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Recovery of Sugar and Nutrients from Algae and *Colocasia esculenta* (Taro) Leaves Using Chemical Hydrolysis

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Abstract: Algal biomass and *Colocasia esculenta* (Taro) leaves are available as waste biomass all over India. These biomasses can be used as renewable and sustainable resources for sugars and nutrients. Recovered nutrients and sugars can be used as cheap raw materials for biofuels and biomaterials production. The hydrolysis of dried algal biomass and *Colocasia esculenta* (Taro) leaves were investigated using 1%, 2%, and 5% solutions of ferric-chloride, nitric acid, and acetic acid for the reaction times of 30 and 60 min at 121 °C and 103.4 kPa (15 psi). 1% and 2% H_2SO_4 treatments were used as the reference. The solid: liquid ratio was kept at 1:10 for all the experiments. For algal biomass, a 5% acetic acid treatment for 60 min was found to be optimum with a total carbohydrate release of 44.2 mg/g biomass (solubilized monomers-0.82 mg/g of biomass) and N and P solubilization of 1.8 mg total nitrogen/g biomass and 7 mg total phosphorus/g biomass. Moreover, for *Colocasia esculenta* (Taro) leaves, the maximum carbohydrate yield of 95 mg/g biomass (solubilized monomers-43.6 mg/g of biomass) and nutrient solubilization of 5.02 mg total nitrogen/g biomass was obtained with 5% ferric chloride treatment for 60 min. The results obtained showed that various hydrolyzing agents used in this study acted differently on different types of biomasses. Acetic acid worked best in hydrolyzing the algal biomass, and for the hydrolysis of Taro leaves, ferric chloride and nitric acid were effective. Statistical analysis showed that the chemical concentration was one of the prime factors for releasing P from algal biomass. For carbohydrate release from Taro leaves, either time or concentration, or both, were the prime factors that affected the carbohydrate release.

Keywords: chemical hydrolysis; algae; *Colocasia esculenta* (Taro) leaves; carbohydrate; nutrient solubilization; acidic treatment

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1. Introduction

In recent years, a surge in research on the production of bio-based products and biofuels is observed in the scientific literature [1,2]. A portion of the petro product and fossil fuel demand is likely to be replaced by these bio-based products and biofuels produced in a biorefinery (similar to an oil refinery) [3]. The most widely used raw material is lignocellulosic biomass, which requires drastic pretreatment to deconstruct lignin–hemicellulose bonds to break cellulose as a simple carbohydrate.

Several chemical reagents ranging from a strong acid and base, a weak acid and base, and various metallic salts (acidic or basic) were used for the pretreatment of lignocellulosic biomass. In addition to the above-mentioned chemicals, ionic liquids, steam, and ammonia explosion techniques have also been used to break the lignin structure of the lignocellulosic biomass [4–7]. Li et al. [8] investigated the effects of several metal oxides on the recovery of sugars from corn stover. These metals worked as catalysts for monomer formation from the complex sugars. However, these metal oxides break the hemicellulose down into its

monomers. Recently, uses of deep eutectic solvents for pretreatment and value-added product recovery from biomass have shown promising results [9].

Similarly, ferric salts (i.e., ferric chloride) also help to solubilize the hemicellulose component of the complex sugars. Organic acids such as acetic acid, oxalic acid, and malic acid have also been used for the solubilization of sugars from biomass. These organic acids depolymerize the cellulose into the oligomers and glucose and enhance the organic nitrogen and phosphorus release from the complex organic matter [10].

The primary sources of lignocellulosic biomass are forest and agricultural residues. Nevertheless, it needs extensive pretreatment strategies to break the lignin—cellulose bonds, ultimately increasing the cost of producing simple sugars [11]. Moreover, Furfural and 5-hydroxymethylfurfural (HMF) are degradation compounds generated during the hydrolysis of lignocellulosic biomass, which distinctly inhibit microbial growth [12]. Feedstock contributes 70–80% of the total production cost, which can be substantially reduced by the utilization of waste and cheaper raw materials [13]. Hence, it is not possible to exploit these resources further to produce value-added products in a considerable amount. So, there is a need to look for waste biomass with a low or minimum content of lignin [14]. In this regard, algae and *Colocasia esculenta* (Taro) leaves are two promising candidates that can thrive in wastewater or marshy lands.

Both biomasses generated from algae and *Colocasia esculenta* (Taro) leaves are amenable to chemical treatment and may not require costly enzymatic hydrolysis for enhanced sugar recovery. Hydrolysates prepared from biomass are ultimately used to produce various value-added chemicals, including fuels using different microbial strains [15,16]. Various water bodies contain a large diversity of algal species, and some of these species can be used to produce value-added chemicals, including fuels [17]. For the production of value-added products, sugar is the main ingredient. However, nutrient contents also play a vital role in the production of value-added products. Chowdhury and his co-workers [18–20] showed that nutrient recovery from biomass and utilization of the same increased biofuel production. The produced biofuel has reduced energy demand and greenhouse gas (GHG) emissions. Hence, nutrient recovery from biomass should also be taken into account to evaluate the efficacy of a hydrolysis procedure. Optimal uses of sunlight for algae and heat production also reduced the cost of the algal biomass produced by wastewater treatment plants [21].

Very few studies are carried out on the hydrolysis of *Colocasia esculenta* (Taro) leaves and algal biomass through chemical treatment. For example, Saenphoom et al. [22] used enzymatic treatment to hydrolyze the Taro leaves and recovered approximately 29.4 mg sugar/g of dry matter. Several researchers also explored the chemical hydrolysis of algal biomass harvested from natural water or wastewater. Most of these hydrolysis procedures used mineral acids. For example, Jain et al. [13] used several acids for hydrolysis procedures and found that digestion in an autoclave with 1% H_2SO_4 was the most effective for releasing nutrients and sugars from algal biomass. Similarly, Castro et al. [15] investigated hydrolysis of algal biomass grown in municipal wastewater and reported that 1 M H_2SO_4 treatment at 121 °C followed by enzymatic treatment released 161 g sugar/kg algal biomass. Previous studies also explored mineral acids at different concentrations and temperatures to check their efficacy in releasing sugars. However, very few studies deliberated on the release of N and P from algae and *Colocasia esculenta* (Taro) leaves.

It is observed that a large percentage of the hydrolyzing agent ultimately ends up in the hydrolysate. Hence, it is of great importance to use the constituents of the hydrolyzing agent as a macro or micronutrient for microbial growth. For the hydrolysis of algal biomass and Taro leaves, three chemicals were chosen. The first one was acetic acid, which showed enhanced cellulose dissolution and TKN (Total Kjeldahl Nitrogen) recovery. Acetic acid has also been used as a carbon source for several microorganisms that produce value-added products [23–25]. Organic acids are less reactive than sulfuric acid. They do not destroy organic materials and produce less undesirable side products than sulfuric acid [10]. The second hydrolyzing reagent used was nitric acid, a vital mineral acid. Nitrate present in

nitric acid can serve as a nitrogen source for microbial growth. In comparison to sulfuric acid, nitric acid causes less equipment corrosion and is more effective for hydrolyzing biomass [26]. The third reagent explored was ferric chloride, which was found to hydrolyze hemicellulose preferentially. Iron also works as a catalyst for converting complex sugars into their monomers. Iron in the hydrolysate can work as a micronutrient, and some studies show that ferric ions positively affect lipid production by various microorganisms [27,28]. $FeCl_3$ has other unique properties such as its low cost, non-toxicity, and abundance, making it a favorable chemical for hydrolysis [29].

Therefore, the present study was designed to find the efficacy of sugar and nutrient release from algal bloom and *Colocasia esculenta* (Taro) leaves, abundantly grown in wastewater or marshy lands with low lignin content (algae contains no lignin). Optimum doses of three different chemicals, namely, ferric chloride, nitric acid, and acetic acid, were sought using various concentrations and time parameters to explore their effects on sugar and nutrient release.

2. Materials and Methods

2.1. Biomass Used

Naturally available mixed algal bloom and *Colocasia esculenta* (Taro) leaves were collected from the stagnant water bodies present near the campus of the Indian Institute of Technology Roorkee, Roorkee, India. Biomass was washed several times with distilled water and dried in a hot-air oven at 80 °C for 24 h before being converted into fine powder.

2.2. Hydrolyzing Reagents

Acetic acid (CH_3COOH, 100% purity, ACS grade), nitric acid (HNO_3, 70% purity, reagent grade), and ferric chloride anhydrous ($FeCl_3$, 98% purity, powder) were used as the hydrolyzing reagents. Sulfuric acid (H_2SO_4, 98% purity, reagent grade) was used as a reference hydrolyzing reagent for comparison. All the chemicals were purchased from Sigma Aldrich (India).

2.3. Hydrolysate Preparation

The powdered forms of algae and *Colocasia esculenta* (Taro) leaves biomass were hydrolyzed separately using 1%, 2%, and 5% concentrations of CH_3COOH, HNO_3, and $FeCl_3$ using an autoclave (at 121 °C and 15 psi) and two time intervals, i.e., 30 min and 1 h. The concentration of the reference hydrolyzing reagent, H_2SO_4 was 1%. The solid: liquid ratio was kept at 1:10 for all experiments. All the hydrolysates prepared were cooled to room temperature, before filtering, using a Whatman filter paper to remove suspended biomass particles. The hydrolysate was then neutralized with 0.1 M NaOH to attain a pH of 7.4.

2.4. Analytical Methods

2.4.1. Characterization of the Hydrolysates

Samples were first centrifuged at 4000 rpm for 10 min, and the supernatant was collected after passing through a 0.22 µm syringe filter. Chemical oxygen demand (COD) was measured using the closed reflux method (colorimetric method) given in the standard methods [30]. The phenol sulphuric acid method [31] was used to estimate the total carbohydrates present in the hydrolysates. Absorbance was measured by a UV-VIS spectrophotometer (DR 6000, HACH, Loveland, CO, USA). Total organic carbon (TOC) was measured in a TOC analyzer (Analytik Jena, multi N/C 3100). Nitrate (NO_3^-), phosphate (PO_4^{-3}), and ammonia (NH_4^+) were measured in an Ion-chromatograph (850 professional IC, Metrohm, Herisau, Switzerland). Metrosep A Supp 5 100/4.0 and Metrosep A Supp 5 100/4.0 columns were used as anion and cation columns, respectively. Total Nitrogen (TN) was measured using the simplified TNT plus simplified TKN sTKNTM- TNT 880 kit (range:0–16 mg/L N) (HACH, USA).

