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# Effect of Enzymatic Beech *Fagus Sylvatica* Wood Hydrolysate on *Chlorella* Biomass, Fatty Acid and Pigment Production

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**Abstract:** This work evaluates the possibility of applying enzymatic beech wood (*Fagus sylvatica*) hydrolysate as a feedstock for *Chlorella sorokiniana* growth, and fatty acid and pigment production. Beech wood solids were pretreated with NaOH at high temperature to partially remove xylose and Klason lignin, and enable production of glucose during subsequent enzymatic hydrolysis. Neutralized wood enzymatic hydrolysate containing glucose (TGP-Enz10), was tested on *Chlorella* growth during heterotrophic cultivation and compared with microalgae growth in a medium containing synthetic glucose (TGP). Results show that enzymatic hydrolysate enabled *Chlorella* growth in the dark for biomass, fatty acid and pigment production due to the presence of glucose, although the productivity obtained was smaller, if compared to heterotrophic cultivation in a synthetic TGP medium. Partial growth inhibition and diminished productivity in wood hydrolysate supplemented *Chlorella* culture was due to the presence of neutralized citrate buffer. Neutralized citrate buffer (TGP-Cit10) was found to partially inhibit heterotrophic growth and also strongly suppress mixotrophic growth in *Chlorella* culture. This buffer was also shown to alter fatty acid composition and to slightly affect $Chl_{Total}/Car_{Total}$ ratio during heterotrophic cultivation. Heterotrophic *Chlorella* cultivation with TGP-Enz10 showed that wood enzymatic hydrolysate can constitute a potential feedstock for microalgae cultivation, although the composition of the buffer used during enzymatic hydrolysis should be taken into consideration.

**Keywords:** wood hydrolysate; microalgae cultivation; bioreactor; fatty acids; pigments

## 1. Introduction

Microalgae are photoautotrophic microorganisms, capable of using light energy to fix $CO_2$ and discharging $O_2$ as a waste product during photosynthesis [1]. Microalgal cells contain valuable biocompounds, such as fatty acids and pigments, which can find applications in many branches of industry [2]. High amounts of microalgal biomass are necessary to produce target biocompounds from microalgae in a feasible process [3]. High microalgae biomass concentrations can be achieved during mixotrophic or heterotrophic cultivation. During mixotrophic or heterotrophic cultivation, microalgae growth can be supported with organic carbon such as glucose, resulting in high microalgal biomass productivity [4].

Glucose can be obtained from cellulose, a carbohydrate polymer found in lignocellulosic materials, upon hydrolysis with the use of cellulosic enzymes [5]. Due to the presence of hemicellulose and

lignin in lignocellulose structure, a pretreatment method needs to be applied prior to enzymatic hydrolysis [6,7]. There are numerous pretreatment methods, such as irradiation, explosion, acid, alkaline, hot water, oxidation and organosolvent processes that can improve, with different efficiencies, enzymatic hydrolysis of cellulose [6–8]. Alkaline pretreatment is a method involving the use of alkaline solutions to remove lignin and hemicellulose and to efficiently increase accesibility of cellulose to enzymes, thereby sharply improving saccharification rate [8].

Nowadays, mostly yeast and bacteria are cultivated on lignocellulosic hydrolysates for fatty acid production [9]. Lignocellulosic hydrolysates also have potential to be used as feedstocks for microalgae growth and biocompound production [10]. Hence, in this study, an enzymatic hydrolysate from alkali pretreated beech *Fagus sylvatica* wood was tested as a feedstock for *Chlorella* cultures. Beech (*Fagus sylvatica*) is a deciduous tree species spread around Europe, also commonly found in Belgium [11], and was a subject of hydrolysis studies in our previous reports [12,13].

Enzymatic hydrolysates from bagasse [14] and straw [15] were tested in terms of their effect on *Chlorella* growth and fatty acid production. However, the effect of the enzymatic hydrolysate from wood on *Chlorella* growth and fatty acid and pigment production was not reported before.

## 2. Materials and Methods

### 2.1. Materials

Beech *Fagus sylvatica* wood material from a region of the Ardennes was collected and ground into 4 mm particles. *Chlorella sorokiniana* (SAG, Culture Collection of Göttingen University) was maintained on agar plates.

