Conception and Development of a Pulsed Microwave Applicator for Exposure of Fresh Microalgae Biomass

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Abstract

This study presents the conception and development of a microwave applicator based on a TM010 cavity and a pulsed microwave source, both custom-designed for evaluation of microwave disruption of microalgae. The objective is the selective heating of microalgae immersed in their environment.

The applicator enables exposing microalgae suspension to microwave in continuous flow mode and monitoring the energy absorbed by the sample. Design was based on dielectric characteristics of microalgae in water but other solvents might be considered. A tuning element ensures efficient microwave coupling into the samples whose relative permittivity ranges from 10 to 80.

The applicator includes a customized power supply operating a commercial magnetron source in pulse mode. The microwave pulses are in the range of multiple kilowatt with adjustable duration in microseconds range. The applicator enables to reach a power density in the microalgae sample beyond $10^{10}~W/m^3$.

Preliminary proof of principle experiments of microwave disruption on fresh microalgae biomass *Auxenochlorella protothecoides* followed by lipid extraction enabled almost complete lipid recovery while yields were negligible on untreated samples. Total energy consumption was below 4.2 MJ per kg of dry microalgae. Finally, the applicator characteristics are promising for future upscaling and deployment in industry.

Index Terms

Microalgae, Biomass Disruption, Microwave Assisted Extraction, Biofuel, Pulse.

I. INTRODUCTION

MICROALGAE are natural organisms that attract a lot of attention because of their ability to produce a wide range of valuable products [1]. For example, production of biofuel from microalgae is still considered as a promising approach to reduce humanity's carbon footprint [2] despite the many challenges that remains to be addressed such as reduction of cultivation costs or improvement of extraction techniques [3]. Additionally, microalgae could become an important source of protein for the food and feed industries and could provide many valuable food additives [4], [5].

For all considered applications, especially the ones focusing on commodities, one of the major problem is the development of an efficient cell disruption technique which enables efficient products recovery at reasonable costs and with minimum energy consumption. Additionally, in a biorefinery concept, disruption techniques should not only enable extraction of one single product but should also enable fractionation of the different valuable components, should not damage valuable thermo-sensitive molecules and should avoid side effects such as production of very fine cell debris that are difficult to separate and can considerably increase further downprocessing costs [6].

A large number of disruption methods are known, but even if some of them are very successful at the lab scale, most of them have serious drawbacks for large scale industrial applications and especially for a microalgae biorefinery [7], [6], [8]. For example high pressure homogenization (HPH) which is a well established technique in the industry and to a certain extend successfull on microalgae [9], [10] tends to produce small cell fragments and stable emulsions. These stable emulsions i.e. mixture of the normally immiscible water and lipid phases, are extremely difficult to separate even with high centrifuge forces which makes therefore the recovery of the different products and the whole downstream processing much more complex [6], [11]. Bead milling has also been shown to be efficient on microalgae with in certain cases reasonable energy consumption [12], [13] and some devices are commercially available. However it has similar drawbacks like HPH. Indeed, up-scaling, cooling efforts, small size of cell debris and maintenance costs are still large concerns for industrial applications [6]. Ultrasound disruption systems are commercially available as well. The ultrasound method can process material with high dry weight

concentration and in some cases with reasonable energy consumption. However, imploding cavitation bubbles can cause local temperatures over $5000°C$ or can lead to a stable emulsion [6] and therefore it remains unsure whether ultrasound treatment is a good choice especially for mild disintegration required in biorefinery concept.

Many other disruption techniques are still at an earlier stage of investigation such as freeze-drying, pulsed electric field treatment [14], [15], microwave (MW) exposure, chemical disruption or enzymatic lysis [16]. Among them, microwave shows promising characteristics for lipid extraction and more generally for implementation in a biorefinery concept [17], [8]. Outside of the microalgae application, microwave is already successfully used at large scales not only as a disruption method but as a mean to improve and accelerate extraction rates. For example in the food industry, MW is used to improve and accelerate extraction of ingredients from fruits, vegetables and aromatic plants. It is especially efficient for the recovery of essential oils, aromas, fats, antioxidants, and food colors [18] [19] [20]. In that case, the raw material is directly treated in the extraction solvent which is chosen depending on the targeted molecules to be extracted. The detailed mechanisms which make microwave assisted extraction (MAE) so successful are not fully understood. In most cases, the biological material to be processed has a higher polarity than the surrounding solvent, due to the high water content and to the presence of other highly polar molecules in the cell structure. Therefore, when MW are applied, the biological sample gets heated more and/or faster than the surrounding environment so that the heat transfer happens from the inside of the biological material to the outside environment i.e. the solvent. Experts of MAE believe that this heat transfer is what favors the diffusion of molecules from the biological material to the solvent since both transfers (heat and molecules) happen in the same direction i.e. that heat transfer synergistically supports mass transfert [21].

Most of the MAE tests performed on microalgae aimed at recovering lipids [22], [23], [24], [25], [26], [27], [28] but other molecules were also targetted such as phycobiliproteines [29], phenolic compounds [30], some functionnal lipophilic compounds [31], pigments [32] or other bioactive metabolite [33].

Based on the previously published studies, MAE could be qualified as successful, since it systematically increased yields or at least extraction speeds. In a couple of studies, microwave treatment was also tested as a pure disruption method in the sense that experiments did not target at extraction of any substances but only focused on percentage of disrupted cells. This confirmed the ability of microwave to induce microalgae cell disruption [34] and not only to enhance extraction.

The efficiency of MW disruption or MAE in terms of energy consumption is however currently difficult to evaluate. Most of the studies have indeed been performed using a batch process in industrial microwave-oven with most of the time a treatment regulation based on the temperature of the sample. Such systems do not guarantee a complete and homogeneous absorption of microwave radiation by the sample and therefore complicate energy evaluation.