2.4.2. Analysis of Reducing Sugars

The estimation of simple sugars (such as glucose, xylose, etc.) and acetic acid was carried out using high-performance liquid chromatography (HPLC). The analysis was performed by injecting filtered hydrolysate into HPLC (water) equipped with a Biorad® Aminex HPX-87H Column (300 × 7.8 mm) maintained at 65 °C. 0.005M H_2SO_4 was used as a mobile phase (flow rate: 0.6 mL/min), and a refractive index detector was used for the detection (temperature 40 °C).

2.4.3. Statistical Analysis

All experiments were performed in replicates. Two factors, i.e., the time of hydrolysis and the concentration of chemicals, were varied in two levels and three levels, respectively (a factorial design approach was undertaken). The obtained data were used to develop models to find the main effects (time and concentration) and interaction effects (time × concentration). The ANOVA and confidence interval values of each effect were estimated. R^2 values of the models generated from the experimental design were estimated. For statistical model building, a 2^2 factorial design was used. Hence, for the concentration, two models were produced (concentration 1 and 2% and 1 and 5%). Details about the experimental design for estimating the effects from the factorial design and associated statistical analysis can be found in [32].

3. Results and Discussion

3.1. Effect of Different Hydrolysis Procedures on Algal Biomass

The algal bloom was hydrolyzed using three different chemicals, i.e., acetic acid, nitric acid, and ferric chloride, to check its efficacy on sugar and nutrient release. Furthermore, 1% H_2SO_4 treatment was used as the reference for the study.

3.1.1. Carbohydrate and COD Solubilisation

Figure 1 shows the number of total carbohydrates in the hydrolysate of algal biomass released by different treatments. For the acetic acid treatment, the carbohydrate released in 30 min was found to be 13, 18, and 40 mg/g of biomass for 1%, 2%, and 5% concentrations, respectively. For 1 h of treatment time, the corresponding values were 18, 18, and 44.2 mg/g of biomass. Due to changes in the concentration of acetic acid from 2 to 5%, the carbohydrate release increased more than two-fold. However, monomer production was no more than 2% of the total carbohydrate produced. Zhang et al. [33] explained that using a weak acid such as acetic acid could help to dissolve the amorphous starch content during the hydrolysis process through acetylation, resulting in a decrease in the molecular packing density by reducing the formation of intermolecular hydrogen bonds. This decrease in packing density increases the acid permeation [23,34]. A 10% acetic acid solution was also investigated for the hydrolysis of algal biomass at reaction times of 30 min and 1 h, releasing 15 and 29.2 mg of sugar/g of dry matter, respectively (Table S2, Supplementary Materials). Compared to the carbohydrate release, the 5% treatment was found to be optimum for the algal biomass hydrolysis. Accordingly, hydrolysate prepared using a 10% concentration of acetic acid was not characterized. Models developed from the factorial design of experiments (time and concentration are the two factors with two levels) showed that none of the effects affected the carbohydrate and glucose release from the algal biomass. Model 2 was a better model if one considered the R^2 value (carbohydrate release) (Table 1). Xylose released by acetic acid treatment showed strong effects of time and concentration. Both models provided a good fit for the experimental data.

Table 1. Statistical analysis of hydrolysate data for algae.

Hydrolysing Agent (Products)	Model 1	Model 2	Statistical Significance
Acetic acid-(carbohydrate)	y = 16.75 + 2.5a + 2.5b − 2.5ab R^2: 0.66	y = 28.8 + 4.6a + 26.6b − 0.4ab R^2: 0.41	None of the effects were significant
HNO_3-(carbohydrate)	y = 1.025 + 0.35a − 0.05b + 0.25ab R^2: 0.14	y = 1.35 + 0.4a + 0.6b + 0.3ab R^2: 0.23	None of the effects were significant
$FeCl_3$-(carbohydrate)	y = 1.15 + 0.3a + 0.1b + 0.1ab R^2: 0.086	y = 4.45 + 0.9a + 6.7b + 0.7ab R^2: 0.6	None of the effects were significant
Acetic acid-(glucose)	y = 0.6 + 0.08a − 0.03b − 0.15ab R^2: 0.37	y = 0.605 + 0.14a − 0.02b − 0.09ab R^2: 0.36	None of the effects were significant
Acetic acid-(xylose)	y = 0.3 + 0.1a − 0.1ab R^2: 0.999	y = 0.25 + 0.1a − 0.1b − 0.1ab R^2: 0.999	Model 1: effect of a and interaction significant, Model 2: all the effects were significant;
Acetic acid-(PO_4-P)	y = 4.45 − 0.1a + 1.4b + 0.4ab R^2: 0.92	y = 7 − 3.5a + 6.5b − 3ab R^2: 0.9	Model 1: effect of b significant; Model 2: all the effects were significant,
HNO_3-(PO_4-P)	y = 4.25 − 0.5a − 0.5b + 0.5ab R^2: 0.93	y = 8.5 − 2a + 8b − ab R^2: 0.9	Model 1: All the effects were significant, Model 2: effect of b significant;
$FeCl_3$-(PO_4-P)	y = 0.75 − 0.1a + 0.6b R^2: 0.82	y = 1.325 + 0.95a + 1.75b + 1.05ab R^2: 0.996	Model 1: effect of b significant, Model 2: all the effects are important

a: Time, b: Concentration, 2^2 factorial tests were conducted. The F test was used for finding the significance of a and b (significance tests at 0.01 and 0.05 levels). Confidence intervals were also determined. R^2 = Sum of square (model)/Sum of the square total. In the first column, the hydrolyzing agent used was given, and in the brackets, (), the name of the product for which the statistical model was developed was depicted. If the significance level is not mentioned, those significance levels are at the 0.05 level.

For the nitric acid treatment, the release of total carbohydrates during the 30 min of reaction time accounted for 1.6, 0.7, and 1.3 mg/g of biomass for the 1%, 2%, and 5% concentrations, respectively. When the treatment time was increased to 1 h, the corresponding values were 1.1, 1.3, and 36.3 mg/g of biomass, respectively. Glucose (29.7 mg/g of biomass) and xylose (6.6 mg/g of biomass) recovery was found in the 5% nitric acid treatment for 1 h. For lower concentrations of nitric acid, the recovery of monomers was low (Figure 1). Moreover, 1% and 2% treatment conditions were not optimum in the production of glucose and xylose. Our results correlated well with the findings of Rodriguez-Chong et al. [35], as they pretreated Jerusalem Artichoke Stalks with HNO_3 and found that 5% HNO_3 (*w*/*v*) was the optimum concentration leading to the recovery of 57 mg of glucose/g of biomass at 121 °C after 60 min treatment. Nitric acid, a strong acid, and a powerful oxidizing agent can produce reducing sugars by breaking the β-1,4 linkages between glucose or xylose monomers. The treatment using nitric acid was not effective for the algal biomass because it does not contain hemicellulose [16,36], which is more prone to mineral acid attack. In this study, only 5% HNO_3 treatment could release monomers, and the lesser concentration was not effective for hydrolysis. Statistical analysis showed that time and concentration did not have any significant effects on the release of carbohydrates or any of the monomers (glucose and xylose) from the algal biomass (Table 1).

Similarly, for the ferric chloride treatment, only the 5% ferric chloride treatment for 1 h could recover glucose (1.35 mg/g of biomass) and xylose (0.55 mg/g of biomass). A lesser concentration was not able to solubilize the carbohydrate (Figure 1). Ferric chloride showed interference with the phenol-sulphuric method because the color of ferric chloride interfered with the colorimetric estimation of carbohydrates. The solution was filtered through a 0.22 µm filter before the estimation of total carbohydrates. The total carbohydrate contents in the ferric chloride treated hydrolysate for 30 min were found to be 1, 1, and 7 mg/g of biomass for 1%, 2%, and 5% concentrations, respectively. For 1 h, the values were 1.2, 1.4, and 8.6 mg/g of biomass. The lesser recovery of xylose could be due to the absence of hemicellulose in the algal biomass. Sun et al. [37] mentioned that hydronium ions derived from ferric chloride salt were responsible for the depolymerization of hemicellulose by selective hydrolysis of glycosidic linkages. Hence, the hydrolysate prepared from algal biomass contained fewer monomers. Statistical analysis showed that time and concentration did not have any significant effects on the release of carbohydrates or any of the monomers (glucose and xylose) from algal biomass (Table 1).

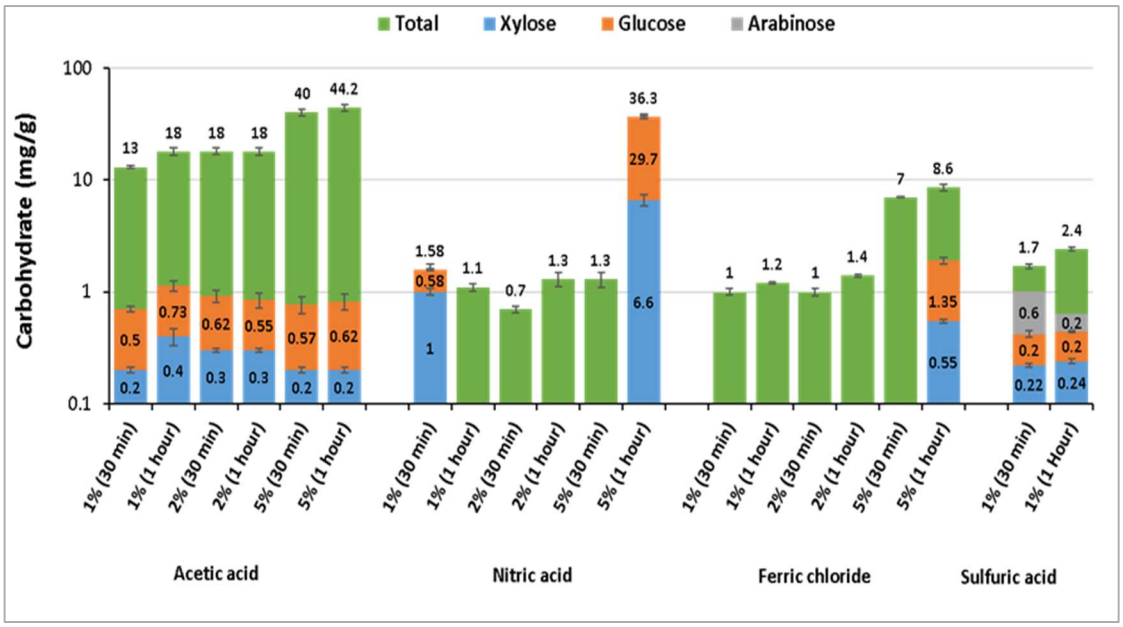

Figure 1. Total carbohydrate and monomer concentrations (mg/g) in different hydrolysates of algae. Number in the figure shows the concentration (mg/g) of individual components (legend). Number on the top of each column shows the total carbohydrate content. Vertical bars show the error in estimation as standard deviation.