### 2.2. Wood Alkaline Pretreatment and Enzymatic Hydrolysis

*Fagus sylvatica* wood particles (4 mm), were soaked in 7% NaOH solution at a liquid/dry matter of 20/1 in a double-necked boiling flask, situated on a heating plate and under cooling column. During 1 h hydrolysis at 100 °C, stirring was continuously applied (250 rpm) and temperature was controlled by an external probe. After 1 h, the hydrolysate was cooled and dark-coloured slurry was filtered under vacuum. Remained solids were rinsed with distilled water (to remove slurry remnants) and collected for content analysis and enzymatic hydrolysis. Wood particles were milled into powder and analyzed for sugar (glucose, xylose) and Klason lignin content, as reported previously [13]. Pretreated wood solids were enzymatically hydrolyzed by Celluclast® 1.5 L (cellulase mixture) and Novozym 188 (cellobiase) (Novozymes A/S Denmark) [16], at a dose level of 0.16 mL cellulase/g pretreated solids and 0.08 mL cellobiase/g pretreated solids. Wood solids were suspended in citrate buffer (0.05 M, pH 4.8) at a concentration of 7.5% dry matter/buffer. Hydrolysis was conducted in 50-mL capped Duran bottles, situated on a magnetic stirrer (250 rpm) and submerged in a water bath at 50 °C for 72 h. Glucose concentration in enzymatic hydrolysates was measured with the use of D-Glucose Assay Kit (© Megazyme International), (GOPOD Format). Saccharification rate (%) was calculated based on glucose content in alkaline pretreated wood solids before enzymatic hydrolysis. Enzymatic hydrolysates were heated in a boiling bath for 5 min, cooled down, filtered and stored in a freezer (−20 °C).

### 2.3. Microalgae Cultivation in Medium Supplemented with Organic Carbon

Experiments with *Chlorella* cultivation were conducted in a multi-tube photobioreactor (Photon Systems Instruments: PSI, Drasov, Czech Republic), (Multi-cultivator MC 1000–OD), with light irradiance set at 0 μE $m^{-2}$ $s^{-1}$ (heterotrophic growth) or 75 μE $m^{-2}$ $s^{-1}$ (mixotrophic growth) and temperature at 25 °C. The medium used for growth was TRIS-minimal medium (TMP), with a composition of: Tris (2.42 g/L), $NH_4Cl$ (0.4 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L), $CaCl_2 \cdot 2H_2O$ (50 mg/L), $K_2HPO_4$ (93.5 mg/L), $KH_2PO_4$ (63 mg/L), $Na_2EDTA$ (50 mg/L), $H_3BO_3$ (11.4 mg/L), $ZnSO_4 \cdot 7H_2O$ (22 mg/L), $MnCl_2 \cdot 4H_2O$ (5.06 mg/L), $FeSO_4 \cdot 7H_2O$ (4.9 mg/L), $CoCl_2 \cdot 6H_2O$ (1.61 mg/L), $CuSO_4 \cdot 5H_2O$

(1.57 mg/L) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (1.1 mg/L). *Chlorella* was cultivated in TMP (pH = 7) medium supplemented with glucose (TGP), glucose-containing 10% neutralized (pH = 7) enzymatic hydrolysate (TGP-Enz10) or glucose with 10% neutralized (pH = 7) citrate buffer (TGP-Cit10). In all profiles, glucose concentration was set at 1 g/L. Additionally, the effect of different loadings (0.5%, 1%, 2%, 5%, 10%, 15%, 20%) of neutralized (pH = 7) citrate buffer (100% = ~0.05 M) on *Chlorella* growth was evaluated. Enzymatic hydrolysate and citrate buffer were neutralized to pH = 7 with concentrated NaOH solution. A list of abbreviations is included in the end of the text.

### 2.4. Growth Rate Measurement

Growth of *Chlorella* was evaluated from $OD_{720}$ measurements during cultivation according to the equation: $\mu = (\mathrm{Ln}x_1 - \mathrm{Ln}x_0)/(t_1 - t_0)$; where $\mu$ equals to growth rate within $t_1 - t_0$ cultivation time ($h^{-1}$), $x_1$ equals to optical density measured at 720 nm at $t_1$ of cultivation (-), $x_0$ equals to optical density measured at 720 nm at $t_0$ of cultivation (-).

