	0.198118	0.170920
3	1:12	0.010072	0.152932		0.004252	0.003690
4	1:9	0.972441	0.198118	0.004252		0.999971
5	1:6	0.951982	0.170920	0.003690	0.999971	

Cell No.	Tukey HSD test; variable C18:1 (Ratio-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = 2.1463, df = 10.000					
	Ratio	{1}	{2}	{3}	{4}	{5}
1	1:18		0.321371	0.002360	0.995137	0.963133
2	1:15	0.321371		0.045881	0.192509	0.128956
3	1:12	0.002360	0.045881		0.001479	0.001069
4	1:9	0.995137	0.192509	0.001479		0.998585
5	1:6	0.963133	0.128956	0.001069	0.998585	

Cell No.	Tukey HSD test; variable C16:0 (Ratio-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00533, df = 10.000					
	Ratio	{1}	{2}	{3}	{4}	{5}
1	1:18		0.937025	0.605121	0.165503	0.084954
2	1:15	0.937025		0.247048	0.451222	0.257874
3	1:12	0.605121	0.247048		0.017653	0.009062
4	1:9	0.165503	0.451222	0.017653		0.990711
5	1:6	0.084954	0.257874	0.009062	0.990711	

Cell No.	Tukey HSD test; variable C18:3 (RS-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = 1.4418, df = 22.000											
	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	2000		1.000000	0.998695	0.930553	0.959715	0.983035	1.000000	0.999562	0.040428	0.074542	0.045997
2	2500	1.000000		0.999959	0.981797	0.991874	0.997783	1.000000	0.994113	0.022726	0.042895	0.025961
3	3000	0.998695	0.999959		0.999913	0.999989	1.000000	0.999861	0.890638	0.006664	0.012892	0.007639
4	3500	0.930553	0.981797	0.999913		1.000000	1.000000	0.971227	0.564909	0.001784	0.003382	0.002031
5	4000	0.959715	0.991874	0.999989	1.000000		1.000000	0.985933	0.640249	0.002314	0.004429	0.002640
6	4500	0.983035	0.997783	1.000000	1.000000	1.000000		0.995520	0.734590	0.003259	0.006293	0.003737
7	5000	1.000000	1.000000	0.999861	0.971227	0.985933	0.995520		0.996973	0.027060	0.050759	0.030880
8	5500	0.999562	0.994113	0.890638	0.564909	0.640249	0.734590	0.996973		0.168261	0.277915	0.187658
9	6000	0.040428	0.022726	0.006664	0.001784	0.002314	0.003259	0.027060	0.168261		1.000000	1.000000
10	6500	0.074542	0.042895	0.012892	0.003382	0.004429	0.006293	0.050759	0.277915	1.000000		1.000000
11	7000	0.045997	0.025961	0.007639	0.002031	0.002640	0.003737	0.030880	0.187658	1.000000	1.000000	

Cell No.	Tukey HSD test; variable C18:1 (RS-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = 4.1087, df = 22.000											
	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	2000		0.999602	0.969838	0.969394	0.954564	0.963108	0.999999	0.996305	0.008374	0.085639	0.015914
2	2500	0.999602		0.999973	0.999971	0.999898	0.999948	0.999999	0.847754	0.001746	0.019041	0.003250
3	3000	0.969838	0.999973		1.000000	1.000000	1.000000	0.997672	0.545258	0.000628	0.005867	0.001070
4	3500	0.969394	0.999971	1.000000		1.000000	1.000000	0.997612	0.543556	0.000625	0.005831	0.001064
5	4000	0.954564	0.999898	1.000000	1.000000		1.000000	0.995262	0.495259	0.000550	0.004883	0.000911
6	4500	0.963108	0.999948	1.000000	1.000000	1.000000		0.996698	0.521272	0.000589	0.005375	0.000993
7	5000	0.999999	0.999999	0.997672	0.997612	0.995262	0.996698		0.960619	0.003732	0.040574	0.007087
8	5500	0.996305	0.847754	0.545258	0.543556	0.495259	0.521272	0.960619		0.063082	0.419275	0.111902
9	6000	0.008374	0.001746	0.000628	0.000625	0.000550	0.000589	0.003732	0.063082		0.988373	1.000000
10	6500	0.085639	0.019041	0.005867	0.005831	0.004883	0.005375	0.040574	0.419275	0.988373		0.999001
11	7000	0.015914	0.003250	0.001070	0.001064	0.000911	0.000993	0.007087	0.111902	1.000000	0.999001	