To the best of our knowledge, only one study was performed on microalgae with a task specific, continuous flow, microwave system i.e. a focusing cavity with a three stub tuner [26]. Unfortunately real energy consumption was also not evaluated in this study and MW was combined with prolonged time in water-bath at $85°C$ to $90°C$, making it difficult to evaluate the specific effect of the MW-treatment. However, the authors could clearly demonstrate in this study that the effect of the MW treatment could not be achieved with pure conventional heating. The strategy that the authors followed in the above mentioned paper is moreover extremely interesting since they could avoid the use of a non-dedicated MW applicator which would necessarily increase largely the overall consumed energy.

One should also point out that energy efficiency of the MW disruption is difficult to evaluate in the current state since all experiments except the one from McMillan and colleagues [34] were performed on dried or at least previously frozen biomass. The microalgae were therefore to a certain extent already pre-treated. Depending on the targeted molecules, drying might be included in the processing of microalgal biomass but in the large majority of applications, drying represents prohibitive energy consumption. Therefore efficiency should be evaluated directly on fresh biomass, either in cultivation medium directly following the harvesting and concentrating step or eventually after resuspension in a specific solvent in case MW is considered not only for disintegration but for MAE.

From a general point of view, microwave as a disruption technique has many attractive features. The volumetric deposition of microwave energy ensures homogeneous handling of the sample in case an appropriate application system is designed [21]. Moreover, a continuous microwave treatment system with microalgae suspension circulating in an adapted MW transparent tube made e.g. of quartz, should not be sensitive to mechanical wear. Easiness of cleaning guarantees less problem related to contaminations. Upscaling is in principal possible especially since high power MW technology is already commercially available. Finally, until now, no creation of debris have been reported like in the case of bead-milling or HPH, an important aspect to facilitate further downstream processing.

Based on all of the above arguments, it appears that MW is interesting for the downprocessing of microalgae and therefore should be studied more in depth. In this context, this study aims at designing an applicator which will allow a more controlled and homogeneous deposition of microwave energy in microalgae biomass. The MW applicator should therefore be adapted to the dielectric properties of microalgae suspensions for different biomass concentration and different solvents. In order to investigate the specific role of the electromagnetic field intensity, setting options like variable power output and variable exposure duration should be available. In regard of future up-scalability for industrial applications a key requirement is the continuous flow processing of the sample. Finally, the applicator should be equipped with appropriate metrology system in order to allow continuous monitoring of deposited MW energy and eventual modification of the absorption because of heating

of the suspension.

II. DIELECTRIC PROPERTIES OF SAMPLES

For a proper design of the microwave applicator, the dielectric properties of microalgae admixed with cultivated medium were investigated. Measurements were performed on fresh microalgae suspension from the specie *Auxenochlorella prototecoides* cultivated mixotrophically in Wu medium (details about cultivation can be found in [14]). The initial microalgae suspension was concentrated by centrifugation and the other concentrations were then obtained by rediluting in cultivation medium to avoid eventual osmotic shocks that could affect the biomass. Dielectric measurements were made for microalgae concentration ranging from 2 g up to 120 g per liter of suspension. The lower value i.e. 2g/L is a typical concentration of microalgae suspension at the end of cultivation for most microalgae type and most cultivation conditions [35]. The upper value i.e. 120 g/L is a standard concentration in order for the microalgae suspension to remain liquid enough to be pumped in a continuous flow equipement [35]. Note that the specific microalgae used in the study can be even more concentrated and nevertheless the suspension remains liquid as can be seen in section V.

Measurements were performed with a network analyzer (*PNA N5224A6, Agilent Technologies*), and a dielectric coaxial probe (*Agilent 85070D Dielectric Probe Kit*) immersed into a cup containing $50\ ml$ of microalgae suspension. Three independent experiments with microalgae batches cultivated on different dates were made. The real permittivity ϵ' and the loss angle $tan\delta$ measured at $2.45\ GHz$ at room temperature are displayed for the different concentrations in Fig. 1. The values obtained for pure medium are displayed on the graph at zero microalgae concentration. Its relative permittivity lies between $\epsilon' = 75$ and $\epsilon' = 78$ at $2.45\ GHz$ i.e. close to typical values of pure water. When the microalgae concentration increases, the real part of permittivity value drops, and the trend line goes towards $\epsilon' = 65$ for a concentration of $120\ g/L$. The loss tangent of pure medium lies between 0.13 and 0.145 and increases with the microalgae concentration. The trend goes towards 0.16 for a concentration of $120\ g/L$.

Based on these experimental values, the microwave applicator was designed for $\varepsilon'_{max} = 80$ as an upper boundary for the dielectric constant. Additionally the lower boundary should be at least $\varepsilon'_{min} = 60$, but taking into account that sample heating might result in a significant permittivity drop since it is mainly made of water [36] and leaving the possibility to work with solvents of lower polarity, the lower boundary was chosen as $\varepsilon'_{min} = 10$. Such a broad range of permittivity will require a tuning element in the applicator as will be developed in section IV-B.

The results additionally indicated that the microalgae cells cause a significant drop of the relative permittivity ε' and an increase of the loss tangent, $tan\delta$. This suggests that the cells are better microwave absorbers than the surrounding water-based medium. Since a large portion of the microalgae cells volume is water and another large portion is made of neutral lipid droplets [14] which are weak absorbers, it can be assumed that small components, probably the cell membrane made of phospholipids or maybe the cell wall, will absorb a large fraction of the microwave energy. This therefore suggests a selective coupling of energy into specific parts of the microalgae cells.
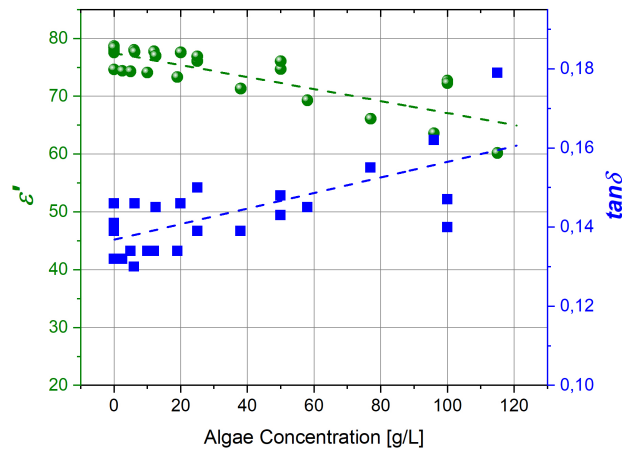

Fig. 1. Real relative permittivity ε' and $tan\delta$ of sample material, *Auxenochlorella Protothecoides* in cultivation medium as a function of concentration at room temperature. Measurements are from 3 independent cultivations of microalgae on three different days.