### 2.5. FAME and Pigment Determination in Microalgae Biomass

Fatty acids in *Chlorella* biomass were extracted and converted into Fatty Acid Methyl Esters (FAMEs) and measured by Gas Chromatography, as reported previously [13]. Content of chlorophyll *a*, chlorophyll *b* and total carotenoids in *Chlorella* biomass was determined spectrophotometrically as reported previously [13]. Fatty acid and pigment content in biomass was expressed on dry weight (%) basis.

## 3. Results and Discussion

### 3.1. Pretreatment and Enzymatic Hydrolysis of Wood

Beech wood material contained 44% ± 2 glucose, 17% ± 1 xylose and 20% ± 1 Klason lignin as it was determined in our previous report, where wood particles were subjected to a diluted acid (3%) treatment ($H_2SO_4$) [13]. However, solids collected after the diluted acid treatment were enzymatically hydrolysed with low (<5%) saccharification rate, and concentration of produced glucose was insufficient as a feedstock for microalgae cultures (data not shown). Therefore, alkaline pretreatment, instead of diluted acid pretreatment, was applied before enzymatic hydrolysis. Alkaline pretreatment with the use of NaOH was reported as an effective method to remove hemicellulose and Klason lignin from birch [17], cedar [18] and eucalyptus [18,19] wood, resulting in the increase in cellulose exposure and improvement of cellulose conversion to glucose during enzymatic hydrolysis [18,19]. Temperature of pretreatment and NaOH concentration are crucial parameters determining removal rate of hemicellulose and lignin from wood, and subsequent cellulose hydrolysis. For example, increase in NaOH concentrations from 1% to 8% improved release of hemicellulose and lignin from birch wood [17]. In another study, increase in temperature (from 25 °C to 100 °C) at 5 N [20%] NaOH, considerably improved the saccharification rate of cedar and eucalyptus [18]. Therefore, in our study, higher NaOH concentration (7%), at higher temperature (100 °C), was applied during pretreatment to facilitate hemicellulose and lignin removal, and to enzymatically produce glucose in the amount sufficient for glucose-based microalgal cultivation in a bioreactor. Hydrolysis of beech wood particles with the use of 7% NaOH, for 1 h at 100 °C caused 57% xylose removal and 11% removal of Klason lignin with no effect on glucose. Alkaline pretreated beech wood contained 63% glucose, 10% xylose and 24% Klason lignin (data not shown). Pretreated wood material was enzymatically hydrolyzed over 72 h to release approximately 10 g/L glucose, which corresponded to an approximately 21% saccharification rate (Figure 1).

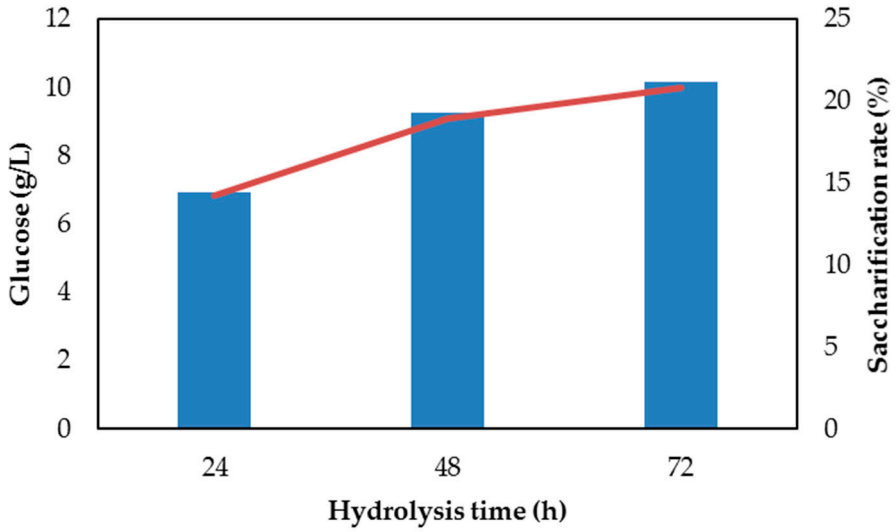


**Figure 1.** Glucose release (line) and saccharification rate (bars) during 72 h enzymatic hydrolysis of alkaline (NaOH) pretreated beech wood solids. Number of independent experiments: 3. Standard error: 5%.

