Removal of sugars in wastewater from food production through heterotrophic growth of *Galdieria sulphuraria*

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1 INTRODUCTION

The heterotrophic cultivation of microalgae is gaining in relevance in algae biotechnology [1]. In contrast to photoautotrophic cultivation, biomass is formed in a significantly shorter time due to higher growth rates. Using the same culture media, pH and temperature, a heterotrophic fed-batch culture of *Neochloris oleoabundans* produced 24 g L\(^{-1}\) cell dry weight, compared with 1 g L\(^{-1}\) under phototrophic conditions in the same time [2]. Additionally no lighting for photosynthesis is required and problems with self-shading being eliminated [3].

The metabolic capabilities of microalgae make them also suitable for treatment of wastewater [4–9]. Efficient treatment of wastewater, predominantly measured on removal of N and P, were achieved mostly with...
Materials and Methods

Strain and growth media

Heterotrophic conversion into biomass. The extremophilic red alga *Galdieria sulphuraria* is capable of chemoheterotrophic metabolism, besides photoautotrophy. Generated biomass of *G. sulphuraria* can be used for the production of the valuable nutraceutical phycocyanin and the depletion of sugars during growth can be a relief for wastewater treatment plants. In this study we demonstrate, that dissolved sugars in wastewater from fruit-salad production can be removed by heterotrophic growth of *G. sulphuraria*.

**PRACTICAL APPLICATION**

Wastewater from food production contains sugars and other dissolved organic carbon sources. In some cases, regulatory discharge levels for chemical oxygen demand are exceeded because of these substances. A removal can be achieved by heterotrophic conversion into biomass. The extremophilic red alga *Galdieria sulphuraria* is capable of chemoheterotrophic metabolism, besides phototrophy. Generated biomass of *G. sulphuraria* can be used for the production of the valuable nutraceutical phycocyanin and the depletion of sugars during growth can be a relief for wastewater treatment plants. In this study we demonstrate, that dissolved sugars in wastewater from fruit-salad production can be removed by heterotrophic growth of *G. sulphuraria*.

2  MATERIALS AND METHODS

2.1  Strain and growth media

*Galdieria sulphuraria* strain SAG 21.92 was obtained from Göttingen algae collection (*Sammlung von Algenkulturen*, Göttingen, Germany). The cells were cultivated in a defined growth medium at pH 2 [29]. Stock cultures were supplemented with 12 g L\(^{-1}\) sucrose, glucose and fructose adding up to a total of 36 g L\(^{-1}\) carbon source and the concentration of ammonium sulfate was increased to 9 g L\(^{-1}\). The resulting C:N ratio was about 7.5:1. The cells were grown in the dark in 250 mL shake flasks containing 50 mL suspension. Temperature and rotational speed were 30°C and 150 rpm (shaking diameter = 25 mm), respectively.

2.2  Shake flask cultivation

Growth of *G. sulphuraria* under different conditions was studied in 500 mL shake flasks containing 100 mL suspension. Temperature was varied between 30°C, 42°C, and 50°C, pH-value between 2.0, 3.0 and 4.0. Sugar concentration was varied in the range between 6 and 60 g L\(^{-1}\), containing equal amounts of the three sugars. All cultivations were carried out under sterile conditions and in the dark at 150 rpm rotational speed (shaking diameter = 25 mm).
Evaporation was determined by weight and compensated with sterile water daily.

### 2.3 Stirred tank reactor cultivation

Growth experiments with *G. sulphuraria* were also conducted in a STR containing 3 L suspension. Reactor temperature was set to 42°C, aeration with synthetic air was 3 L·min⁻¹ (1vvm) and the stirrer speed was 150 rpm, respectively. Cultivations were done in defined growth media [29] and in diluted wastewater from fruit salad production. All experiments were conducted under non-sterile conditions. Evaporation was compensated with water before sampling.