For the reference study (1% H_2SO_4), the total carbohydrate content for the 30 min treatment was estimated as 1.7 mg/g of biomass, which included more than 50% monomers (glucose—0.22 mg/g of biomass, xylose—0.2 mg/g of biomass, and arabinose—0.6 mg/g of biomass). For the 1 h treatment, the recovery of total carbohydrate was 2.4 mg/g of biomass with glucose, 0.2 mg/g of biomass with xylose, and 0.2 mg/g of biomass with arabinose. Compared to the other treatments used in this study, only acetic acid could solubilize the carbohydrates in a low concentration (1% and 2%). Hence, acetic acid proved to be an effective treatment method for the hydrolysis of algal biomass.

COD was estimated after subtracting COD generated from the hydrolyzing reagent present in the hydrolysate. Hence, the estimated COD was due to the sugar and other constituents generated from biomass present in the hydrolysate. A significant increase in the solubilization of COD was noted for the 5% treatment of acetic acid. However, if one assumes that all the soluble sugar is present as glucose (glucose imparts the highest COD among all the sugars), the estimated COD was higher than that imparted by sugars.

Hence, it appears that there are several other soluble compounds present in the hydrolysate, which also contributed to the COD. Lin et al. [38] also reported that many organic compounds were present in the hydrolysate they prepared. TOC (Total Organic Carbon) was also estimated to deduce the prevalent oxidation state of the resultant hydrolysate. It was observed that the COD/TOC ratio of the hydrolysate prepared by the acetic acid treatment ranged from 2–2.5 for the 5% acetic acid treatment. However, the COD/TOC ratio of the most reduced form of sugar (glucose) is 2.7. Hence, it is most likely that some organic compounds are present in the hydrolysate in a less reduced form than glucose (in glucose C has an oxidation state of 0). These organic compounds imparted the unexplained part of the estimated COD. The COD of one-mole glucose is 192 g/L, whereas the TOC of one-mole glucose is 12 × 6= 72 g/L. Hence, the COD/TOC: is 2.67 (Table S1, Supplementary Materials).

In contrast to the acetic acid treatment, soluble chemical oxygen demand (sCOD) in the nitric-acid-treated hydrolysate was found to be lower (Figure 2). Low COD obtained in the nitric acid treatment was most likely due to the fact that nitric acid is a strong acid and might have degraded the solubilized COD to CO_2.

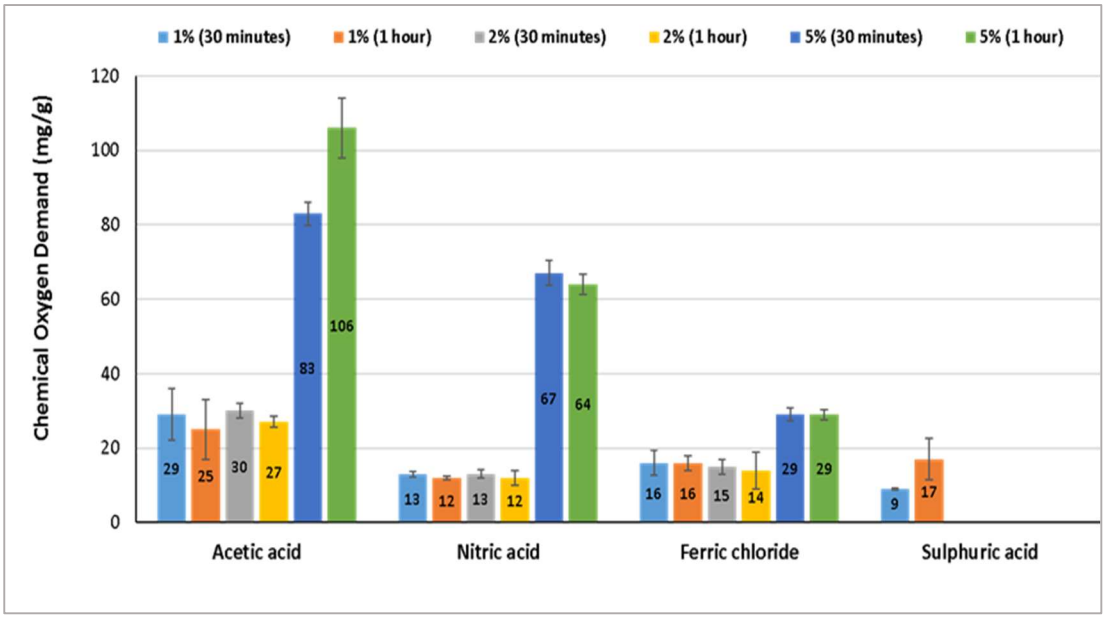

Figure 2. COD (mg/g) in different hydrolysates of algal biomass. Number in the figure shows the COD concentration (mg/g). Vertical bars show the error in estimation as standard deviation.

Ferric chloride treatment was also found to be less efficient for the solubilization of COD than other hydrolyzing reagents in the case of algal biomass (Figure 2). For the 30-min treatment, the amount of COD was estimated to be 16, 15, and 27 mg/g of biomass for 1%, 2%, and 5%, respectively. For the 1 h treatment, the corresponding values were 16, 14, and 29 mg/g of biomass, respectively. Thus, the 5% treatment showed a better result than the lower concentrations. However, a change in the time of hydrolysis did not have a significant effect on the COD solubilization. Therefore, from Figures 1 and 2, it was concluded that only the 5% treatment is effective for the solubilization of organic matter from the algal biomass in the ferric-chloride-treated hydrolysate. For 1% H_2SO_4, COD solubilization was 9 with 17 mg/g of biomass for reaction times of 30 min and 1 h, respectively.

3.1.2. Nutrient Solubilisation (TN and TP)

Nitrogen and phosphorous present in a culture medium play a significant role in the growth of microorganisms [39] as nitrogen acts as the building block for the synthesis of proteins and nucleic acid. Moreover, phosphorous is necessary for the cellular uptake of nitrate present in the media. As shown in Figures 3 and 4, the total nitrogen (TN) and orthophosphate contents were higher in all the hydrolysates compared to the reference hydrolysate (1% H_2SO_4 treatment). This observation indicated that the hydrolyzing reagents used in the present study were able to elevate the extraction of nutrients from the algal biomass.

The total nitrogen in this study (Figure 3) comprises Nitrate-N and TKN-N. TKN (TKN is used to express TKN-N from this point onward) was estimated only for samples that showed enhanced sugar recoveries. The nitrate-N content for the acetic acid-treated hydrolysate was very low, i.e., between 0.2 and 0.3 mg/g of biomass, and TKN was estimated to be approximately 2 mg/g of biomass. For nitric acid, the value of nitrate was found to be higher in all hydrolysates because of the presence of nitrogen (as nitrate) in the hydrolyzing reagent itself. Therefore, TKN analysis was not performed for the nitric-acid-treated hydrolysates.

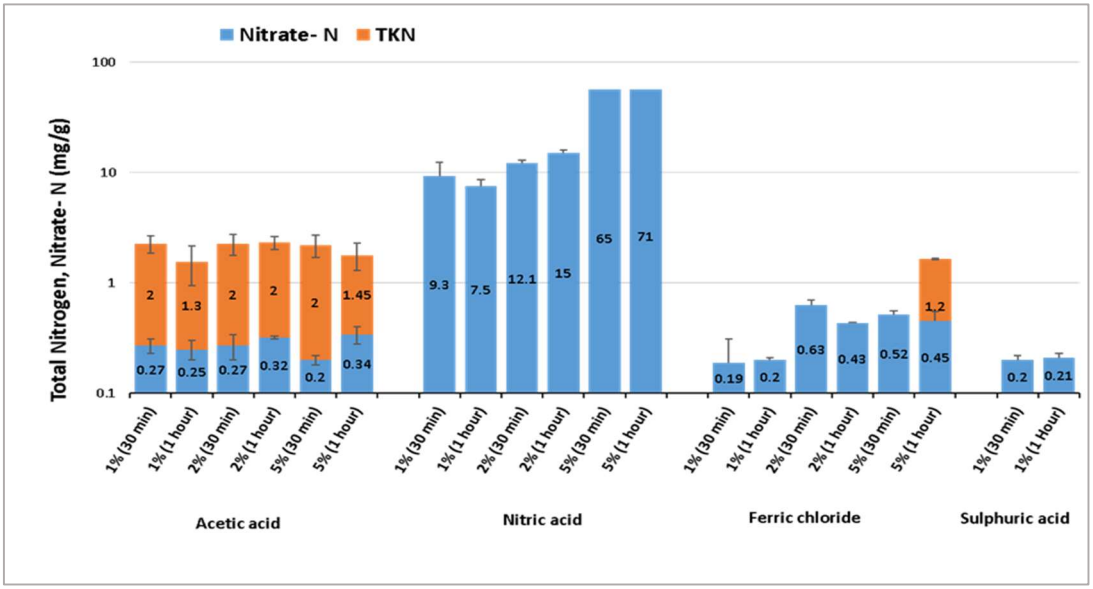

Figure 3. Total nitrogen and nitrate-N (mg/g) present in different hydrolysates of algal biomass. Number in the figure shows the concentration (mg/g) of individual components (lagend). Vertical bars show the error in estimation as standard deviation.

For the ferric chloride treatment, the nitrate content was between 0.2 and 0.6 mg/g of biomass in all the hydrolysates for 30 min and 1 h of reaction time. TKN was estimated to be 1.2 mg/g of biomass for the 5% treatment (1 h). The nitrate values were lower in this case as compared to the nitric acid treatment. As compared to the 1% H_2SO_4 treatment (reference hydrolyzing agent), which resulted in nitrate recovery of 0.2 mg/g of biomass for both the 30 min and 1 h treatments, the hydrolysate produced using acetic acid had a higher amount of nitrate content. The concentration of hydrolyzing reagents and reaction time did not have a significant effect on the TN recovery.