### 3.2. Effect of Neutralized Citrate Buffer on Chlorella Growth

Citrate buffer is a constituent of wood enzymatic hydrolysate, as this buffer is used to keep pH at a level (4.8) optimal for activity of cellulosic enzymes. The addition of neutralized (pH = 7) wood enzymatic hydrolysate into microalgal cultures also involves adding citrate buffer. Therefore, the effect of neutralized (pH = 7) citrate buffer on *Chlorella* growth in TGP (1 g/L glucose) during heterotrophic and mixotrophic cultivation was evaluated. The results of our study show that neutralized citrate buffer can exert an inhibitory effect on *Chlorella* growth (Figure 2). Inhibition of *Chlorella* growth during heterotrophic cultivation increased with the increase in the neutralized citrate buffer loading from 0.5% to 10%, although further loading increases (15%, 20%) did not alter inhibition rate, which remained as for a 10% loading. Citrate buffer contains citric acid, which has been reported to be inhibitory to bacteria [20] and fungi [21]. Citric acid has also been reported to exert positive [22] or negative [23] effects on *Chlorella* growth. Citric acid improved biomass production during *Chlorella vulgaris* cultivation, because it was able to be utilized as a carbon source [22]. In contrast, *Chlorella ellipsoidea* failed to grow in the presence of citric acid, and the inhibitory effect of citric acid was suggested to be due to feedback inhibition of citrate synthase activity or the blockage of DNA synthesis [23]. Besides citric acid, neutralized buffer also contains sodium ions, and it was reported that the occurrence of salt stress in *Chlorella* cells was associated with the presence of $Na^+$ ions [24]. Therefore, the inhibitory effect of $Na^+$ ions accumulated in medium towards microalgae should not be excluded in the case of applying enzymatic hydrolysate at high loadings on industrial scale.

In our study, heterotrophic growth of *Chlorella* was partially inhibited, but the inhibitory effect of neutralized citrate buffer was much more pronounced during mixotrophic cultivation. Figure 2 shows that light-driven growth of mixotrophic conditions is almost completely inhibited in the presence of neutralized citrate buffer, with a growth rate for TGP-Cit10 (L-Light) much smaller than for TGP (L-Light), and nearly at the same level as for TGP-Cit10 (D-Dark).

Based on the facts that the obtained wood enzymatic hydrolysate contains ~10 g/L glucose, and that neutralized citrate buffer seems to block the photosynthetic part of mixotrophic growth, a 10% loading of neutralized wood hydrolysate (TGP-Enz10) was used to obtain the 1 g/L glucose sufficient for *Chlorella* growth during heterotrophic cultivation, to avoid inhibition of light-based growth.

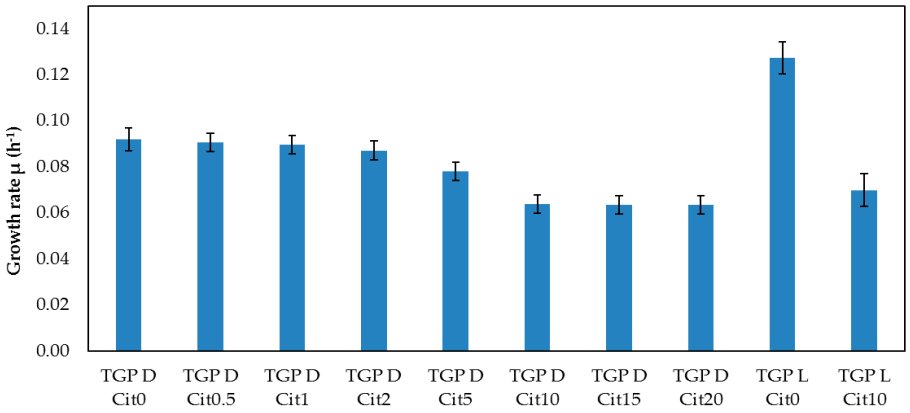


**Figure 2.** Effect of neutralized (pH = 7) citrate buffer on *Chlorella* growth in TGP (1 g/L glucose) medium. Different neutralized citrate buffer loadings: 0%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20% are marked: Cit0, Cit0.5, Cit1, Cit2, Cit5, Cit10, Cit15, Cit20, where 100% neutralized citrate buffer is ~0.05 M. Heterotrophic (D-Dark) growth in photobioreactor at 0 μE $m^{-2}$ $s^{-1}$. Mixotrophic (L-Light) growth in photobioreactor at 75 μE $m^{-2}$ $s^{-1}$. Temperature of cultivation: 25 °C. Number of independent experiments: 3.