III. APPLICATOR DESIGN

A. ESTIMATION OF HEAT TRANSPORT CHARACTERISTIC TIME

According to the dielectric measurement results presented in the previous section, the microalgae cells will absorb more microwave radiation than their environment. This results in more efficient heating of the microalgae cells as compared with the surrounding medium. However, due to the small size of the cells, the temperature in the suspension will reach equilibrium very fast. We suppose that the disruption of cells will happen if the temperature gradient across the wall of the cell will reach a level sufficient for the resulting mechanical stress to break (fully or partly) the cell wall. In order to reach such a high temperature gradient, the microwave energy needs to be delivered during a period of time much shorter than the temperature equilibrium time.

To estimate the characteristic temperature equilibrium time, a simplified heat transfer problem which is commonly used [37] [38] consists in considering that a microalgae cell in a medium can be represented as a sphere of water surrounded by water. Initially, the sphere of water, representing the microalgae cell, has a homogeneous temperature of $100°$ C in a whole volume. Then, it is placed instantly in surrounding water environment whose temperature is of $20°C$. Thus, at time $t = 0$ the temperature gradient at the interface between the microalgae and the medium is of $\Delta T_{max} = 80°C$. Next, we evaluate how the temperature inside the microalgae volume will relax to the temperature of the surrounding medium due to convection heat transfer at the microalgae/medium interface. We assume for the simplicity, that at any time, the temperature gradient inside the microalgae sphere is negligible and the temperature of the surrounding medium T_s is always $20°C$. The rate of change of stored energy E_{st}, within the microalgae cell can be approximated by equation 1 where V_{algae} is the volume of the microalgae cell, $\varrho = 1000 \ kg \cdot m^{-3}$ is the water density and $c_p = 4184 \ J \cdot kg^{-1} \cdot K^{-1}$ is the water heat capacity.

$$\frac{dE_{st}}{dt} = \varrho \cdot V_{algae} \cdot c_p \cdot \frac{dT}{dt} \tag{1}$$

This change of stored heat energy within the cell is balanced by energy flowing out of the sphere boundary, E_{conv}, due to heat convection in surrounding media, which can be expressed with equation 2. The coefficient h_{water} is the convection heat coefficient of water, which for forced convection may range between 1 and 10 $kW \cdot m^{-2} \cdot K^{-1}$, A_{algae} is the surface of microalgae sphere.

$$\frac{dE_{conv}}{dt} = h_{water} \cdot A_{algae} \cdot (T(t) - T_s) \tag{2}$$

It can be shown that the solution of equations 1 and 2 for the temperature inside microalgae, $T(t)$, exponentially decreases as follow:

$$T(t) = T_s + \Delta T_{max} exp(-t/\tau_b) \tag{3}$$

with a characteristic time, τ_b, given by equation 4 [39].

$$\tau_b = \frac{\varrho_{water} \cdot V_{algae} \cdot c_p}{h_{water} \cdot A_{algae}} \tag{4}$$

Thus, for a radius of microalgae cell of $r_{algae} = 5 \ \mu m$, the characteristic temperature equilibration time can range between 700 and 7000 μs depending on the value of the convection coefficient. Based on this estimation, the microwave applicator should deliver the microwave in the form of pulses with duration sufficiently shorter than 700 μs. Assuming that the microalgae consists of a 50/50 mixture of water and oil, and since plant oils have a heat capacity close to the half of the heat capacity of water, the estimation of τ_b would be approximately 25% lower. Note that an exact calculation of the temperature equilibrium time constant would require the knowledge of thermal characteristic of the microalgae cell and of the cell wall structures. Unfortunatly such microscopic data are not available and only some macroscopic homogeneized measurements of microalage suspension can be found [40]

B. POWER DENSITY ESTIMATION

The absorbed power density in the microalgae suspension for one single pulse, is defined by the desired temperature increase and the MW pulse duration, τ_h. For the physical model considered in previous section, the guaranteed steep temperature gradient at the microalgae cell wall may be reached if the microwave pulse length is several times shorter than the obtained estimation of relaxation time. For estimation we take a microwave pulse duration of $\tau_h = 100\mu s$ (seven times shorter than the minimum relaxation time estimated above) and a temperature increase $\Delta T = 80°C$. Thus, for water density ρ_{water} and heat capacity c_p, the absorbed power density p inside the microalgae cells suspension can be estimated according to equation 5. Thus, for water density ϱ_{water} and heat capacity c_p, the required power density p inside the microalgae cells can be estimated according to equation 5.

$$p = \frac{\Delta T \cdot \varrho_{water} \cdot c_p}{\tau_h} \simeq 3 \cdot 10^{12} \ W/m^3 \tag{5}$$

Note, that in the above calculation we neglect the heat loss to the surroundings during the heating phase. Also, due to selective heating properties of microwave, the power absorbance in the microalgae cells is larger than in the medium and is certainly not homogeneous within the cell. The calculated power density value is therefore an upper limit for the macroscopic power density in the sample. It is worth to be mentioned that to reach such a high power density in the treated material in the system equipped with the microwave sources available on the market (which are ranging from 1 to 10 kW), the microwave power needs to be concentrated within a sample volume of about $1 \ ml$. This point will be discussed further in the section IV-B. It should be noted that previously published microwave assisted extraction studies on microalgae have shown promising results using commercial kitchen microwave ovens with a power density on the order of $p = 5 \cdot 10^4 \ W/m^3$ [34] which is eight orders of magnitude less than the value calculated above. Therefore, a reasonable trade-off needs to be considered between system costs and sample size to get a power density well above $5 \cdot 10^4 W/m^3$ and close to $3 \cdot 10^{12} W/m^3$.