Tukey HSD test; variable C16:0 (RS-T2FD microalgae biodiesel_FA extraction efficiency)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .00497, df = 22.000

Cell No.	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	2000		0.999922	1.000000	0.998493	0.996654	0.999943	0.989943	0.822981	0.015949	0.015826	0.003138
2	2500	0.999922		1.000000	0.928516	0.901043	0.980254	0.843174	0.474479	0.004253	0.004220	0.000914
3	3000	1.000000	1.000000		0.987047	0.978140	0.998442	0.954221	0.676053	0.008856	0.008787	0.001775
4	3500	0.998493	0.928516	0.987047		1.000000	1.000000	1.000000	0.998010	0.092908	0.092270	0.020080
5	4000	0.996654	0.901043	0.978140	1.000000		1.000000	1.000000	0.999166	0.109633	0.108896	0.024182
6	4500	0.999943	0.980254	0.998442	1.000000	1.000000		0.999987	0.984831	0.054787	0.054390	0.011263
7	5000	0.989943	0.843174	0.954221	1.000000	1.000000	0.999987		0.999855	0.143252	0.142326	0.032850
8	5500	0.822981	0.474479	0.676053	0.998010	0.999166	0.984831	0.999855		0.403887	0.401948	0.120009
9	6000	0.015949	0.004253	0.008856	0.092908	0.109633	0.054787	0.143252	0.403887		1.000000	0.999512
10	6500	0.015826	0.004220	0.008787	0.092270	0.108896	0.054390	0.142326	0.401948	1.000000		0.999531
11	7000	0.003138	0.000914	0.001775	0.020080	0.024182	0.011263	0.032850	0.120009	0.999512	0.999531	

Tukey HSD test; variable C18:3 (RS-CC-FACTORIAL-T2FD microalgae biodiesel_FA extraction efficiency)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 1.4033, df = 16.000

Cell No.	Cat Conc. [%]	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	0	2000		0.981322	0.303902	0.256527	0.000175	0.000175	0.000175	0.000177
2	0	3000	0.981322		0.798727	0.738157	0.000181	0.000177	0.000179	0.000190
3	0	4000	0.303902	0.798727		1.000000	0.000340	0.000248	0.000287	0.000506
4	0	5000	0.256527	0.738157	1.000000		0.000380	0.000266	0.000313	0.000588
5	0.5	2000	0.000175	0.000181	0.000340	0.000380		0.999868	0.999999	0.999942
6	0.5	3000	0.000175	0.000177	0.000248	0.000266	0.999868		0.999999	0.992456
7	0.5	4000	0.000175	0.000179	0.000287	0.000313	0.999999	0.999999		0.998812
8	0.5	5000	0.000177	0.000190	0.000506	0.000588	0.999942	0.992456	0.998812	

Tukey HSD test; variable C18:1 (RS-CC-FACTORIAL-T2FD microalgae biodiesel_FA extraction efficiency)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 1.7726, df = 16.000

Cell No.	Cat Conc. [%]	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	0	2000		0.537413	0.012071	0.004756	0.313989	0.939678	0.974707	0.395692
2	0	3000	0.537413		0.374053	0.180915	0.999833	0.989109	0.968089	0.999995
3	0	4000	0.012071	0.374053		0.999595	0.612286	0.101771	0.074111	0.512654
4	0	5000	0.004756	0.180915	0.999595		0.344131	0.041812	0.029894	0.269676
5	0.5	2000	0.313989	0.999833	0.612286	0.344131		0.905782	0.834652	1.000000
6	0.5	3000	0.939678	0.989109	0.101771	0.041812	0.905782		1.000000	0.953018
7	0.5	4000	0.974707	0.968089	0.074111	0.029894	0.834652	1.000000		0.903173
8	0.5	5000	0.395692	0.999995	0.512654	0.269676	1.000000	0.953018	0.903173	