### 2.4 Biomass determination

Optical density (OD) was measured at 750 nm (Specord 40; Analytic Jena AG, Germany) to monitor algal growth. If necessary, samples were diluted below 0.4 with deionized water.

To determine cell dry weight (DW) 900 μL cell suspension were filled into dried, pre-weighted glass tubes and centrifuged at 13,000 rpm. The supernatant was collected and frozen for determination of ammonia. The cell pellet was dried for 24 h at 100°C. Measurements were always done in quadruples.

### 2.5 Measurement of substrate concentration

Sucrose, glucose and fructose were used as substrate in all experiments and the only sugars present in the wastewater samples. Concentration of these three sugars was measured using high-pressure liquid chromatography. A Prominence UFLC system (shimadzu corp., Japan) with a PL Hi-Plex Ca²⁺ 8 μm column was used. Samples of 50 μL were eluted with 0.5 mL·min⁻¹ purified water at 80°C, respectively. Sugars were detected with a refraction index detector.

### 2.6 Measurement of ammonia concentration

Ammonia concentration was determined photometrically using an ammonia cuvette test kit (Spectroquant® NH₄-N, Or.-No. 114752, Merck Millipore). Cell-free supernatant from the biomass determination was treated as instructed and absorbance was measured at 600 nm.

### 3 RESULTS

#### 3.1 Characterization of the used wastewater and composition of a synthetic media

All wastewater samples contained sucrose, glucose and fructose, as expected for the procession of raw fruits (see Table 1). Based on these results a model substrate was created containing equal amounts of the three sugars. The term “synthetic media” is used for describing culture media supplemented with the model substrate as carbon source [29]. Total amount of added sugar varied depending on process parameters. Amount of ammonium sulfate was adjusted to C:N-ratio of 7.5:1. Besides the mentioned sugars, no other substances in relevant concentrations could be detected. The water samples contained no biocidal products or detergents, since cleaning solutions were collected separately. Due to the relatively high sugar concentrations, only the water from the apple-cleaning bath would be suitable for cultivation without any further dilution.

#### 3.2 Hydrolysis kinetic due to pH effects

It is already reported, that microalgae have poor capabilities for disaccharide uptake and lack a transportation system for sucrose in general [5,37]. *Lepocinclis sp.*, *Euglena mutabilis*, *Chlorella sacharophila* and *G. sulphuraria* are known to grow on sucrose and it is proposed, that these species produce extracellular hydrolases in order to utilize polysaccharides [29,38,39]. However, hydrolysis of sucrose is also catalyzed by protons, which is estimated to mainly contribute to the rate of cleavage. In Figure 1, the hydrolysis rate of sucrose in synthetic media is shown at different pH values. Growth experiments with *G. sulphuraria* showed, that at pH 3 and 4 substrate availability can be limited if glucose and fructose are depleted and sucrose is not completely hydrolyzed (see Figure 2B). Higher cultivation temperatures had a positive effect on sucrose cleavage rate, as expected. At pH 2 and temperatures above 30°C sucrose hydrolysis had no influence on growth, which is in good accordance with previous studies [32].

#### 3.3 Optimization of process conditions in shake-flask scale

The maximum specific growth rate $\mu_{\text{max}}$ of *G. sulphuraria* SAG 21.92 was $1.53 \pm 0.09$ day⁻¹. It was measured during exponential phase at 42°C, pH 2 and 36 g·L⁻¹ substrate concentration (see Table 2). Cultivation
Figure 1  Hydrolysis of sucrose in culture media at 42°C and pH 2 (○), pH 3 (△) and pH 4 (□) Experiment was conducted in 500 mL shake flasks. Error bars represent standard deviation for two experiments.