IV. EXPERIMENTAL SETUP

A. MICROWAVE SOURCE

As discussed in previous sections, the microwave source must be able to generate microwave pulses on the order of microseconds and must be sufficiently powerful at reasonable costs. Magnetrons have a very good power to cost ratio, especially in comparison with solid state devices and are highly reliable. The designed applicator was powered by the air-cooled magnetron, *Philips 2M246*, with nominal output frequency of $2.46 \ GHz$ and a microwave power output of $1 \ kW$ in DC mode. The nominal electrical specifications are: cathode potential $-4 \ kV$, filament voltage $3.15 \ V$, average anode current $380 \ mA$ dc and $59 \ \%$ electrical efficiency in dc mode. The conventional magnetron voltage supply was modified to meet both the technical specification and the experimental requirements i.e. operation in pulsed mode. For that purpose, a custom designed half-wave boost converter was added to the conventional magnetron supply for pulse triggering. The circuit diagram is shown in Fig. 2. The two transformers enable to adjust filament potential and filament voltage. Additionally, the trigger signal enables to generate pulses with varying lengths between 1 and 1000 μs.
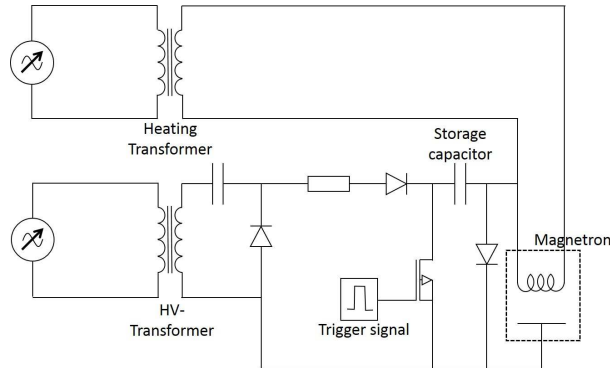

Fig. 2. Microwave source voltage supply principle circuit diagram

The generated microwave signal for a pulse length of $100 \ \mu s$ was recorded with a digital Oscilloscope and its spectral properties analysed with $Matlab^{\circledR}$ (see Fig. 3). The time domain representation (Fig. 3a) shows that the microwave modulating pulse is, as expected, rectangularly shaped with steep raising and falling edges. It takes less then $100 \ ns$ for the power level to build up and about $500 \ ns$ for the power ripples to disappear. These ripples are presumed to be partially originated from the transient behavior of the magnetron itself and partially from the transient behavior of the voltage supply and trigger mechanism. The output spectrum is centered around $2.4665 \ GHz$ i.e. slightly higher than nominal, with a $3 \ dB$ bandwidth of $700 \ kHz$, which is much narrower than the specification for the dc mode. Additionally, a shift of the central frequency of about $380 \ kHz$ is observed during the $100 \ \mu s$ pulse, that results from a continuous drop of the voltage at the storage capacitor array and therefore at the cathode. Thus, the effective bandwidth during a $100 \ \mu s$ pulse is of approximately $1 \ MHz$.

The magnetron background generation before and after the pulse (without applied high voltage) has some deviation in frequency as compared with the wave generated within the pulse (see Fig. 3b).

B. MICROWAVE APPLICATOR

The dedicated microwave applicator was designed to enable efficient coupling of the microwave energy into the microalgae sample. The sample is a liquid suspension and should be treated in continuous flow. For practical reasons the quartz tube was chosen as the sample holder, since quartz is mechanically stable and transparent for microwaves. The cylindrical geometry of
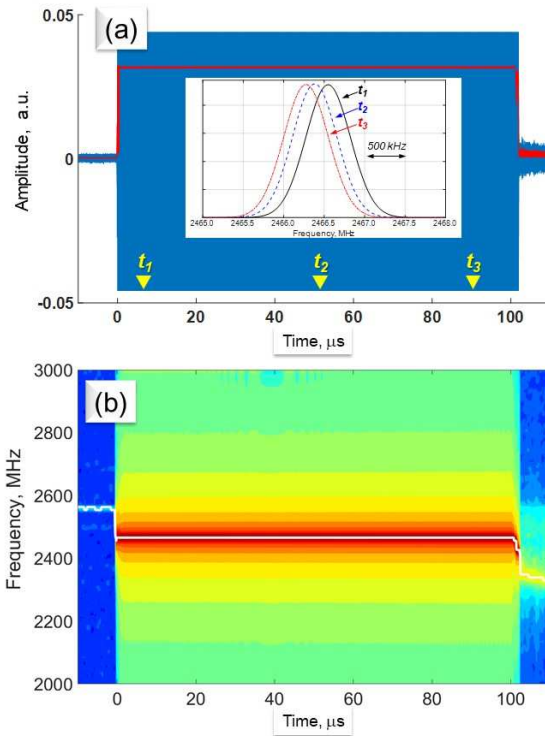
Fig. 3. (a) Time trace of magnetron wave generated as a $100 \mu s$ pulse (blue) and its RMS value (red). In the inlet the spectra at three times of pulse t_1, t_2 and t_3 are shown, which indicate the decrease of magnetron frequency in time by about $380\ kHz$. (b) Spectrogram of magnetron wave. The features of wave frequency in the pre-pulse phase (no high voltage applied) and post-pulse phase (high voltage relaxation) are different.

sample holder and the microwave wavelength, λ, which amounts to $122\ mm$ for frequency $2.46\ GHz$, defines at most the choice of the geometry of the applicator. First, the choice between a single-mode (more compact with a volume of $\approx \lambda^3$) and multi-mode resonator was made. Assuming that the permittivity of the microalgae sample varies both during the microwave treatment and from sample to sample because of different algae concentration and different type of solvent used, the single mode resonator is more preferential since it provides a more robust and predictable wave pattern in the sample. In the multimode resonator, the permittivity variation of the sample may lead to the variation in the spectrum of excited modes and energy distribution between them. As a result, the wave pattern in the material sample is much less predictable. The disadvantage is that the length of the sample, which is flowing throw the cavity and is exposed to the microwave, is shorter than it would be in multi-mode resonator which has a larger size by definition. For our experiment, a single-mode cylindrical cavity with the resonance TM_{010} mode at a frequency of $2.46\ GHz$ was chosen. The amplitude of this mode is homogeneous both in the azimuthal and axial directions and decreases with the radius. To accommodate the sample at the maximum of electric field, the quartz tube needs to be positioned at the axis of the cylindrical cavity.