$PO_4{}^{-3}$-P content (Figure 4) for acetic-acid-treated hydrolysates at 1%, 2%, and 5% concentrations was 4, 5, and 13.5 mg/g of biomass, respectively, for the 30 min reaction time. For the 1 h treatment, the corresponding values were 3.5, 5.3, and 7 mg/g of biomass. A higher amount of phosphorus was found in the 5%-treated hydrolysate, and it was reduced when the treatment time was increased from 30 min to 1 h. Statistical analysis also revealed that for model 1, concentration had a significant influence on phosphorus solubilization. Model 2 depicted that all effects were significant for phosphorus solubilization. However, time and interaction terms had a negative effect on P solubilization (Table 1).

In the case of nitric acid, the phosphate-*p* values (Figure 4) were found to be higher for the 5% concentration with 14 and 11 mg/g of biomass for 30 min and 1 h treatment, respectively. The pattern of the results was similar to the acetic-acid-treated hydrolysates as the maximum amount of phosphorus was obtained in the 5% nitric-acid-treated hydrolysate. Statistical analysis also revealed that concentration was the main parameter that affected the P solubilization (Table 1).

Ferric chloride was the least efficient chemical for the recovery of phosphorus from the algal biomass. For ferric chloride treatment, neither the concentration nor time positively affected phosphorus recovery from the biomass. Estimated P in the ferric-chloride-treated hydrolysates was 0.5, 1.1, and 1.2 mg/g of biomass for 1%, 2%, and 5%, respectively, for the 30 min reaction. For the 1 h reaction, the corresponding values were 0.4, 1, and 3.2 mg/g of biomass. Statistical analysis showed that concentration and other effects were important depending on the model. Model 2 provided a better estimate of variance (R^2) as compared to model 1 (Table 1).

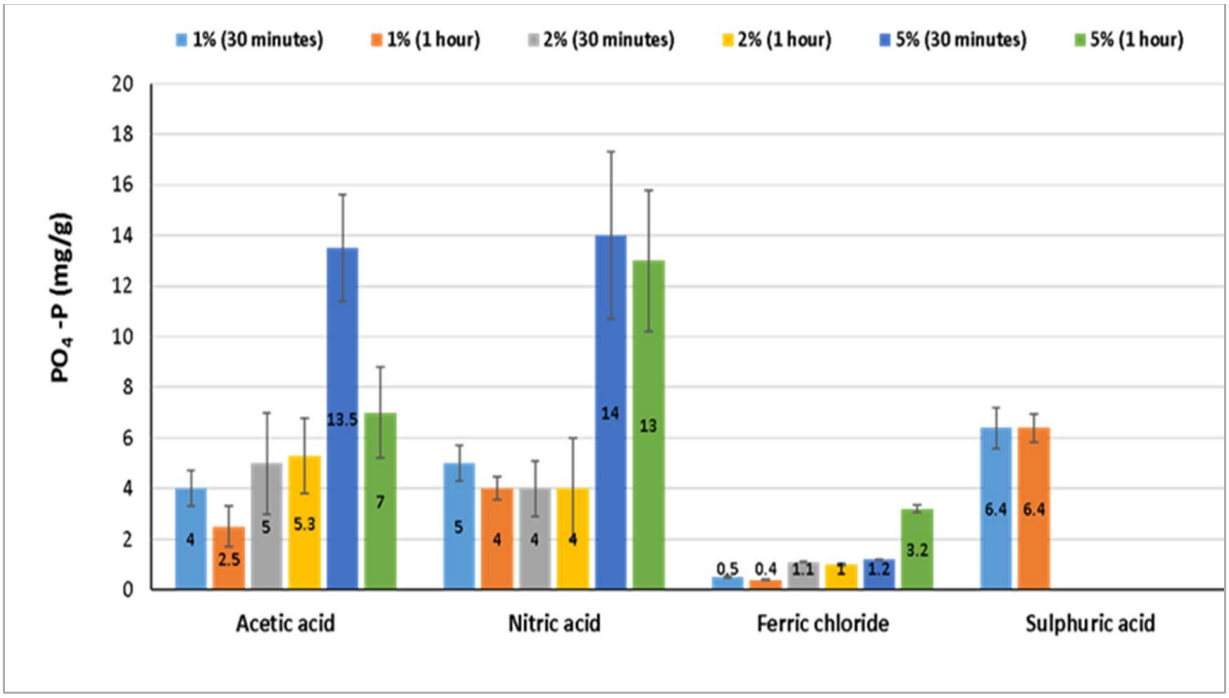

Figure 4. Phosphate-P content (mg/g) in hydrolysates prepared from algal biomass. Numbers in the figure show the PO_4-P concentration (mg/g). Vertical bars show the error in estimation as standard deviation.

For the reference study (1% H_2SO_4), the orthophosphate content was 6.4 mg/g of biomass for both the 30 min and 1 h treatments.

Solubilization of phosphorus bound in biomass was higher in the 5% acetic-acid- and nitric-acid-treated hydrolysates than in the H_2SO_4-treated hydrolysate. Ferric chloride was inefficient for the recovery of phosphorus even in the higher concentration. The low concentration of phosphate found in the hydrolysate could be due to the formation of insoluble iron phosphate [40]. For P solubilization, concentration was the most critical parameter. However, in some statistical models, concentration and time were the significant parameters for P release (Table 1).

3.2. Comparison of Hydrolysates Prepared from Algal Biomass

Several researchers have tried to hydrolyze the algal biomass through various chemical treatments. A comparative study of sugar release was carried out to evaluate the attractiveness of the hydrolyzing reagents used in the present study compared to other chemicals reported in the literature (Table 2). In the present study, 44 mg of carbohydrate/g biomass was obtained for acetic acid treatment, and COD solubilization was also high. However, as these studies did not provide the recovery of other nutrients, a decision to select the best chemical for hydrolysis could not be deduced. In this study, the 5% nitric acid treatment resulted in the highest TN and TP content; however, the carbohydrate solubilization and the soluble CODs were low, which were essential for using a hydrolysate as a growth medium for microorganisms. A low concentration (1% and 2%) of acetic acid treatment could hydrolyze the algal biomass and release more carbohydrates and COD than other chemical treatments. Among the hydrolysates, acetic acid proved to be the best treatment for the hydrolysis of algal biomass. The optimal treatment condition was the 5% acetic acid treatment for 60 min.

Table 2. Comparison of sugar recovery from algal biomass using various chemical treatments reported in the literature vs. present study.

Reference	Treatment	Result
Wu et al. [41]	5% H_2SO_4	51.6 mg/g of glucose
Castro et al. [15]	10% H_2SO_4	sugar yield was 161.1 mg/g of biomass
Jain et al. (2017) [13]	1% H_2SO_4	42.4 mg COD/g, 10.6 mg TOC/g, 0.086 mg TP/g 39 mg TN/g
Dziekonska-Kubczak et al. (2018) [26]	Treated Jerusalem Artichoke Stalks with 5% HNO_3 (w/v) at 121 °C in 60 min.	57 mg of glucose/g of biomass
Our study	5% HNO_3 5% Acetic acid	36.3 mg/g 40–44 mg/

3.3. Characteristics of Hydrolysate Prepared from Taro Leaves

The hydrolysates prepared via the chemical treatment of Taro leaves are given below.

3.3.1. Carbohydrate and COD Solubilization for Taro Leaves

The cell wall of Taro leaves is different from algal biomass as it mainly contains cellulose (1,4-β D-glucans) and non-cellulosic polysaccharides (galacturonorhamnans, galactomannan, glucomannan, and arabinoxylan) with β-1,4 linkages [42]. It was observed from this study that the same chemicals acted differently for different biomass. Figure 5 shows the carbohydrate and monomer content in the hydrolysate of *Colocasia esculenta* (Taro) leaves. Acetic acid has been reported to decrease cellulose crystallinity by altering the cellulose structure with high hydrogen bond basicity (>1.0). Pin et al. [43] summarized that acetate anions can permeate into lignin and break the O-4 bonds between carbohydrates (cellulose and hemicelluloses) and lignin to improve the overall hydrolysis efficiency. In the present study, monomers (mg/g biomass) in the 1% acetic-acid-treated hydrolysate were 15.4 for glucose and 21.8 for xylose for the 30 min treatment, and 6.58 for glucose and 8.58 for xylose for the 1 h treatment. By increasing the concentration of acetic acid to 2%, the percentage of monomers was almost 100% of the recovered carbohydrate. For the 5% acetic acid treatment, sugars (mg/g biomass) were found to be low, i.e., 7.93 for glucose and 10.12 for xylose for the 30 min treatment and 7.17 for glucose and 8.05 for xylose for the 1 h treatment. The amount of reduced sugar started decreasing with an increase in the concentration of chemicals, which indicated that increasing the chemical concentration led to the oxidation of the monomers in the resultant hydrolysate. Statistical analysis of carbohydrate data showed that the concentration and all the effects were significant for hydrolysis prepared by acetic acid. For monomers, in most of the models, all the effects were also statistically significant. R^2 values were also more than 0.9 for most models (Table 3).

Results obtained in the present study were similar to those reported by [31], in which rice straw was treated with 4.5% of acetic acid for 1 h and the recovery of sugars was as follows: Glucose—4.6 mg/g, xylose—5.6 mg/g, and arabinose—13.4 mg/g. As compared to the other chemical treatments (Figure 5), being a weak acid, acetic acid could not break the lignin–carbohydrate bonds to facilitate carbohydrate solubilization and was not effective for the hydrolysis of *Colocasia esculenta* (Taro) leaves.