Tukey HSD test; variable C16:0 (RS-CC-FACTORIAL-T2FD microalgae biodiesel_FA extraction efficiency)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .01417, df = 16.000

Cell No.	Cat Conc. [%]	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	0	2000		0.999612	0.999856	0.997199	0.007722	0.000518	0.000197	0.000181
2	0	3000	0.999612		1.000000	1.000000	0.003104	0.000312	0.000182	0.000177
3	0	4000	0.999856	1.000000		0.999996	0.003514	0.000330	0.000184	0.000177
4	0	5000	0.997199	1.000000	0.999996		0.002259	0.000270	0.000180	0.000176
5	0.5	2000	0.007722	0.003104	0.003514	0.002259		0.760196	0.135690	0.046911
6	0.5	3000	0.000518	0.000312	0.000330	0.000270	0.760196		0.871365	0.553684
7	0.5	4000	0.000197	0.000182	0.000184	0.000180	0.135690	0.871365		0.998579
8	0.5	5000	0.000181	0.000177	0.000177	0.000176	0.046911	0.553684	0.998579	

Cell No.	Tukey HSD test; variable C18:3 (Water-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = .48137, df = 10.000					
	Water content [%]	{1}	{2}	{3}	{4}	{5}
1	67		0.000198	0.000183	0.000177	0.000179
2	75	0.000198		0.931621	0.199139	0.699018
3	80	0.000183	0.931621		0.529696	0.983335
4	85	0.000177	0.199139	0.529696		0.813295
5	90	0.000179	0.699018	0.983335	0.813295	

Cell No.	Tukey HSD test; variable C18:1 (Water-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = .67650, df = 10.000					
	Water content [%]	{1}	{2}	{3}	{4}	{5}
1	67		0.000176	0.000176	0.000176	0.000176
2	75	0.000176		0.837221	0.044394	0.101596
3	80	0.000176	0.837221		0.209160	0.422234
4	85	0.000176	0.044394	0.209160		0.981115
5	90	0.000176	0.101596	0.422234	0.981115	

Cell No.	Tukey HSD test; variable C16:0 (Water-T2FD microalgae biodiesel_FA extraction efficiency) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00411, df = 10.000					
	Water content [%]	{1}	{2}	{3}	{4}	{5}
1	67		0.223202	0.982398	0.856240	0.060094
2	75	0.223202		0.440231	0.691747	0.002086
3	80	0.982398	0.440231		0.989635	0.026566
4	85	0.856240	0.691747	0.989635		0.013224
5	90	0.060094	0.002086	0.026566	0.013224	

Table 5: Tukey test of FA to FAME conversion efficiencies of the DT of wet biomass of *Chloroparva pannonica* in T^2FD operated in continuous extraction mode.

Tukey HSD test; variable FA to FAME conversion efficiency [%] (CC-T2FD microalgae biodiesel)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 48.865, df = 14.000

Cell No.	Catalyst concentration [%wt./v]	{1}	{2}	{3}	{4}	{5}	{6}	{7}
1	0		0.000174	0.000174	0.000174	0.000174	0.000174	0.000186
2	1	0.000174		0.999421	0.999999	0.995792	1.000000	0.000310
3	3	0.000174	0.999421		0.996933	0.999996	0.997762	0.000234
4	5	0.000174	0.999999	0.996933		0.986822	1.000000	0.000349
5	7	0.000174	0.995792	0.999996	0.986822		0.989464	0.000217
6	9	0.000174	1.000000	0.997762	1.000000	0.989464		0.000340
7	12	0.000186	0.000310	0.000234	0.000349	0.000217	0.000340	

Tukey HSD test; variable FA to FAME conversion efficiency [%] (Water-T2FD microalgae biodiesel)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 1.1222, df = 10.000