Figure 2  (A) Example for a cultivation of G. sulphuraria SAG 21.92 in the STR at 42°C and 15 g L⁻¹ substrate concentration. Measurement of DW (□), OD (●), concentration of substrate (△) and available nitrogen in ammonia (◊) over the course of 24 h. Substrate represents the sum of the three contained sugars sucrose, glucose and fructose. Error bars represent standard deviation. Number of determinations: DW n = 4, OD n = 2, substrate n = 2, ammonia n = 3 (B) Depletion of sucrose (◇), glucose (△) and fructose (○) contained in substrate. Error bars represent standard deviation for two determinations.
temperatures showed the strongest impact on growth of *G. sulphuraria*. Specific growth rate at 42°C was 216% higher than at 30°C and almost 250% higher than at 50°C (at pH 2 and 6 g L⁻¹ substrate concentration). Substrate concentration and pH-value also affected growth. Compared to pH 2, growth occurred 3% slower at pH 3 and 12% slower at pH 4 on average. At pH 2 the highest growth rates were observed at 36 g L⁻¹ at all three temperatures. Above 36 g L⁻¹ growth rate declined. Similar behavior was observed with *G. sulphuraria* 074G grown on glucose [20]. The highest impact of substrate concentration on growth rate could be observed at 30°C. Compared to 6 g L⁻¹ the specific growth rate at 36 g L⁻¹ (pH 2) was 66% higher (see Table 2).

Glucose was depleted before fructose. If glucose is present, it is prioritized over fructose by *G. sulphuraria* SAG 21.92 (see Figure 2B).

Variation of biomass substrate yield Yₓ/S during cultivation experiments was minimal. Higher substrate concentrations seem to lower the biomass yield at 30°C and 50°C, but at 42°C, this trend could not be observed. The lowest yields were measured at 50°C.

Specific substrate consumption rate qₛ was determined to compare efficiency of sugar depletion. Since the biomass substrate yield nearly remained on the same level, the specific substrate consumption rate showed the same trends as the growth rate. Higher growth rates resulted in higher substrate consumption per g biomass. The maximum specific substrate consumption rate of 2.41 ± 0.14 gₘₒₛₐₑ₧⁻¹⋅d⁻¹ was measured at 42°C, pH 2 with 36 g L⁻¹ substrate.

### 3.4 Scale-up study in a stirred tank bioreactor

In shake flasks the fastest growth of *G. sulphuraria* was observed at 42°C, 36 g L⁻¹ and pH 2. However, for a whole bioreactor a considerable amount of sulphuric acid is needed to lower the pH-value of the culture media to the desired level. To minimize the amount of sulphuric acid used for cultivation in the STR, experiments were conducted at pH 3 rather than pH 2. On average a reduction of 3% of the maximum specific growth rate at pH 3 compared to pH 2 was expected, according to data from shake-flask cultivation. A procession of wastewater in a cycle of 24 h was desirable. In order to achieve complete substrate depletion in the course of 24 h, maximum substrate concentration was limited to 30 g L⁻¹ and starting biomass 8 g L⁻¹ DW was chosen. The specific growth rate and sugar consumption rate were determined at 30 and 15 g L⁻¹ substrate concentration in synthetic media, respectively.

The measured specific growth rate and sugar consumption rate in the STR were not comparable with the data from shake flask cultivation. Both were significantly lower in the STR.

Similar to shake flask cultivation the specific growth rate increased with higher substrate concentration. In the STR the maximum specific growth rate and specific substrate consumption rate of 1.26 ± 0.16 day⁻¹ and 2.16 ± 0.19 gₘₒ₄ₐₑ₧−¹⋅d⁻¹, respectively, were measured at 30 g L⁻¹ substrate concentration (see Table 3). Biomass substrate yield and biomass nitrogen yield were higher at 15 g L⁻¹ substrate concentration, compared to 30 g L⁻¹ and ranged between 0.59–0.62 gDW−¹⋅gₘₒ₄ₐₑ₧−¹ and 14.83–15.48 gDW−¹⋅gNH₄−¹.