Since the dielectric factor of the sample, ε', is expected to vary between 10 and 80, the shift of resonance frequency in the loaded cavity is expected to be quite large. As the frequency of magnetron is fixed to $2.45\ GHz$, the resonance frequency of the reactor needs to be tuned back to the frequency of the microwave source. This is the reason why the reactor has to be equipped with a frequency tuner. The tuner was designed as a hollow metallic cylinder which is positioned at the cavity axis and enters the cavity from its upper side. To block the microwave leakage through the tuner a choke structure filled with Teflon was designed. The electromagnetic design of the full system and its optimization is performed with the aid of $CST\ Microwave\ Studio^{\circledR}$. Fig. 4 shows the designed CST model of the full system which includes the port (1), the taper (2), the cavity (3), the quartz tube holder (4), the tuner (5) and the filter (6).

The microwave power enters the system through the waveguide port (1). The taper (2) provides broadband, low reflection impedance matching between the feeding *WR 340* waveguide and the cavity. The design of a single mode resonant cavity (3) was aimed to get the best matching of the magnetron wave at of $2.46\ GHz$ when the cavity is loaded with the quartz tube filled with microalgae suspension. With $2\ cm$ in height and $4.2\ cm$ in radius, this cavity enables the symmetric TM_{010} mode
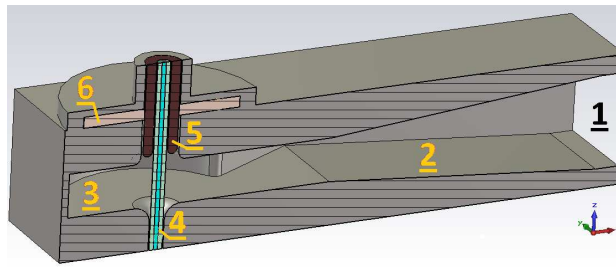
Fig. 4. cavity based applicator cross section: **1** waveguide port; **2** taper; **3** TM_{010} resonator; **4** quartz tube; **5** tuning pin; **6** filter

with the maximal electric field strength at the cavity's axis. Simulation results of the electric field distribution inside the cavity and the sample are shown in Fig. 5.
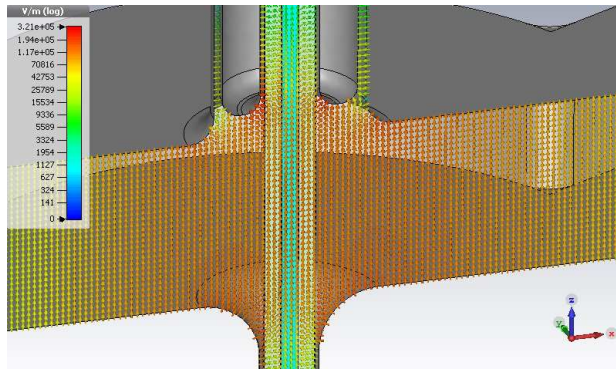

Fig. 5. Electrical field vector distribution in the cavity and sample with $\varepsilon' = 80$ at $f = 2.46\ GHz$. The input power is $4000\ W$.

A quartz tube with inner and outer diameters of $2\ mm$ and $6\ mm$ respectively is used. The effective volume of the sample in the cavity is therefore of $\approx 6.2 \cdot 10^{-8}\ m^3$. Assuming the input microwave power of $4\ kW$ is fully absorbed in the microalgae sample, we can estimate the upper bound of the absorbed power density in the sample as $4000\ W/6.2 \cdot 10^{-8}\ m^3 \approx 6.4 \cdot 10^{10}\ W/m^3$. The full-wave simulation in *CST Microwave Studio* has showed that the absorbed power density in the sample is quite homogeneous (Fig. 6) and has a maximum of $2.4\ \cdot\ 10^{10}\ W/m^3$. It is in quite good agreement with the estimate made above taking the Ohmic losses in the cavity's walls and back reflection (not perfect matching) into account.
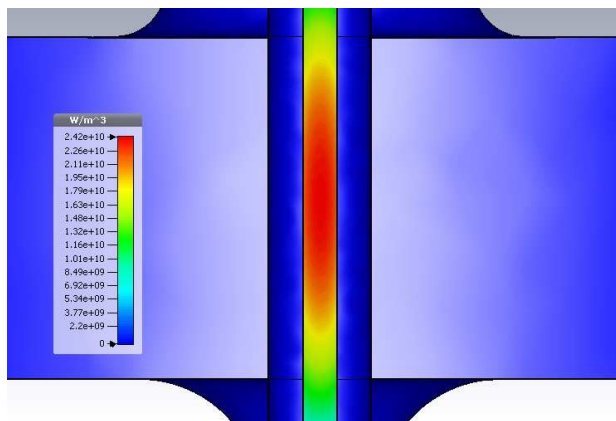

Fig. 6. Absorbed power density distribution in the sample with $\varepsilon' = 80$ at $f = 2.46\ GHz$. The input power in the simulation is $4000\ W$.

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The coaxial tuning element (5 on Fig. 4) allows to compensate the resonance frequency shift caused by changing sample permittivity in the range of $10 \leq \varepsilon' \leq 80$. By moving the tuning element in and out of the cavity the resonance peak may be tuned over $350\ MHz$. This allows to match the resonance frequency of the cavity loaded with dielectric sample to the microwave source frequency thus keeping the maximum efficiency.