Cell No.	water content [wt. %]	{1}	{2}	{3}	{4}	{5}
1	67		0.183541	0.000334	0.000470	0.001444
2	75	0.183541		0.004912	0.009350	0.046816
3	80	0.000334	0.004912		0.990689	0.584197
4	85	0.000470	0.009350	0.990689		0.824089
5	90	0.001444	0.046816	0.584197	0.824089	

Tukey HSD test; variable FA to FAME conversion efficiency [%] (FR-T2FD microalgae biodiesel)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 18.336, df = 10.000

Cell No.	flow rate [mL/min]	{1}	{2}	{3}	{4}	{5}
1	1		0.004637	0.001518	0.007751	0.007875
2	2	0.004637		0.908477	0.995979	0.995485
3	3	0.001518	0.908477		0.748070	0.742217
4	4	0.007751	0.995979	0.748070		1.000000
5	5	0.007875	0.995485	0.742217	1.000000	

Tukey HSD test; variable FA to FAME conversion efficiency [%] (RS-0%-T2FD microalgae biodiesel)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 8.1991, df = 8.0000

Cell No.	Rotational speed [rpm]	{1}	{2}	{3}	{4}
1	2000		0.200442	0.119039	0.022457
2	3000	0.200442		0.980282	0.442846
3	4000	0.119039	0.980282		0.646466
4	5000	0.022457	0.442846	0.646466	

Tukey HSD test; variable FA to FAME conversion efficiency [%] (RS-1%-T2FD microalgae biodiesel)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 5.3713, df = 22.000

Cell No.	Rotational speed [rpm]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	2000		0.651104	0.999972	0.952472	0.268571	0.478624	0.987713	0.564284	0.952931	0.606170	0.990269
2	2500	0.651104		0.915210	0.999773	0.999724	1.000000	0.131785	0.014752	0.999767	0.017136	0.140473
3	3000	0.999972	0.915210		0.998912	0.556796	0.795314	0.857627	0.273826	0.998935	0.304429	0.872170
4	3500	0.952472	0.999773	0.998912		0.948251	0.995688	0.397307	0.061985	1.000000	0.071131	0.416331
5	4000	0.268571	0.999724	0.556796	0.948251		0.999998	0.032967	0.003216	0.947762	0.003740	0.035475
6	4500	0.478624	1.000000	0.795314	0.995688	0.999998		0.076331	0.007888	0.995614	0.009174	0.081744
7	5000	0.987713	0.131785	0.857627	0.397307	0.032967	0.076331		0.990600	0.398452	0.994309	1.000000
8	5500	0.564284	0.014752	0.273826	0.061985	0.003216	0.007888	0.990600		0.062250	1.000000	0.988110
9	6000	0.952931	0.999767	0.998935	1.000000	0.947762	0.995614	0.398452	0.062250		0.071431	0.417504
10	6500	0.606170	0.017136	0.304429	0.071131	0.003740	0.009174	0.994309	1.000000	0.071431		0.992610
11	7000	0.990269	0.140473	0.872170	0.416331	0.035475	0.081744	1.000000	0.988110	0.417504	0.992610	

Tukey HSD test; variable FA to FAME conversion efficiency [%] (RS-0and0.5%-T2FD microalgae biodiesel)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 7.4312, df = 16.000

Cell No.	Rotational speed [rpm]	Catalyst concentration [%wt./v]	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	2000	0.0		0.000175	0.340571	0.000175	0.186419	0.000175	0.018918	0.000175
2	2000	0.5	0.000175		0.000175	0.999818	0.000175	0.999848	0.000175	1.000000
3	3000	0.0	0.340571	0.000175		0.000175	0.999891	0.000175	0.714455	0.000175
4	3000	0.5	0.000175	0.999818	0.000175		0.000175	1.000000	0.000175	0.999843
5	4000	0.0	0.186419	0.000175	0.999891	0.000175		0.000175	0.902419	0.000175
6	4000	0.5	0.000175	0.999848	0.000175	1.000000	0.000175		0.000175	0.999870
7	5000	0.0	0.018918	0.000175	0.714455	0.000175	0.902419	0.000175		0.000175
8	5000	0.5	0.000175	1.000000	0.000175	0.999843	0.000175	0.999870	0.000175	

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