Complete depletion of substrate after 24 h could not be achieved with 8 g L⁻¹ starting-biomass for 15 and 30 g L⁻¹ substrate concentration (see Figure 2A). Besides slower growth of *G. sulphuraria* in the STR, the pH-value affected substrate availability. As mentioned before, the hydrolysis of sucrose is slower at pH 3 compared to pH 2 (see Figure 1). As a result, after a 24 h cultivation in the STR with 15 g L⁻¹ substrate primarily sucrose was remaining (see Figure 2B).

*G. sulphuraria* SAG 21.92 was successfully grown on wastewater from a fruit-salad production facility. Wastewater from filling process (see Table 1) was diluted to 15 g L⁻¹ total sugar concentration, micronutrients and ammonia where added accordingly. The specific growth rate in wastewater exceeded growth rate in synthetic media at the same substrate concentration by over 30% and the sugar consumption rate by over 24%. Due to slow hydrolysis rate of sucrose at pH 3, the complete depletion of dissolved sugars could not be achieved. Like in synthetic media, glucose and fructose were almost depleted after 20 h of cultivation and after 24 h, primarily sucrose remained in the wastewater. As a result, specific substrate consumption dropped significantly after 20 h of cultivation in wastewater.

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**Table 1** Sugar content of three different wastewater samples from fruit-salad production process

| Sample                             | Sucrose [g L⁻¹] | Glucose [g L⁻¹] | Fructose [g L⁻¹] | Total substrate [g L⁻¹] |
|------------------------------------|-----------------|-----------------|-----------------|-------------------------|
| Sugar-fond                         | 31.12±1.67      | 39.02±2.84      | 32.88±2.65      | 103.02±7.16            |
| Wastewater from filling process    | 35.22±1.85      | 42.67±2.29      | 36.33±1.85      | 114.22±5.99            |
| Apple-cleaning bath                | 7.33±0.54       | 5.50±0.63       | 10.33±1.58      | 23.17±2.75             |