As compared to cellulose, hemicelluloses are made of C_5 (xylose and arabinose) and C_6 sugars (mannose, glucose, and galactose) [22]. Hemicellulose has an amorphous and heterogeneous structure. Such a structure makes it susceptible to hydrolysis [44]. During the initial phase of acid pretreatment, oligomers of various chain lengths were produced due to acid-mediated hydrolysis. Later, these oligomers of varying degrees of polymerization continued to break down into monomers [45]. In the present study, the reduced sugars for nitric acid treatment were the highest at a 5% concentration (Figure 5). Sun et al. [37] also used nitric acid (6%) to treat sugarcane bagasse and recovered 18.6 mg of xylose/g, 20.4 mg of arabinose/g, and 28.7 mg of glucose/g. If one compared the results reported by Sun et al. [37], the recovery of arabinose was lower in our study but glucose and xylose

recovery were almost similar. Statistical analysis of carbohydrates released during HNO_3 treatment indicated that depending on the treatment, either time or concentration, or both, were significant parameters that affected the carbohydrate or monomer's release. These models also showed that, most often, these significant parameters provided positive effects on carbohydrate release (Table 3).

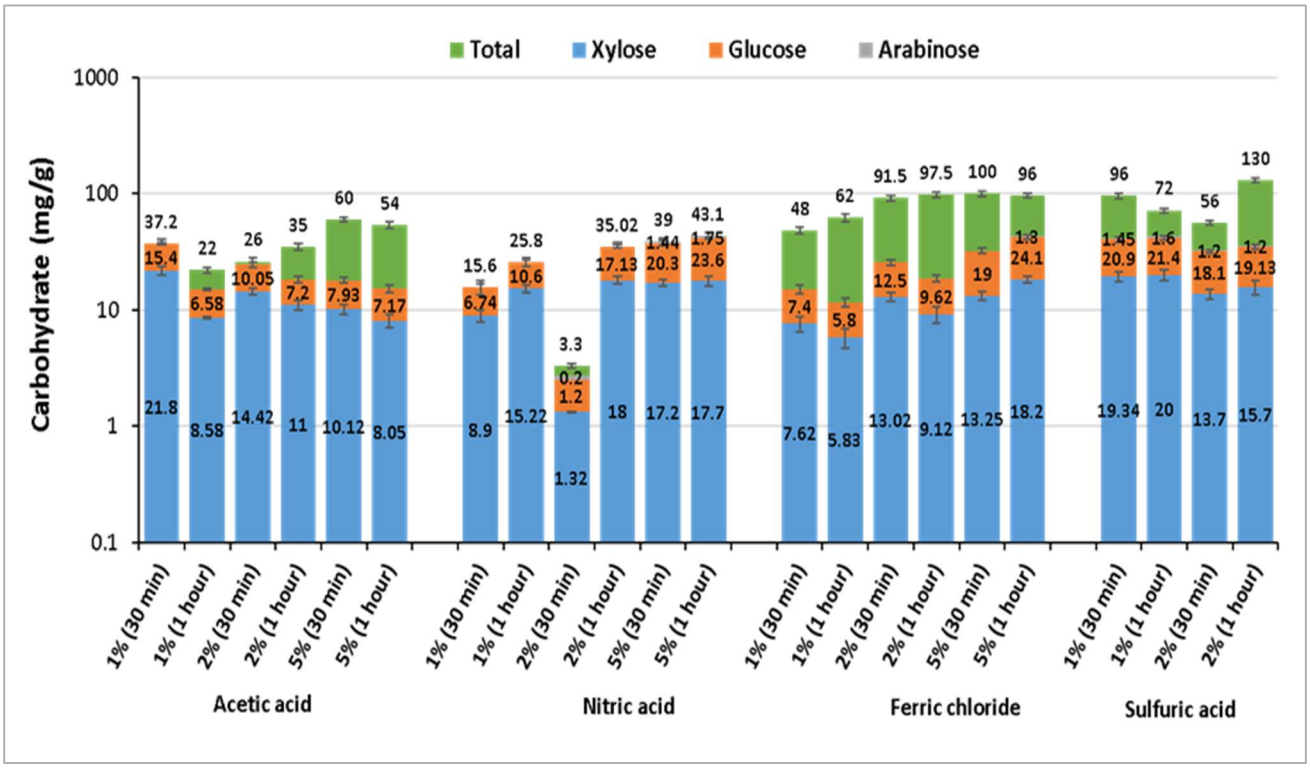

Figure 5. Total carbohydrate and its monomers (mg/g) in the hydrolysates of *Colocasia esculenta* (Taro) leaves. Numbers in the figure show the concentration (mg/g) of individual components (lagend). Numbers on the top of each column show the total carbohydrate content. Vertical bars show the error in estimation as standard deviation.

As these biomass feedstocks have cellulose and its breakdown produces monomers, the acid hydrolysis of pure cellulose using the above chemicals was explored. The main goal was to examine the hydrolysis of cellulose into its monomers, and the results obtained were compared with the hydrolysis of biomass (algae and Taro leaves) to determine the efficiency of the reagents used in this study. The glucose content obtained by the hydrolysis of biomass (Figure 5) was higher than the pure cellulose powder (Figure S1), which indicated that hemicellulose (present in the Taro leaves) solubilization led to the formation of a glucose monomer along with xylose. Hence, the glucose content observed in Figure 5 was due to the dissolution of both cellulose and hemicellulose. Acetic acid was not effective in the solubilization of pure cellulose (Figure S1), so the monomers obtained in Figure 5 must be due to the solubilization of hemicellulose present in the biomass of Taro leaves.

Metal salt $FeCl_3$ was reported to enhance hemicellulose solubilization with low sugar degradation and less inhibitory compound formation [46,47]. In the present study, the carbohydrate release was the highest in the ferric-chloride-treated hydrolysate (Figure 5). The ferric chloride's strength directly affected the hydrolysis rate as the sugar solubilization increased with an increase in the ferric chloride concentration. The best treatment condition for the solubilization of carbohydrates was 5% ferric chloride treatment for 60 min.

Table 3. Statistical analysis of hydrolysate data for Taro leaves.

Hydrolysing Agent (Products)	Model 1	Model 2	Statistical Significance
Acetic acid-(carbohydrate)	y = 30.05 − 3.1a + 0.9b + 12.1ab R^2: 0.96	y = 43.3 − 10.6a + 27.4b + 4.6ab R^2: 0.99	Model 1: interaction effect significant, Model 2: main effects and interaction are significant, In model 2 confidence interval is significant for concentration
HNO_3-(carbohydrate)	y = 19.78 + 21.26a + 1.84b + 11.06ab R^2: 0.89	y = 30.875 + 7.15a + 20.35b − 3.05ab R^2:0.82	Model 1: Effect of a was significant, Model 2: b significant;
$FeCl_3$-(carbohydrate)	y = 74.75 + 10a + 39.5b − 4ab R^2: 0.94	y = 76.5 + 5a + 43b − 9ab R^2: 0.91	b significant for both the models,
H_2SO_4-(carbohydrate)	y = 88.5 + 25a + 9b + 49ab R^2: 0.9	none	Interaction effect significant (F 0.01)
Acetic acid –(glucose)	y = 9.8075 − 5.835a − 2.365b + 2.985ab R^2: 0.96	y = 9.2775 − 4.775a − 3.425b + 4.045ab R^2: 0.95	All the effects were significant (model 1 and model 2)
HNO_3-(glucose)	y = 13.765 + 18.09a + 10.19b + 14.23ab R^2: 0.9	y = 15.16 + 3.48a + 12.98b − 0.38ab R^2: 0.96	Model 1: a and interaction effects were significant, Model 2: b significant,
$FeCl_3$-(glucose)	y = 8.22 − 1.01a + 3.23b + 0.58ab R^2: 0.56	y = 14.077 + 1.755a + 14.945b + 3.345ab R^2: 0.91	Model 1: none of the effects was significant, Model 2: b was significant,
H_2SO_4-(glucose)	y = 19.885 + 0.77a − 2.54b + 0.26ab R^2: 0.234	none	None of the effects was significant
Acetic acid-(xylose)	y = 13.93 − 8.335a − 2.515b + 4.885ab R^2: 0.96;	y = 12.14 − 7.645a − 6.105b + 5.575ab R^2: 0.95	Model 1: a and interaction effects were significant, Model 2: all effects were significant,
HNO_3-(xylose)	y = 10.855 + 11.49a − 2.39b + 5.19ab R^2: 0.99;	y = 14.75 + 3.4a + 5.4b − 2.9ab R^2: 0.95	Model 1: a and ab effects were significant; Model 2: all the effects were significant,
$FeCl_3$-(xylose)	y = 9.2475 − 3.545a + 5.045b − 1.755ab R^2: 0.91	y = 11.2275 + 1.535a + 9.005b + 3.325ab R^2: 0.92	Model 1: main effects were significant, Model 2: effect of b was significant,
H_2SO_4-(xylose)	y = 17.14 + 1.24a − 4.88b + 0.76ab R^2: 0.42	none	None of the effects was significant;
Acetic acid-(PO_4-P)	y = 0.8 − 0.1a − 0.15b − 0.11ab; R^2: 0.53	y = 1.07 + 0.01a + 0.39b; R^2: 0.17	None of the effects was significant;
HNO_3-(PO_4-P)	y = 0.1325 + 0.145a − 0.045b − 0.015ab R^2: 0.76	none	Effects of a was significant,
$FeCl_3$-(PO_4-P)	y = 0.5625 − 0.495a − 0.065b + 0.115ab R^2: 0.82,	y = 0.545 − 0.35a − 0.1b + 0.26ab R^2: 0.88	Model 1: effects of a was significant; Model 2: a and interaction term were significant,
H_2SO_4-(PO_4-P)	y = 0.3925 + 0.155a + 0.295b + 0.145ab; R^2: 0.67	none	None of the effects was significant;

a: Time, b: Concentration, 2^2 factorial tests were conducted. The F test was tested for finding the significance of a and b (significance tests at 0.01 and 0.05 levels). R^2 = Sum of square (model)/Sum of the square total. In the first column, the hydrolyzing agent used was given and, in the brackets (), the name of the product for which the statistical model was developed was depicted. If the significance level is not mentioned, those significance levels are at the 0.05 level.

The reaction mechanism of $FeCl_3$ in biomass degradation is related to its Lewis acid character and is mainly involved in hemicellulose solubilization [48]. According to the data obtained from hemicellulose hydrolysis, it is hypothesized that hemicellulose was categorized into two fragments: Fast-reacting xylan and slow-reacting xylan [49]. This hypothesis was built on the fact that the hydrolysis rate decreased dramatically after conversion reached approximately 70%. It is observed that xylan located in the cell wall is easily accessible by the hydrolyzing reagent. This hemicellulose is fast-reacting, while the remaining xylan is located at a greater depth and is firmly retained within cellulose chains. The other part of the slow-reacting xylan is embedded within or attached to the lignin by lignin–carbohydrate bonds [50].