### *3.3. Effect of Enzymatic Hydrolysate on Chlorella Biomass, Fatty Acid and Pigment Productivity*

Wood hydrolysates were reported to possess negative or positive effects on microalgae growth. Wood hydrolysate from pulp and paper mills [25] or wood hydrolysate from diluted acid treatment [13] inhibited *Chlorella* growth, if applied at high loadings, and supported *Chlorella* growth when applied at low loadings. In our previous study [13], neutralized beech wood diluted acid hydrolysate at a 12% loading partially inhibited *Chlorella* growth, when compared to a synthetic organic carbon-based medium. In the literature, lignocellulosic hydrolysates contain a range of compounds that inhibit the metabolism of yeast [26,27], and can also negatively affect microalgae growth [10]. In the present study, neutralized enzymatic beech wood hydrolysate at 10% loading (TGP-Enz10) also inhibited *Chlorella* growth. However, inhibitory activity of this hydrolysate seems to be attributed solely to the presence of neutralized citrate buffer (TGP-Cit10). Our results show (Tables 1 and 2) that the inhibitory effect of enzymatic hydrolysate (TGP-Enz10) is not greater that the inhibitory effect of a synthetic medium containing glucose and citrate buffer (TGP-Cit10). This suggests that 10% enzymatic beech wood hydrolysate does not contain lignocellulose-derived compounds that could inhibit microalgae growth, and that the only inhibitory substances are those added externally in order to produce wood hydrolysate. Moreover, inhibition in enzymatic hydrolysate containing neutralized citrate buffer (TGP-Enz10) seems to be partially alleviated when compared to TGP-Cit10. As a result, the following biomass productivities were achieved: 403 ± 20 (mg $L^{-1}$ $d^{-1}$) for TGP, 345 ± 20 (mg $L^{-1}$ $d^{-1}$) for TGP-Enz10 and 279 ± 15 (mg $L^{-1}$ $d^{-1}$) for TGP-Cit10 (Tables 1 and 2).

Therefore, enzymatic wood hydrolysate shows the potential to be used as a feedstock for microalgal biomass production. Moreover, enzymatic wood hydrolysate should contain only glucose as a final product from cellulose hydrolysis. Cellobiose was tested in terms of its effect on *Chlorella* growth, and was found to be neutral, as this sugar neither supported nor inhibited *Chlorella* growth (data not shown). However, cellobiose should be hydrolyzed to glucose in order to improve utilization of sugar for microalgae growth. In our study, the presence of cellobiose in wood enzymatic hydrolysate was not detected (data not shown).

The effect of wood enzymatic hydrolysate on fatty acid production in heterotrophic *Chlorella* culture, was evaluated (Table 1). Fatty acid compositions in *Chlorella* biomass were different during different cultivation profiles. A remarkable change in fatty acid composition was observed between *Chlorella* cultivated in TGP medium and TGP-Enz10 or TGP-Cit10. In TGP medium, the primary

fatty acid was linolenic acid (C18:3), followed by palmitic acid (C16:0) and linoleic acid (C18:2). In TGP-Enz10 and TGP-Cit10, C18:2 was a primary fatty acid (a remarkable increase in % composition), followed by C16:0 and C18:3 (a remarkable drop in % composition). Results suggest that the neutralized citrate buffer present in enzymatic hydrolysate and synthetic TGP medium causes a shift in fatty acid composition. The following fatty acid productivities were achieved: 14.5 ± 0.72 (mg $L^{-1}$ $d^{-1}$) for TGP, 12.1 ± 0.6 (mg $L^{-1}$ $d^{-1}$) for TGP-Enz10 and 10.32 ± 0.52 (mg $L^{-1}$ $d^{-1}$) for TGP-Cit10.