Superscript numbers indicate the number of determinations. Error ranges represent standard deviation.
| Substrate [g L\(^{-1}\)] | pH | \(\mu_{\text{max}}\) [day\(^{-1}\)] | \(Y_{X/S}\) [gDW g\(_{\text{Sub}}\)^{-1}] | \(q_S\) [gSub gDW\(^{-1}\) day\(^{-1}\)] | \(\mu_{\text{max}}\) [day\(^{-1}\)] | \(Y_{X/S}\) [gDW g\(_{\text{Sub}}\)^{-1}] | \(q_S\) [gSub gDW\(^{-1}\) day\(^{-1}\)] | \(\mu_{\text{max}}\) [day\(^{-1}\)] | \(Y_{X/S}\) [gDW g\(_{\text{Sub}}\)^{-1}] | \(q_S\) [gSub gDW\(^{-1}\) day\(^{-1}\)] |
|-----------------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 6               | 2 | 0.67±0.01\(^3\) | 0.62±0.09\(^3\) | 1.14±0.14\(^3\) | 1.44±0.04\(^3\) | 0.63±0.08\(^3\) | 2.28±0.11\(^3\) | 0.58±0.05\(^3\) | 0.60±0.08\(^3\) | 0.96±0.07\(^3\) |
| 6               | 3 | 0.69±0.05\(^3\) | 0.65±0.12\(^3\) | 1.08±0.08\(^3\) | 1.39±0.04\(^3\) | 0.63±0.06\(^3\) | 2.23±0.09\(^3\) | 0.57±0.04\(^3\) | 0.61±0.07\(^3\) | 0.93±0.05\(^3\) |
| 6               | 4 | 0.68±0.02\(^3\) | 0.61±0.08\(^3\) | 1.12±0.08\(^3\) | 1.17±0.03\(^3\) | 0.63±0.04\(^3\) | 1.86±0.05\(^3\) | 0.48±0.03\(^3\) | 0.62±0.08\(^3\) | 0.80±0.07\(^3\) |
| 12              | 2 | 0.86±0.07\(^3\) | 0.64±0.05\(^3\) | 1.35±0.09\(^3\) | 1.48±0.09\(^3\) | 0.62±0.07\(^3\) | 2.39±0.14\(^3\) | 0.53±0.03\(^3\) | 0.60±0.08\(^3\) | 0.88±0.07\(^3\) |
| 12              | 3 | 0.84±0.04\(^3\) | 0.63±0.05\(^3\) | 1.33±0.06\(^3\) | 1.39±0.09\(^3\) | 0.64±0.06\(^3\) | 2.36±0.12\(^3\) | 0.54±0.05\(^3\) | 0.59±0.07\(^3\) | 0.92±0.07\(^3\) |
| 12              | 4 | 0.80±0.03\(^3\) | 0.60±0.05\(^3\) | 1.34±0.08\(^3\) | 1.16±0.04\(^3\) | 0.65±0.04\(^3\) | 1.78±0.06\(^3\) | 0.50±0.04\(^3\) | 0.58±0.05\(^3\) | 0.87±0.04\(^3\) |
| 18              | 2 | 0.92±0.01\(^3\) | 0.66±0.09\(^3\) | 1.34±0.11\(^3\) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 30              | 2 | 0.92±0.07\(^3\) | 0.63±0.05\(^3\) | 1.44±0.10\(^3\) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 36              | 2 | 1.10±0.09\(^3\) | 0.65±0.09\(^3\) | 1.70±0.11\(^3\) | 1.53±0.09\(^3\) | 0.64±0.06\(^3\) | 2.41±0.14\(^3\) | 0.67±0.06\(^3\) | 0.52±0.05\(^3\) | 1.29±0.10\(^3\) |
| 60              | 2 | 0.79±0.06\(^3\) | 0.63±0.05\(^3\) | 1.25±0.07\(^3\) | 1.43 | 0.61 | 2.32 | 0.62±0.08\(^3\) | 0.52±0.04\(^3\) | 1.18±0.10\(^3\) |
| 60              | 3 | 0.74±0.04\(^3\) | 0.59±0.03\(^3\) | 1.15±0.07\(^3\) | n.d. | n.d. | n.d. | 0.55±0.01\(^2\) | 0.49±0.04\(^2\) | 1.14±0.05\(^2\) |
| 60              | 4 | 0.70±0.03\(^3\) | 0.59±0.04\(^3\) | 1.20±0.07\(^3\) | n.d. | n.d. | n.d. | 0.55±0.01\(^2\) | 0.43±0.03\(^2\) | 1.26±0.04\(^2\) |

Resulting specific substrate consumption rate \(q_S\) was added. Substrate stands for the sum of sucrose, glucose and fructose in equal amounts. Superscript numbers indicate number of experiments. Error ranges represent standard deviation.

4 Discussion

The fastest growth of \(G.\ sulphuraria\) SAG 2192 was observed at 42°C and pH 2, which is in accordance with results of Schmidt and colleagues [20, 30-32, 34]. The determined specific growth rate of 1.53 day\(^{-1}\) at 42°C was on average 3% lower at pH 3 than at pH 2. For strain O74A a decline in specific growth rate of about 14% was reported [40]. To reduce the amount of sulphate and needed for cultivation the elevation of pH value might thus be reasonable. Above 36 g L\(^{-1}\) - substrate concentration a slight decrease in growth rate was observed in all three temperatures. Similar behavior was observed at all three cultivation temperatures. Other studies observed lower growth rates at 50 g L\(^{-1}\) - substrate concentration compared to 5 g L\(^{-1}\) - glucose for strain OAG [30, 31]. However, the decrease in growth rate was observed at all three cultivation temperatures. Other studies observed lower growth rates at 50 g L\(^{-1}\) - substrate concentration compared to 5 g L\(^{-1}\) - glucose for strain OAG [30, 31]. In contrast Schmidt and colleagues reported a decrease of the growth rate starting above 16 g L\(^{-1}\) - glucose for strain O74A [20]. O74A has been shown to grow well in wastewater containing higher concentrations of sulphate [32]. The variation of pH value also revealed problems with the growth of \(G.\ sulphuraria\) SAG 2192 (see Table 4). For specific growth rates of 13.6 day\(^{-1}\) at 36 g L\(^{-1}\) substrate concentration a decrease of the pH value had less impact on growth than a decrease of the growth rate starting above 16 g L\(^{-1}\) - glucose for strain O74A [30]. In contrast Schmidt and colleagues reported a decrease of the growth rate starting above 16 g L\(^{-1}\) - glucose for strain O74A [20]. O74A has been shown to grow well in wastewater containing higher concentrations of sulphate [32]. The variation of pH value also revealed problems with the growth of \(G.\ sulphuraria\) SAG 2192 (see Table 4).
TABLE 3 Specific maximum growth rate $\mu_{\text{max}}$, biomass substrate yield $Y_{X/S}$ and biomass nitrogen yield $Y_{X/N}$ of $G. sulphuraria$ SAG 21.92 in the STR at 42°C