In addition to the hydrolysis of hemicellulose, diluted acid pretreatment decreases the degree of polymerization of cellulose by improving its susceptibility to enzymatic degradation. Pretreatment with ferric chloride has been successfully used in earlier studies for different lignocellulosic biomass such as sugarcane bagasse [29,46], olive tree biomass [51], corn stover [52], and barley straw [53]. In total, 300–350 mg of sugar/g of biomass was released when $FeCl_3$-pretreated biomass was further hydrolyzed using an enzyme.

In the present study, COD solubilization (Figure 6) for the ferric-chloride-treated hydrolysate was 75, 106.3, and 135.3 mg/g of biomass within a 30 min reaction. For the 1 h treatment, the corresponding values were 87.8, 100.3, and 119.7 mg/g of biomass, respectively. There is a significant increase in the COD solubilization with an increase in the ferric chloride concentration. However, in the acetic acid-treated hydrolysate, there was no

significant increase in the solubilization of the COD with an increase in the concentration of the chemical or time parameters.

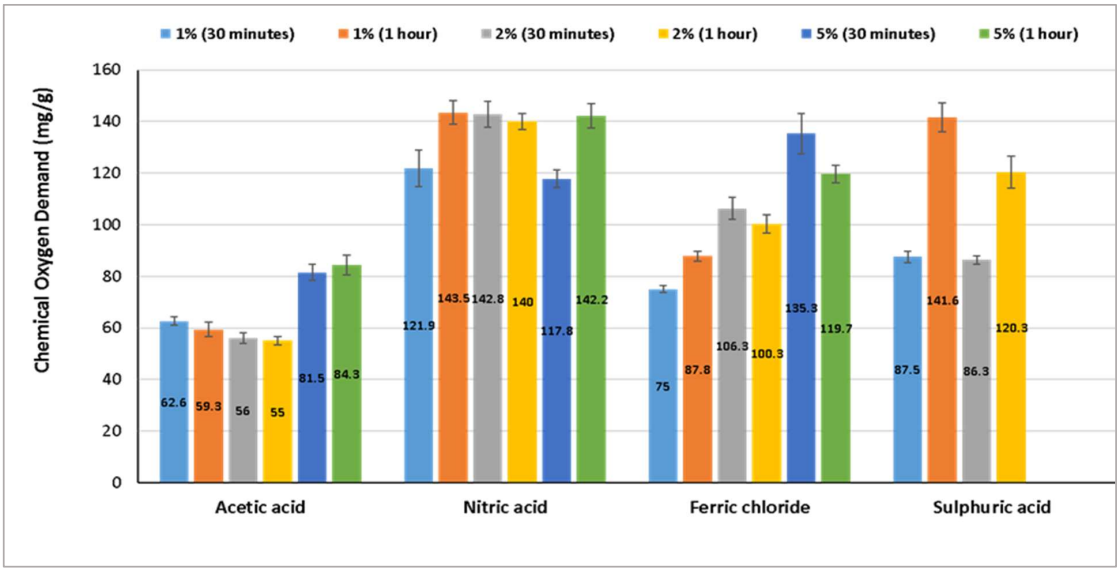

Figure 6. COD (mg/g) in the different hydrolysates of *Colocasia esculenta* (Taro) leaves. Numbers in the figure show the COD concentration (mg/g). Vertical bars show the error in estimation as standard deviation.

In the nitric-acid-treated hydrolysate, COD was very high, which showed that nitric acid was very effective in the dissolution of COD compared to other chemicals such as acetic acid and ferric chloride (Figure 6). Higher soluble COD illustrates higher solubilization of organic matter [54]. For the reference study, the soluble COD (Figure 6) did not show any significant change with an increase in the reaction time. Results of the present study correlated well with Pin et al. [43], who examined the effect of different pretreatment times (between 1 h and 3 h) on the hydrolysis of water hyacinth and concluded that there were no significant differences in the yield due to the changes in the duration of pretreatment.

According to the results obtained, it was observed that contrary to the algal biomass treatment, nitric acid and ferric chloride proved to be the better hydrolyzing reagents for *Colocasia esculenta* (Taro) leaves, and acetic acid treatment was found to be the least effective.

Several studies have been carried out on the hydrolysis of Taro leaves mostly using the enzymatic treatment. However, in the present study, chemical hydrolysis was conducted (Table 4). The sugar yield in this study was higher than those reported in the literature. Hence, for better carbohydrate release, chemicals used in the present study showed better carbohydrate recovery, especially with 5% HNO_3 and 5% $FeCl_3$, for which a release of 43.6 and 42.7 mg of sugar/g of dry matter was obtained, respectively. From the results, it is evident that the Taro leaf is one of the attractive resources for the recovery of sugars and nutrients.

Table 4. Comparison of sugar recovery from *Colocasia esculenta* (Taro) leaves by various chemical treatments reported in literature vs. present study.

Reference	Treatment Reagent	Reducing Sugar Yield
Saenphoom et al. [22]	Enzyme treatment {Hemicellulase} (1% *w*/*v*) at 60 °C for 24 h leaves of Taro leaves were treated	29.4 mg/g
Yan et al. [55]	Enzymatic treatment {Cellulase} (0.04 g/g) at 45 °C for 48 h, Roots of Taro plants were treated	165 mg/g
Our study	5% HNO_3 5% $FeCl_3$ (leaves of Taro were treated)	43.6 mg/g 42.7 mg/g

3.3.2. Nutrient Solubilisation (TN and TP)

Nutrient recovery from biomass would reintroduce these nutrients as fertilizers. Such recovery also helps to reduce the load on the limited phosphorus reserve and also helps to reduce the unintended release of these nutrients in water and soil [40]. Figure 7 shows the total nitrogen and nitrate (mg/g) present in the hydrolysates of Taro leaves prepared using different treatments. TKN was performed only for those samples, which showed enhanced sugar recoveries. Figure 8 shows the orthophosphate content present in the hydrolysates of Taro leaves.

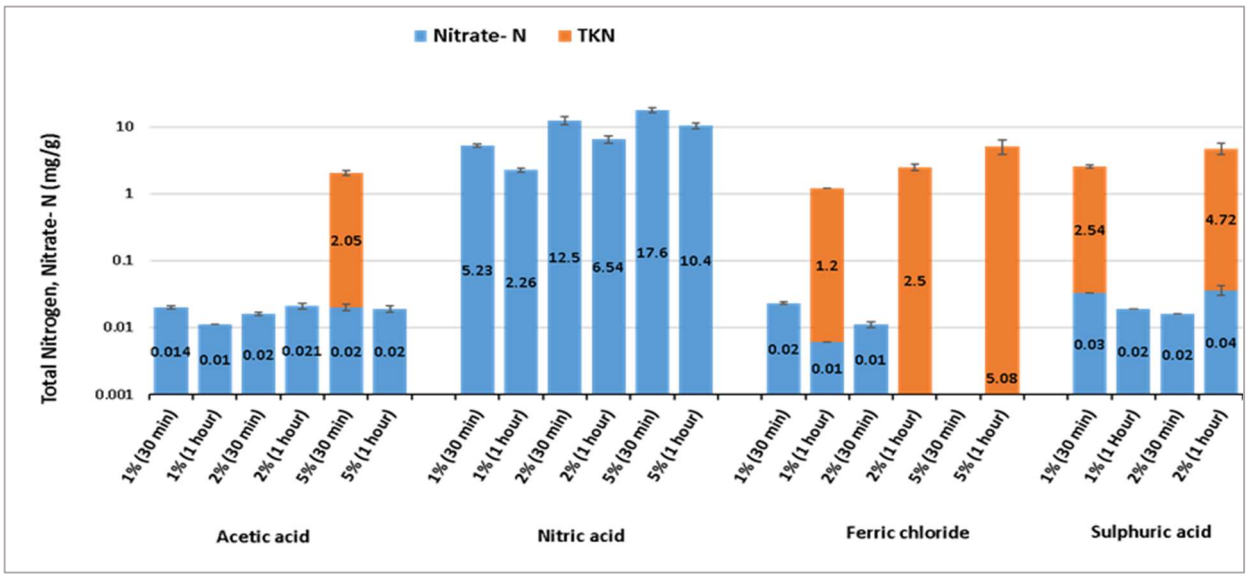

Figure 7. Total nitrogen and nitrate (mg/g) in different hydrolysates of *Colocasia esculenta* (Taro) leaves. Numbers in the figure show the concentration (mg/g) of individual components (lagend). Vertical bars show the error in estimation as standard deviation.

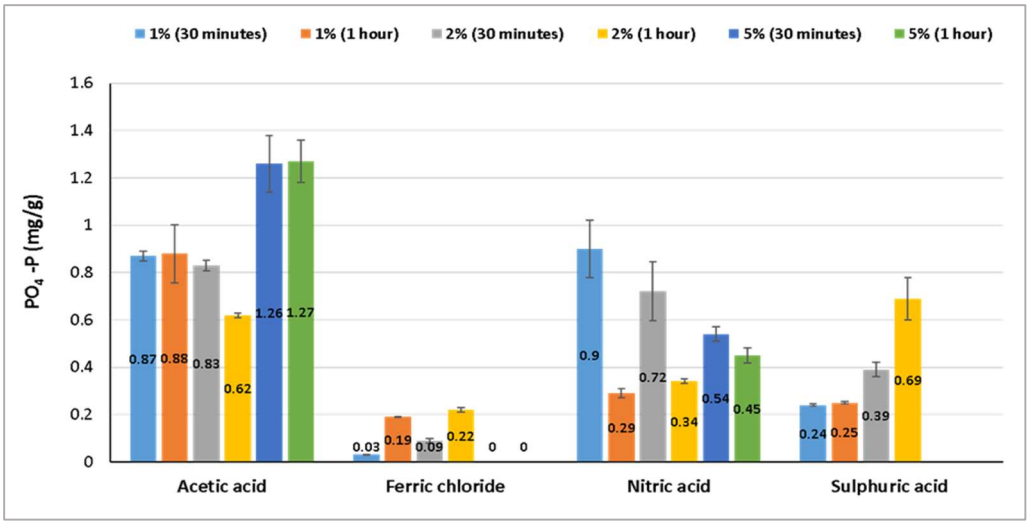

Figure 8. Phosphate-P (mg/g) in different hydrolysates of *Colocasia esculenta* (Taro) leaves. Number in the figure shows the PO_4-P concentration (mg/g). Vertical bars show the error in estimation as standard deviation.