**Table 1.** Biomass productivity, total fatty acid (FA) content, fatty acid productivity and fatty acid composition in *Chlorella* cultures cultivated on 1 g/L glucose (TGP), wood enzymatic 10% hydrolysate containing 1 g/L glucose (TGP-Enz10), and 1 g/L glucose supplemented with 10% neutralized citrate buffer loading (TGP-Cit10). Cultivation conditions: photobioreactor under heterotrophic mode (0 µE $m^{-2}$ $s^{-1}$) at 25 °C. Number of independent experiments: 3.

| Fatty Acid Production | TGP (36 h) | TGP-Enz10 (42 h) | TGP-Cit10 (52 h) |
|---|---|---|---|
| Biomass productivity (mg $L^{-1}$ $d^{-1}$) [A] | 403 ± 20 | 345 ± 20 | 279 ± 15 |
| Total fatty acid content (% d.w.) | 3.6 ± 0.05 | 3.5 ± 0.1 | 3.7 ± 0.1 |
| Fatty acid productivity (mg $L^{-1}$ $d^{-1}$) | 14.5 ± 0.72 | 12.1 ± 0.6 | 10.32 ± 0.52 |
| Fatty acid (FA) composition (% of total fatty acids) | | | |
| C14:0 | 1.00 ± 0.1 | 1.27 ± 0.1 | 1.26 ± 0.23 |
| C16:0 | 29 ± 1.5 | 29.15 ± 0.63 | 28.3 ± 0.56 |
| C18:1 | 5.85 ± 0.5 | 8.31 ± 0.56 | 8.75 ± 0.78 |
| C18:2 | 26.1 ± 0.5 | 41.28 ± 0.39 | 45.33 ± 0.52 |
| C18:3 | 38.4 ± 1.2 | 19.85 ± 1.62 | 16.25 ± 0.49 |

[A] Average daily biomass productivity after specific cultivation time (24+ h). d.w. means dry weight.

The effect of wood enzymatic hydrolysate on pigment production in heterotrophic *Chlorella* culture was evaluated (Table 2). The total chlorophyll content and Chl *a*/*b* was the same in all tested profiles, although the presence of neutralized citrate buffer seemed to decrease $Chl_{Total}/Car_{Total}$ ratio. The following $Chl_{Total}$ and $Car_{Total}$ productivities were achieved: 4.89 ± 0.25 and 1.19 ± 0.06 (mg $L^{-1}$ $d^{-1}$) for TGP, 4.278 ± 0.21 and 1.08 ± 0.054 (mg $L^{-1}$ $d^{-1}$) for TGP-Enz10 and 3.59 ± 0.18 and 0.94 ± 0.047 (mg $L^{-1}$ $d^{-1}$) for TGP-Cit10.

**Table 2.** Chlorophyll ($Ch_{Total}$) content, carotenoid ($Car_{Total}$) content and pigment productivity in *Chlorella* cultures cultivated on 1 g/L glucose (TGP), wood enzymatic 10% hydrolysate containing 1 g/L glucose (TGP-Enz10), and 1 g/L glucose supplemented with 10% neutralized citrate buffer loading (TGP-Cit10). Cultivation conditions: photobioreactor under heterotrophic mode (0 µE $m^{-2}$ $s^{-1}$) at 25 °C. Number of independent experiments: 3.

| Pigment Production | TGP (36 h) | TGP-Enz10 (42 h) | TGP-Cit10 (52 h) |
|---|---|---|---|
| Biomass productivity (mg $L^{-1}$ $d^{-1}$) [A] | 403 ± 20 | 345 ± 20 | 279 ± 15 |
| $Chl_{Total}$ content (% d.w.) | 1.215 ± 0.065 | 1.24 ± 0.03 | 1.288 ± 0.108 |
| $Ch_{Total}$ productivity (mg $L^{-1}$ $d^{-1}$) | 4.89 ± 0.25 | 4.278 ± 0.21 | 3.59 ± 0.18 |
| Chl *a*/*b* ratio (-) | 3.67 ± 0.09 | 3.645 ± 0.085 | 3.857 ± 0.337 |
| $Car_{Total}$ content (% d.w.) | 0.297 ± 0.016 | 0.313 ± 0.008 | 0.337 ± 0.03 |
| $Car_{Total}$ productivity (mg $L^{-1}$ $d^{-1}$) | 1.19 ± 0.06 | 1.08 ± 0.054 | 0.94 ± 0.047 |
| $Chl_{Total}/Car_{Total}$ ratio (-) | 4.09 ± 0.05 | 3.96 ± 0.11 | 3.82 ± 0.18 |

[A] Average daily biomass productivity after specific cultivation time (24+ h). d.w. means dry weight.