| Sugar [g L$^{-1}$] | $\mu_{\text{max}}$ [day$^{-1}$] | $Y_{X/S}$ [gDW gSub$^{-1}$] | $q_S$ [gSub gDW$^{-1}$ day$^{-1}$] | $Y_{X/N}$ [gDW gNH$_4^{-1}$] |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| 15 (synthetic media) | 1.03±0.19$^1$ | 0.62±0.05$^2$ | 1.52±0.11$^2$ | 15.48±0.62$^2$ |
| 30 (synthetic media) | 1.26±0.16$^2$ | 0.59±0.04$^2$ | 2.16±0.19$^2$ | 14.83±0.46$^2$ |
| 15 (wastewater) | 1.21 | 0.64 | 1.88 | 15.19 |

Resulting specific substrate consumption rate $q_S$ was added. Synthetic media contained sucrose, glucose and fructose in equal amounts. Superscript numbers indicate number of experiments. Error ranges represent standard deviation.

TABLE 4 Comparison of specific growth rate and biomass substrate yield of $G. sulphuraria$ strain 074G with strain SAG 21.92 (this study) on different substrates

| Substrate | Conc. [g L$^{-1}$] | Temp. [$^\circ$C] | Reactor system | $\mu_{\text{max}}$ [day$^{-1}$] | $Y_{X/S}$ [gDW gSub$^{-1}$] | $Y_{X/N}$ [gDW gNH$_4^{-1}$] | Reference |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|
| Glucose   | 4.5             | 42              | 500 mL shake flask | 1.10 | 0.48 | 11.90 | [32] |
| Fructose  | 4.5             | 42              | 500 mL shake flask | 1.08 | 0.54 | 11.10 | [32] |
| Glucose   | 5               | 42              | 500 mL shake flask | 1.22 | 0.55 | 11.70 | [34] |
| Glucose + sucrose | 52.5 | 42 | 500 mL shake flask | 1.44 | 0.42 | 11.00 | [32] |
| Glucose + fructose + sucrose | 6 | 42 | 500 mL shake flask | 1.44 | 0.63 | n.d. | This study |
| Glucose + fructose + sucrose | 36 | 42 | 500 mL shake flask | 1.53 | 0.64 | n.d. | This study |
| Glucose   | 10              | 42              | 3 L STR         | 1.26 | 0.46 | n.d. | [31] |
| Glucose   | 50              | 42              | 3 L STR         | 1.11 | 0.55 | 23.4 | [31] |
| Glucose + fructose + sucrose | 15 | 42 | 3 L STR         | 1.03$^*$ | 0.62$^*$ | 15.48$^*$ | This study |
| Glucose + fructose + sucrose | 30 | 42 | 3 L STR         | 1.26$^*$ | 0.59$^*$ | 14.83$^*$ | This study |

All cultivations were conducted at pH 2, except data marked with *, which were obtained at pH 3.