For acetic acid treatment, nitrate-N (Figure 7) was found to be 0.01–0.02 mg/g of biomass for both 30 min and 1 h reaction times. TKN was estimated to be 2.05 mg/g of biomass for the 5% treatment for 30 min. There was an insignificant change in the

nitrate recovery with changes in the concentration and time. For ferric chloride treatment, the nitrate-N (Figure 7) values decreased with an increase in the chemical concentration irrespective of the time parameter. For the 30 of treatment, nitrate-N was 0.02, 0.01, and 0 mg/g of biomass for 1%, 2%, and 5% concentrations, respectively. For the 1 h treatment, the corresponding values were 0.01, 0, and 0 mg/g of biomass. TKN was measured for 1 h of treated hydrolysate and it was found to be 1.2, 2.5, and 5.08 mg/g of biomass for 1%, 2%, and 5% concentrations. Soluble TKN increased due to the production of intermediate compounds such as protein, amino acids, and other nitrogenous components [56].

Nitrate-N (Figure 7) for nitric acid treatment was higher than the other chemical treatment due to the presence of nitrate in the hydrolyzing reagent itself. For the reference treatment (1% and 2% H_2SO_4), nitrate-N was between 0.02 and 0.04 mg/g of biomass for 30 min and 1 h, respectively. TKN was approximately 2.54 mg/g of biomass for 1% treatment for 30 min, and for the 2% treatment, TKN was 4.72 mg/g of biomass for the 1 h reaction.

The solubilization of orthophosphate-P (Figure 8) for acetic-acid-treated hydrolysates was higher than the other chemical treatments and ranged from 0.88–1.27 mg/g of biomass. There was a significant change in the solubilization of phosphate with an increase in the concentration. For ferric-chloride-treated hydrolysates, orthophosphate-P solubilization was found to be much lower, and the content decreased with an increase in the concentration (Figure 8). Ferric chloride leads to the formation of Iron phosphate and its oxyhydroxide complexes, and these complexes have their minimum solubility at a pH between 4 and 6. This could be the reason behind the low phosphorus recovery in the hydrolysate prepared from both biomasses. To remove phosphorus through chemical precipitation, one needs to maintain alkalinity, pH, and hardness at the proper level. These are the main controlling factors behind phosphorus precipitation [40].

The orthophosphate-P (Figure 8) content was between 0.29 and 0.9 mg/g of biomass for nitric-acid-treated hydrolysates. There was an insignificant change in the phosphate recovery with a change in concentration and time for nitric-acid-treated hydrolysates. The orthophosphate-P (Figure 8) release for 1% H_2SO_4 treatment was 0.24 and 0.25 for 30 min and 1 h reactions, respectively. The corresponding values for 2% H_2SO_4 treatment were 0.39 and 0.69 mg/g of biomass, respectively. According to Kim et al. [57], using chemicals for hydrolysis procedures can also form trace amounts of soluble hydroxide and phosphate complexes. However, the concentrations of these complexes did not interfere with precipitation. To understand the attractiveness of each biomass, the carbohydrate, N, and P release from the best hydrolyzing reagent were compared. It was observed that for algae, the carbohydrate release was less than that of Taro leaves (a ratio of algae: Taro of 0.44). However, the release of N and P was much higher for algal biomass (4 to 11 times higher in algae). Hence, it can be deduced that Taro leaves may be a good source of sugars compared to algae, whereas algae would be the best source to recover nutrients.

4. Conclusions

Algae and *Colocasia esculenta* (Taro) leaves can be utilized as low-cost waste biomass to recover sugars and nutrients. Among the chemicals used for hydrolyzing the biomass, different chemicals acted differently for the respective biomass. Acetic acid was found to be the best for algal biomass hydrolysis with a sugar yield of 44.2 mg/g biomass (solubilized monomers—0.82 mg/g of biomass), and for *Colocasia esculenta* (Taro) leaves, ferric chloride treatment was the best with a sugar yield of 95 mg/g biomass (solubilized monomers—43.6 mg/g of biomass). PO_4-P release from algal biomass was significantly affected by the concentration of the hydrolyzing agents. Statistical models revealed that in most cases, an increase in concentration would also increase the P release. However, for carbohydrate release, in most cases, no such significant effect was observed. The statistical model indicated that carbohydrate release from Taro leaves was affected by the time and concentration for most of the chemical hydrolyzing agents used in this study. Based on the results of this study, in the future, composting followed by chemical treatment will be explored to improve the sugar and nutrient yield.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142416383/s1, Figure S1: Carbohydrate (mg/g) in the different hydrolysates of pure cellulose powder. *Number in the figure shows the concentration (mg/g) of individual components (lagend). Number on the top of each column shows the total carbohydrate content*; Table S1: COD and TOC data obtained from various hydrolysis procedures for algae, Table S2: Characteristics of hydrolysate prepared from algae using various hydrolysing agents.

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References

1. Show, K.Y.; Yan, Y.; Ling, M.; Ye, G.; Li, T.; Lee, D.J. Hydrogen production from algal biomass-advances, challenges and prospects. *Bioresour. Technol.* **2018**, *257*, 290–300. [CrossRef] [PubMed]
2. Dahiya, S.; Chowdhury, R.; Tao, W.; Kumar, P. Biomass and Lipid Productivity by Two Algal Strains of *Chlorella sorokiniana* grown in Hydrolysate of Water Hyacinth. *Energies* **2021**, *14*, 1411. [CrossRef]
3. Chew, K.W.; Yap, J.Y.; Show, P.L.; Suan, N.H.; Juan, J.C.; Ling, T.C.; Chang, J.S. Microalgae biorefinery: High value products perspectives. *Bioresour. Technol.* **2017**, *229*, 53–62. [CrossRef]
4. Bundhoo, Z.M.; Mohee, R. Ultrasound-assisted biological conversion of biomass and waste materials to biofuels: A review. *Ultrason. Sonochem.* **2018**, *40*, 298–313. [CrossRef] [PubMed]
5. Kim, D.Y.; Yim, S.C.; Lee, P.C.; Lee, W.G.; Lee, S.Y.; Chang, H.N. Batch and continuous fermentation of succinic acid from wood hydrolysate by *Mannheimia succiniciproducens* MBEL55E. *Enzym. Microb. Technol.* **2004**, *35*, 648–653. [CrossRef]
6. Park, J.Y.; Shiroma, R.; Al-Haq, M.I.; Zhang, Y.; Ike, M.; Arai-Sanoh, Y.; Ida, A.; Kondo, M.; Tokuyasu, K. A novel lime pretreatment for subsequent bioethanol production from rice straw-calcium capturing by carbonation process. *Bioresour. Technol.* **2010**, *101*, 6805–6811. [CrossRef]
7. Mohan, M.; Balaji, C.; Goud, V.V.; Banerjee, T. Thermodynamic insights in the separation of cellulose/hemicellulose components from lignocellulosic biomass using ionic liquids. *J. Solut. Chem.* **2015**, *44*, 538–557. [CrossRef]
8. Li, J.; Zhang, M.; Li, J.; Wang, D. Corn stover pretreatment by metal oxides for improving lignin removal and reducing sugar degradation and water usage. *Bioresour. Technol.* **2018**, *263*, 232–241. [CrossRef]
9. Mehariya, S.; Fratini, F.; Lavecchia, R.; Zuorro, A. Green extraction of value-added compounds form microalgae: A short review on natural deep eutectic solvents (NaDES) and related pre-treatments. *J. Environ. Chem. Eng.* **2021**, *9*, 105989. [CrossRef]
10. Srinivasan, A.; Nkansah-Boadu, F.; Liao, P.H.; Lo, K.V. Effects of acidifying reagents on microwave treatment of dairy manure. *J. Environ. Sci. Health* **2014**, *49 Pt B*, 532–539. [CrossRef]
11. Ruan, Z.; Zanotti, M.; Wang, X.; Ducey, C.; Liu, Y. Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. *Bioresour. Technol.* **2012**, *110*, 198–205. [CrossRef]
12. Huang, C.; Chen, X.F.; Xiong, L.; Ma, L.L.; Chen, Y. Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. *Biotechnol. Adv.* **2013**, *31*, 129–139. [CrossRef] [PubMed]
13. Jain, P.; Arora, N.; Mehtani, J.; Pruthi, V.; Majumder, C.B. Pretreated algal bloom as a substantial nutrient source for microalgae cultivation for biodiesel production. *Bioresour. Technol.* **2017**, *242*, 152–160. [CrossRef]
14. Espi, E.; Ribas, I.; Díaz, C.; Sastron, O. Feedstocks for Advanced Biofuels. In *Sustainable Mobility*; IntechOpen: London, UK, 2020; pp. 39–60.
15. Castro, Y.A.; Ellis, J.T.; Miller, C.D.; Sims, R.C. Optimization of wastewater microalgae saccharification using dilute acid hydrolysis for acetone, butanol, and ethanol fermentation. *Appl. Energy* **2015**, *140*, 14–19. [CrossRef]
16. Moon, M.; Kim, C.W.; Farooq, W.; Suh, W.I.; Shrivastav, A.; Park, M.S.; Mishra, S.K.; Yang, J.W. Utilization of lipid extracted algal biomass and sugar factory wastewater for algal growth and lipid enhancement of *Ettlia* sp. *Bioresour. Technol.* **2014**, *163*, 180–185. [CrossRef]
17. Dahiya, S.; Shilpie, A.; Balasundaram, G.; Chowdhury, R.; Kumar, P.; Mishra, A.K. Diversity of algal species present in waste stabilisation ponds and different factors affecting its enrichment and phototaxis. *Chem. Ecol.* **2021**, *37*, 515–529. [CrossRef]