In Fig. 7 the power reflection factor S_{11} for three samples with the dielectric factor $\varepsilon' = 80$, $\varepsilon' = 45$ and $\varepsilon' = 10$ and three corresponding tuner positions are presented. Note that the minimal reflection ($-13\ dB$ or 5% of the input power) is achieved at $\varepsilon' \sim 45$. For the samples with $\varepsilon' = 10$ and $\varepsilon' = 80$, the coupling is less efficient: 22% of the input power is reflected.
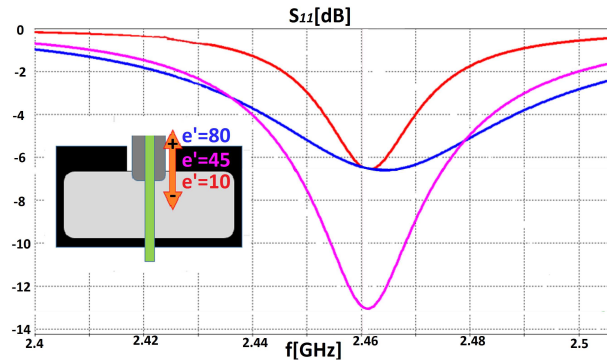

Fig. 7. Simulation of S_{11} power reflection parameter of the tuned cavity with permittivity of sample material: $\varepsilon' = 80$, $\varepsilon' = 45$ and $\varepsilon' = 10$

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The cavity (see Fig. 8) was manufactured from buck aluminum using a CNC milling machine (see Fig. 8). It consists of two almost symmetrical top and bottom pieces, which are connected with screws. The tuning pin is cut from a brass alloy. The designed filter prevents efficiently the parasitic leakage through the tuning structure with an attenuation estimated at $-72\ dB$ from CST simulation, thus allowing a safe operation at full range of input power.
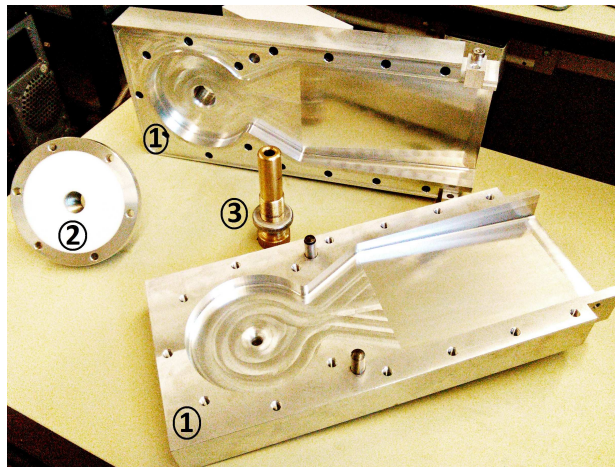

Fig. 8. Fabricated microwave cavity: **1** cavity top and bottom pieces; **2** choke filter; **3** tuning pin.

C. POWER MEASUREMENT

For the characterization of the microwave assisted disruption of microalgae an accurate power measurement is necessary. To estimate the microwave power absorbed in microalgae suspension simultaneous measurements of both input (s_i) and reflected (s_r) power are required. Since the cavity has only one port and parasitic leakage as well as wall losses are negligible, the power absorbed in the microalgae suspension can be evaluated as the difference between power coupled in and power coupled out of the cavity.

The microwave circuitry between source and load is made from a *WR-340* standard waveguide network and includes transmission lines, a circulator, a matched load for the absorption of reflected power and a bi-directional coupler (*MUEGGE MW7009A-260EC*) placed between the cavity and circulator (Fig. 9). In the scheme we distinguish four points to calibrate: the magnetron port *(M)*, the cavity port*(C)* and the two oscilloscope ports *(P1, P2)* connected to the bi-directional coupler (see Fig. 9). For the power calibration, the transmission coefficients between the above four ports are determined using the network analyzer *E5071C ENA Series, Agilent Technologies*. The signals (s_{P1}) and (s_{P2}) are measured at the oscilloscopes ports $P1$ and $P2$. They are always the sum of weighted input (s_i) and reflected (s_r) signals as expressed in equation 6 and 7 where the coefficients S_{1M}, S_{2M}, S_{1C}, S_{2C} are the power transmission factors from the source (M) and cavity (C) to two measuring ports of the oscilloscope.
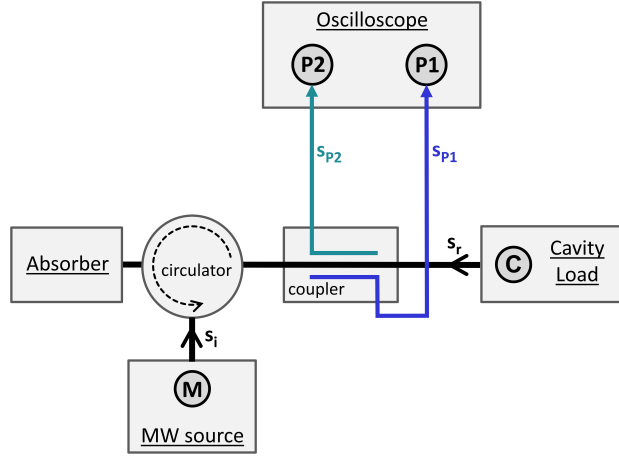
Fig. 9. Power calibration setup with the four ports **C**, **M**, **P1**, **P2**

$$s_{P1} = s_i \cdot S_{1M} + s_r \cdot S_{1C} \tag{6}$$

$$s_{P2} = s_i \cdot S_{2M} + s_r \cdot S_{2C} \tag{7}$$

The equations 6 and 7 yield the *4-term* equations for finding of the input s_i and the reflected s_r signals (equations 8 and 9).

$$s_i = \frac{s_{P1} \cdot S_{2C} - s_{P2} \cdot S_{1C}}{S_{1M} \cdot S_{2C} - S_{2M} \cdot S_{1C}} \tag{8}$$

$$s_r = \frac{s_{P2} \cdot S_{1M} - s_{P1} \cdot S_{2M}}{S_{1M} \cdot S_{2C} - S_{2M} \cdot S_{1C}} \tag{9}$$

Due to the high directivity ($\approx 25dB$) of the bidirectional coupler the S_{2M} and S_{1C} terms in the equations 8 and 9 may be neglected, thus leading to simplified *2-term* equations (equations 10 and 11).

$$s_i = \frac{s_{P1}}{S_{1M}} \tag{10}$$

$$s_r = \frac{s_{P2}}{S_{2C}} \tag{11}$$

The signals from both bidirectional coupler ports can be measured simultaneously with a *LeCroy WaveRunner 640Zi* $4\,GHz$ digital oscilloscope.