## 4. Conclusions

This work shows, for the first time, that wood enzymatic hydrolysate containing glucose can be used as a feedstock to produce *Chlorella* biomass, fatty acids and pigments in the dark. In the literature, high microalgal cell densities can be achieved during heterotrophic cultivation in scaled-up bioreactors [28]. Hence, application of this wood hydrolysate to microalgae cultivation in the dark could be interesting in terms of industrial production in large-volume closed tanks [25]. However, the

hydrolysate used in this study was shown to suppress *Chlorella* growth during heterotrophic cultivation, when compared to synthetic TGP medium. A constituent of wood hydrolysate that possesses inhibitory activity was found to be a neutralized citrate buffer. Neutralized citrate buffer caused a partial inhibition of *Chlorella* heterotrophic growth, and also drastically reduced light-supported growth during mixotrophic cultivation. Moreover, the presence of neutralized citrate buffer caused an alteration in fatty acid (FA) composition, and also slightly decreased $Chl_{Total}/Car_{Total}$ ratio during heterotrophic cultivation. In our previous work [13], neutralized dilute-acid wood hydrolysate was used to support microalgae growth, and in the present work, neutralized enzymatic hydrolysate was used to support microalgae growth. Both hydrolysates possess their advantages and disadvantages. Diluted acid hydrolysate can be prepared in a single hydrolysis step, but the inhibitory effect of this hydrolysate is attributed both to the presence of molecules released from lignocellulose and inorganic compounds used for hydrolysis [13]. Enzymatic hydrolysate requires two steps of preparation, alkaline treatment and subsequent enzymatic hydrolysis, but the inhibitory effect of this hydrolysate seems to be attributed only to the presence of compounds used for hydrolysis.

Our study shows that neutralized citrate buffer exerts a negative effect on microalgae growth and a modulating effect on microalgal metabolism. A promising replacement for citrate buffer could be a sodium acetate buffer, which is also used during enzymatic hydrolysis of lignocellulose biomass [18,19]. This buffer contains acetate, which was shown in our previous study to greatly improve microalgae growth [13], and therefore can constitute an additional carbon source for microalgae cultivated on lignocellulosic hydrolysates. On the other hand, citric acid was also reported to exert positive effect on microalgae culture [22], showing that the citric acid effect is strain specific. Nethertheless, the type of buffer used for enzymatic hydrolysis should be taken into consideration when applying lignocellulosic enzymatic hydrolysates to microalgae cultivation medium.

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**Author Contributions:** Krystian Miazek carried out all experiments and wrote manuscript. Aurore Richel provided expertise in lignocellulose hydrolysis. Claire Remacle provided expertise in microalgal cultivation. Dorothee Goffin developed ideas and designed structure of manuscript. All authors approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

| | |
|---|---|
| TMP | TRIS minimal medium. |
| TGP | TMP medium containing synthetic glucose (1 g/L). |
| TGP-Enz10 | TMP medium supplemented with enzymatic hydrolysate at a 10% loading, containing 1 g/L glucose (from hydrolysate) and neutralized citrate buffer at a 10% loading (from hydrolysate). |
| TGP-Cit10 | TMP medium containing synthetic glucose (1 g/L) and neutralized citrate buffer at 10% loading, where 100% citrate buffer equals to 0.05 M. |
| L-Light | cultivation conditions under light irradiance: 75 $\mu E\ m^{-2}\ s^{-1}$. |
| D-Dark | cultivation conditions without light supplied: 0 $\mu E\ m^{-2}\ s^{-1}$. |
| Cit0 | neutralized citrate buffer at a 0% loading. |
| Cit0.5 | neutralized citrate buffer at a 0.5% loading. |
| Cit1 | neutralized citrate buffer at a 1% loading. |
| Cit2 | neutralized citrate buffer at a 2% loading. |
| Cit5 | neutralized citrate buffer at a 5% loading. |
| Cit10 | neutralized citrate buffer at a 10% loading. |
| Cit15 | neutralized citrate buffer at a 15% loading. |
| Cit20 | neutralized citrate buffer at a 20% loading. |

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