18. Chowdhury, R.; Freire, F. Bioenergy production from algae using dairy manure as a nutrient source: Life cycle energy and greenhouse gas emission analysis. *Appl. Energy* **2015**, *154*, 1112–1121. [CrossRef]
19. Chowdhury, R.; Franchetti, M. Life cycle energy demand from algal biofuel generated from nutrients present in the dairy waste. *Sustain. Prod. Consum.* **2017**, *9*, 22–27. [CrossRef]
20. Chowdhury, R.; Sadhukhan, J.; Traverso, M.; Keen, P.L. Effects of residence time on life cycle assessment of bioenergy production from dairy manure. *Bioresour. Technol. Rep.* **2018**, *4*, 57–65. [CrossRef]
21. Verma, S.; Chowdhury, R.; Das, S.K.; Franchetti, M.J.; Liu, G. Sunlight intensity, photosynthetically active radiation modelling and its application in algae-based wastewater treatment and its cost estimation. *Sustainability* **2021**, *13*, 11937. [CrossRef]
22. Saenphoom, P.; Chimtong, S.; Phiphatkitphaisan, S.; Somsri, S. Improvement of taro leaves using pre-treated enzyme as prebiotics in animal feed. *Agric. Agric. Sci. Procedia* **2016**, *11*, 65–70. [CrossRef]
23. Zhao, R.; Zhang, Z.; Zhang, R.; Li, M.; Lei, Z.; Utsumi, M.; Sugiura, N. Methane production from rice straw pretreated by a mixture of acetic–propionic acid. *Bioresour. Technol.* **2010**, *101*, 990–994. [CrossRef] [PubMed]
24. Cimini, D.; Argenzio, O.; D'Ambrosio, S.; Lama, L.; Finore, I.; Finamore, R.; Pepe, O.; Faraco, V.; Schiraldi, C. Production of succinic acid from *Basfia succiniciproducens* up to the pilot scale from *Arundo donax* hydrolysate. *Bioresour. Technol.* **2016**, *222*, 355–360. [CrossRef] [PubMed]
25. Xavier, M.C.A.; Coradini, A.L.V.; Deckmann, A.C.; Franco, T.T. Lipid production from hemicellulose hydrolysate and acetic acid by *Lipomyces starkeyi* and the ability of yeast to metabolize inhibitors. *Biochem. Eng. J.* **2017**, *118*, 11–19. [CrossRef]
26. Dziekońska-Kubczak, U.; Berłowska, J.; Dziugan, P.; Patelski, P.; Pielech-Przybylska, K.; Balcerek, M. Nitric acid pretreatment of *Jerusalem artichoke* stalks for enzymatic saccharification and bioethanol production. *Energies* **2018**, *11*, 2153. [CrossRef]
27. Yeesang, C.; Cheirsilp, B. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. *Bioresour. Technol.* **2011**, *102*, 3034–3040. [CrossRef] [PubMed]
28. Zhang, H.; Zhang, S.; Yuan, H.; Lyu, G.; Xie, J. $FeCl_3$-catalyzed ethanol pretreatment of sugarcane bagasse boosts sugar yields with low enzyme loadings and short hydrolysis time. *Bioresour. Technol.* **2018**, *249*, 395–401. [CrossRef] [PubMed]
29. Zhang, Y.; Nielsen, J.; Liu, Z. Engineering yeast metabolism for production of terpenoids for use as perfume ingredients, pharmaceuticals and biofuels. *FEMS Yeast Res.* **2017**, *17*, fox080. [CrossRef]
30. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association (APHA): Washington, DC, USA, 2005.
31. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.T.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356. [CrossRef]
32. Montgomery, D.C. *Design and Analysis of Experiments*; John Wiley & Sons: New York, NY, USA, 2005.
33. Zhang, R.; Lu, X.; Sun, Y.; Wang, X.; Zhang, S. Modeling and optimization of dilute nitric acid hydrolysis on corn stover. *J. Chem. Technol. Biotechnol.* **2011**, *86*, 306–314. [CrossRef]
34. Vinogradov, V.V.; Mizerovskii, L.N.; Akaev, O.P. Reaction of cellulose with aqueous solutions of orthophosphoric acid. *Fibre Chem.* **2002**, *34*, 167–171. [CrossRef]
35. Rodrıguez-Chong, A.; Ramirez, J.A.; Garrote, G.; Vázquez, M. Hydrolysis of sugar cane bagasse using nitric acid: A kinetic assessment. *J. Food Eng.* **2004**, *61*, 143–152. [CrossRef]
36. Wang, R.; Tian, Y.; Xue, S.; Zhang, D.; Zhang, Q.; Wu, X.; Cong, W. Enhanced microalgal biomass and lipid production via co-culture of *Scenedesmus obliquus* and *Candida tropicalis* in an autotrophic system. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 1387–1396. [CrossRef]
37. Sun, S.; Chen, W.; Tang, J.; Wang, B.; Cao, X.; Sun, S.; Sun, R.C. Synergetic effect of dilute acid and alkali treatments on fractional application of rice straw. *Biotechnol. Biofuels* **2016**, *9*, 217. [CrossRef] [PubMed]
38. Lin, Y.C.; Shangdiar, S.; Chen, S.C.; Chou, F.C.; Lin, Y.C.; Cho, C.A. Microwave irradiation with dilute acid hydrolysis applied to enhance the saccharification rate of water hyacinth (*Eichhornia crassipes*). *Renew. Energy* **2018**, *125*, 511–517. [CrossRef]
39. Kim, I.; Hwan, Y. Co-production of bioethanol and biodiesel from corn stover pretreated with nitric acid. *Fuel* **2015**, *143*, 285–289. [CrossRef]
40. Sengupta, S.; Nawaz, T.; Beaudry, J. Nitrogen and phosphorus recovery from wastewater. *Curr. Pollut. Rep.* **2015**, *1*, 155–166. [CrossRef]
41. Wu, C.H.; Chien, W.C.; Chou, H.K.; Yang, J.; Lin, H.T.V. Sulfuric acid hydrolysis and detoxification of red alga *Pterocladiella capillacea* for bioethanol fermentation with thermotolerant yeast *Kluyveromyces marxianus*. *J. Microbiol. Biotechnol.* **2014**, *24*, 1245–1253. [CrossRef]
42. Jiang, G. The non-starch polysaccharides of taro (*Colocasia esculenta*). Ph.D. Thesis, The University of Hong Kong, Hong Kong, China, 1999. [CrossRef]
43. Pin, T.C.; Nakasu, P.Y.; Mattedi, S.; Rabelo, S.C.; Costa, A.C. Screening of protic ionic liquids for sugarcane bagasse pretreatment. *Fuel* **2019**, *235*, 1506–1514. [CrossRef]
44. Mohan, M.; Deshavath, N.N.; Banerjee, T.; Goud, V.V.; Dasu, V.V. Ionic liquid and sulfuric acid-based pretreatment of bamboo: Biomass delignification and enzymatic hydrolysis for the production of reducing sugars. *Ind. Eng. Chem. Res.* **2018**, *57*, 10105–10117. [CrossRef]
45. Shi, N.; Liu, Q.Y.; Wang, T.J.; Zhang, Q.; Ma, L.L.; Cai, C.L. Production of 5-hydroxymethylfurfural and furfural from lignocellulosic biomass in water-tetrahydrofuran media with sodium bisulfate. *Chin. J. Chem. Phys.* **2015**, *28*, 650. [CrossRef]
46. Zhao, J.; Zhang, H.; Zheng, R.; Lin, Z.; Huang, H. The enhancement of pretreatment and enzymatic hydrolysis of corn stover by $FeSO_4$ pretreatment. *Biochem. Eng. J.* **2011**, *56*, 158–164. [CrossRef]

47. Romero, I.; Lopez-Linares, J.C.; Moya, M.; Castro, E. Optimization of sugar recovery from rapeseed straw pretreated with $FeCl_3$. *Bioresour. Technol.* **2018**, *268*, 204–211. [CrossRef] [PubMed]
48. Kamireddy, S.R.; Li, J.; Tucker, M.; Degenstein, J.; Ji, Y. Effects and mechanism of metal chloride salts on pretreatment and enzymatic digestibility of corn stover. *Ind. Eng. Chem. Res.* **2013**, *52*, 1775–1782. [CrossRef]
49. Kobayashi, T.; Sakai, Y. Hydrolysis rate of pentosan of hardwood in dilute sulfuric acid. *Bull. Agric. Chem. Soc. Jpn.* **1956**, *20*, 1–7. [CrossRef]
50. Conner, A.H. Kinetic modeling of hardwood prehydrolysis. Part I. Xylan removal by water prehydrolysis. *Wood Fiber Sci.* **2007**, *16*, 268–277.
51. Lopez-Linares, J.C.; Romero, I.; Moya, M.; Cara, C.; Ruiz, E.; Castro, E. Pretreatment of olive tree biomass with $FeCl_3$ prior enzymatic hydrolysis. *Bioresour. Technol.* **2013**, *128*, 180–187. [CrossRef]
52. Liu, L.; Sun, J.; Li, M.; Wang, S.; Pei, H.; Zhang, J. Enhanced enzymatic hydrolysis and structural features of corn stover by $FeCl_3$ pretreatment. *Bioresour. Technol.* **2009**, *100*, 5853–5858. [CrossRef]
53. Kim, S.B.; Lee, J.H.; Oh, K.K.; Lee, S.J.; Lee, J.Y.; Kim, J.S.; Kim, S.W. Dilute acid pretreatment of barley straw and its saccharification and fermentation. *Biotechnol. Bioprocess Eng.* **2011**, *16*, 725. [CrossRef]
54. Barua, V.B.; Raju, V.W.; Lippold, S.; Kalamdhad, A.S. Electrohydrolysis pretreatment of water hyacinth for enhanced hydrolysis. *Bioresour. Technol.* **2017**, *238*, 733–737. [CrossRef]
55. Yan, H.Z.; Fu, H.Y.; Su, G.X.; Zhao, D.; Wu, Y.C.; Liu, J.F.; Gao, P.F.; Huang, Y.T. Research on the Cellulase hydrolysis of *Colocasia antiquorumin* in producing Ethyl alcohol. *Earth Environ. Sci.* **2018**, *192*, 012057. [CrossRef]
56. Lo, K.V.; Tan, H.; Tunile, I.; Burton, T.; Kang, T.; Srinivasan, A.; Liao, P.H. Microwave enhanced advanced oxidation treatment of municipal wastewater sludge. *Chem. Eng. Process. Process Intensif.* **2018**, *128*, 143–148. [CrossRef]
57. Kim, Y.; Kreke, T.; Ko, J.K.; Ladisch, M.R. Hydrolysis-determining substrate characteristics in liquid hot water pretreated hardwood. *Biotechnol. Bioeng.* **2015**, *112*, 677–687. [CrossRef] [PubMed]