For the absorbed power estimations the oscilloscope signals are calibrated in terms of power using a power meter *Anritsu ML2488B*. The recorded oscilloscope data are processed to find the absolute power absorbed in the cavity.

Such an on-line monitoring of input and absorbed power is necessary since the cavity resonance frequency depends on microalgae concentration, sample temperature and formation of gas bubbles in the sample. Moreover, the generated microwave frequency and power may vary due to the variation of temperature of the magnetron and fluctuations in supply voltage.

D. *EXPERIMENTAL SETUP OVERVIEW*

The final experimental setup consists of the pulsed microwave source, a waveguide network including circulator and matched load (absorber), a bidirectional coupler and the loaded cavity. For the measurement of the microwave signals the oscilloscope is connected to the ports of the bidirectional coupler to measure the reflected and transmitted microwave power (Fig. 10).

The feed suspension is contained in a glass vessel placed on a magnetic stirrer which prevents sedimentation of the microalgae (A in Fig. 10). A peristaltic pump with adapted connectors provides a continuous flow of the microalgae suspension material from the feed container, through the quartz tube inside the cavity, to the collecting container (B in Fig.10). The pump flow rate can be adjusted very precisely. Typical working flow rate was chosen at $0.125\ ml/min$.
Thermocouple sensors introduced into the quartz tube inside the tuning pin and inside the feeding tank enable to control the material temperature before and after the microwave treatment.
With switched off microwave source, a network vector analyzer connected to the circulator port instead of the absorber can be used for pre-tuning of the resonance frequency of the reactor.
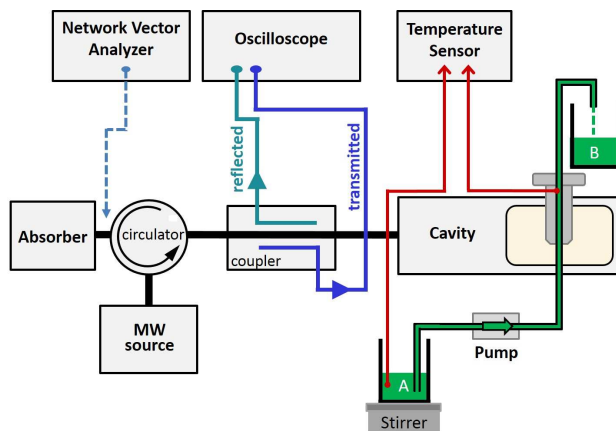
Fig. 10. Experimental setup overview

V. PROOF OF PRINCIPLE

A proof of principle experiment was designed in order to test the complete setup. Fresh microalgae of the strain *Auxenochlorella prototothecoides* were cultivated mixotrophically. Details about cultivation can be found in [14]. The microalgae were harvested in the stationary phase and concentrated by centrifugation to a concentration $C = 200~g_{DW}/l$ i.e. 200g of microalgae dry weight (DW) per liter of suspension. Indeed, for this microalgae, the viscosity of the suspension remains low even at such a high concentration and the suspension can still be easily pumped. A high concentration is a good approach to reduce the energy required per kilogram of microalgae dry weight and therefore the highest possible concentration was used for this first test. The microalgae suspension was pumped through the system at a flow rate of $\dot{V} = 0.4~ml/min$ and exposed to microwave pulses of $T = 270~\mu s$ applied with a repetition rate of $f_{rep} = 3~Hz$. The output power generated by magnetron was set at the maximum value of $Q_{output} = 4.2~kW$ and the absorbed power was on average of $Q_{absorbed} = 3.6~kW$, as evaluated from the oscilloscope traces. The parameters were chosen in order to have an energy input around 1.5-2.0 MJ per kilogram of microalgae dry weight, since we know from our other work, that this level of energy is efficient on this microalgae in case pulsed electric field (PEF) is used as a pre-treatment. MW and PEF are very different and have completely different mode of action but this level of energy was used as a starting point [14] [15].

The specific absorbed energy $E_{absorbed}$ and the specific output energy E_{output} are given by equations 12 and 13. Numerical applications indicate a specific absorbed energy of $E_{absorbed} = 2.2~MJ/kg_{DW}$ and a specific output energy of $E_{output} = 2.5~MJ/kg_{DW}$. Considering that the electrical efficiency of the magnetron is in the range of 60%, this corresponds to a total consumed energy of about 4.2 MJ/kg_{DW}. Control samples were pumped through the cavity but not submitted to microwave treatment.

$$E_{absorbed} = \frac{f_{rep}TQ_{absorbed}}{\dot{V}C} \tag{12}$$

$$E_{output} = \frac{f_{rep}TQ_{output}}{\dot{V}C} \tag{13}$$

After microwave exposure, a lipid extraction was performed according to the protocol described in [14]. In brief, samples were centrifuged and supernatant was removed. The microalgae pellet was then resuspended in a mixture of ethanol and n-hexane and left in the dark with constant agitation. After 20 hours, samples were centrifuged. Fig. 11 illustrate the aspect of the samples after this step. One can note that the microalgae pellet of the control sample is very green in comparison to the treated microalgae pellet which significantly bleached. Additionally the ethanol/hexane mixture has a light yellow color for the control sample and is intense green for the microwave treated samples.