of sucrose. Since the hydrolysis rate of sucrose is temperature dependent, a procession of wastewater containing considerable amounts of sucrose is not recommended at 30°C without any further treatment. To overcome problems of sucrose cleavage a storage tank for wastewater should be established. In advance of the cultivation with $G. sulphuraria$, the wastewater is collected and treated with sulphuric acid at 42°C to induce sucrose hydrolysis. Determined hydrolysis rate of sucrose was lower in cell-free media in shake-flasks compared to cultivation in the STR. The amount of hydrolyzed sucrose after 24 h cultivation in the STR was on average 79% higher (data not shown) than in the cell-free synthetic media in shake-flasks. Besides acceleration of hydrolysis due to acidification of the culture media caused by ammonia uptake, the presence of extracellular hydrolyases could be an explanation [40].

Expected growth rates from shake flask cultivation could not be reached in the STR. Since oxygen supply was sufficient (data not shown), deficits could be caused by cells aggregating on the reactor wall due to foaming. Cultivations with wastewater exceeded growth rates in synthetic media with 15 g L$^{-1}$ total sugar concentration in the STR. The synthetic media contained equal amounts of the three sugars, whereas the wastewater from filling process contained more glucose than sucrose (see Table 1). Sucrose needed to be hydrolyzed before consumption. At 15 g L$^{-1}$ substrate concentration this cleavage reaction was growth limiting. Because of the lower sucrose concentration in the wastewater compared to the synthetic media, $G. sulphuraria$ SAG 21.92 could grow faster in the wastewater.

Determined specific growth rate of 1.21 day$^{-1}$ of strain SAG 21.92 in wastewater supplemented with micronutrients and ammonia was in good accordance with previous studies with strain 074G in hydrolyzed restaurant waste (also supplemented with micronutrients and ammonia), where a specific growth rate of 1.22 ± 0.01 day$^{-1}$ was obtained [34]. Resulting sugar consumption rates were...
almost identical. Utilization of hydrolyzed potato starch with \textit{G. sulphuraria} was also shown recently, where a specific growth rate of 0.82 day$^{-1}$ was attained [41]. The mentioned growth rates were measured at pH 2 in contrast to this study, where comparable growth rates could be achieved at pH 3 with strain SAG 21.92. In process scale, this could save a significant amount of sulphuric acid. Recovery of sulphuric acid from used wastewater is possible, but optimization has to be addressed in further investigations. To date, no other studies have investigated biomass production of \textit{G. sulphuraria} with waste streams containing high loads of organic carbon.

However, other microalgae like \textit{Chlorella zofingiensis} or \textit{C. protothecoides} could already be cultivated in waste molasses for pigment and biofuel production [42,43]. Also dairy effluent has proven to be a suitable carbon source for \textit{Chlorococcum sp} [44]. The authors propose that \textit{G. sulphuraria} could also harness the carbon sources in the mentioned waste streams. \textit{G. sulphuraria} is also able to produce the pigment phycocyanin. A phycocyanin production process based on organic carbon in wastewaters from food production process with \textit{G. sulphuraria} will be investigated in future studies.

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\section*{Conflict of Interest}
The authors have declared no conflict of interest.

\section*{Data Availability Statement}
The data that support the findings of this study are available from the corresponding author upon reasonable request.

\section*{Nomenclature}

\begin{table}[h]
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\begin{tabular}{|c|c|}
\hline
Symbol & Description & Unit & \hline
$\mu_{\text{max}}$ & Maximum specific growth rate during exponential phase & [day$^{-1}$] & \hline
$Y_{X/S}$ & Biomass substrate yield & [gDW gSub$^{-1}$] & \hline
$Y_{X/N}$ & Biomass nitrogen yield & [gDW gNH$_4^{-}$] & \hline
$q_S$ & Specific substrate consumption rate & [gSub gDW$^{-1}$ day$^{-1}$] & \hline
\end{tabular}
\end{table}

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