Oil dissolved in the ethanol/hexane phase was recovered using standard phase transfer method. The average oil yields obtained from duplicates was of 6% of microalgae cell dry weight (CDW) for the control sample versus 35% for sample treated with microwave. Absolute oil content of the microalgae was evaluated using bead-milling and soxhlet extraction and was mesured at 38% of CDW (for details see [14]). The macroscopic material temperature at the measurement point after treatment stayed below $40°C$, eventhought the generation of some local hot spots during the process cannot be excluded. Nevertheless, the treated material kept its typical green color, thus suggesting the non-destruction of the chlorophyll and therefore a mild extraction process.

Fig. 11. Pictures of control and MW-treated samples after 20h of extraction in ethanol/hexane mixture. Details of MW-treatment and extraction protocol are given in the text.

VI. CONCLUSION AND DISCUSSION

In this study, a novel applicator for microwave treatment of microalgae was designed, built and tested by performing preliminary experiments on fresh microalgae suspension. The innovative concept of the applicator consists in delivering high microwave power to the sample during a time interval shorter than the millisecond. This enables to take advantage of microwave selectivity and to achieve high temperature inside the cells, generating therefore higher temperature and maybe pressure gradients. Since the suspension is processed in continuous flow, temperature rapidly decreases as soon as the suspension exits the microwave cavity. Such a temperature profile should be beneficial to preserve thermo-sensitive molecules.

The first proof-of-principle experiment demonstrated that applying pulsed microwave energy, was successful to induce some disruption of fresh microalgae cells. Indeed, solvent extraction of oil on MW-treated biomass results in yields of 35% normalised to the microalgae dry weight, which represents 92% of the total lipid content while it was much less efficient on unprocessed biomass. The level of energy required in this first test was 4.2 MJ/kg of dry microalgae. As a comparison, the energies for pre-treatment mentionned in the litterature range between one and several hundreds of megajoule per kilogram dry weight [8]. The results obtained therefore suggest that pulsed microwave could compete (after optimisation) with other more established pretreatment methods such as bead-milling or high pressure homogenization which in optimized condition require energy input ranging from 1 to 4 MJ/kg dry weight [13], [41]. In the future, it will be interesting to optimize the microwave power delivery and other parameters such as biomass concentration or flow rate in order to precisely quantify the minimum requested energy for efficient disruption. Keeping the energy consumption as low as possible will be of highest importance in case the microalgae should be used for energetical application such as biodiesel. Indeed, the energy spent along the whole production process should not exceed the energy stored in the microalgae which ranges between 20 and 27 MJ/kg dry weight [42], especially bearing in mind that the cultivation and harvest of the microalgae are already energy demanding with reported values ranging between 1.8 and 11 MJ/kg dry weight [43]. Keeping energy consumption as low as possible might however be less important in case other applications are considered which are not related to the energy application. This can be for example cosmetics, food or feed, pharmaceuticals. From a pure scientific point of view, it will be extremely valuable to compare for the same amount of delivered energy, two different modes of delivery i.e. intense pulsed microwave power during a few tens or hundreds of microseconds versus a more standard delivery of much lower microwave power but in a continuous way during several minutes. These kind of experiments could indeed help to better understand the mechanisms of cell disruption by microwave.

Demonstrating the efficiency at the laboratory scale is the preliminary step to any further development but such a study only makes sense if up-scaling is possible. In order to increase the flow rate of such a system, several options can be considered. Increasing the cross section of the tube is a first possibility. This will require an increase of the power of the MW generator in case it is important to keep the power density constant. For magnetrons, sources up to 20 kW (at 2.45 GHz) are readily available which already enables an increase of the cross section of the tube by a factor 5 (currently our magnetron delivers 4kW). If the experiments which will be performed in the future suggest that the power density can be reduced, it will be possible to even further increase the cross section. Additionally it can also be considered to work at lower ISM-band frequencies such as 915 MHz or 433 MHz especially since magnetrons for those frequencies have higher efficiency i.e. around 85-90% and higher output powers. Another possibility to increase the throughput of the system is simply to increase the velocity and to apply the MW pulses with higher repetition rates. In the paper, the magnetron was operated with a repetition rate of 3 Hz for pulses of $270\mu s$ which translates into a duty cycle smaller than 10^{-3} and therefore can largely be increased. Finally, parallelization of several single mode cavities or the use of a multimode cavity is a last option to even further increase the total amount of material that can be processed per unit of time. However, in a multimode cavity, tailoring the electric field pattern in such

a way to couple microwave energy with a maximum efficiency will represent a new challenge. Food industry which already uses microwaves for cooking or sterilization of large volumes of water-based material has many solutions available to propose that could be adapted or serve as inspiration [19]. The multimode resonance cavities approach in particular, is a strategy often used in the food industry to treat large volumes [20]. An additional advantage of microwave as a pre-treatment is that microalgae suspension is never in contact with metallic parts such as ventils, beads or electrodes. This could largely reduce eventual mechanical wear over time when large volumes have to be processed and would therefore decrease maintenance costs. Moreover, since the sample is confined in a quartz tube and the system is operated at atmospheric pressure, no additional MW windows are required in the waveguide and in the cavity. Therefore, any contamination in the waveguide part of the system, or in the applicator and on the outer surface of the tube is not possible. The contamination of the inner surface of the quartz tube, which is in contact with the liquid sample, is also unlikely since quartz is chemically very stable. A possible risk of deposition of some solid particles on the inner surface of the tube (e.g. due to non-appropriate temperature and flow regime) cannot be excluded [44]. In that case some cleaning approaches might be helpful. Reciprocally, the fact that the sample is confined in the quartz tube prevents any contamination of the treated suspension by small metallic particles which could be problematic in case the targeted molecules to be extracted e.g. therapeutic agents are under strict regulation rules. Finally, microwave technology is already largely accepted by consumers and therefore social acceptance would also not be a challenge for dissemination of final products.

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