



Article Hydrolysis Optimization of By-Products from the Potato Processing Industry and Biomethane Production from Starch Hydrolysates

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(NPV) 37.5% higher than that of the current utilization scenario.

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Abstract: This study aims to investigate the use of Fenton reagents for the efficient hydrolysis of starch, an industrial by-product obtained from the potato processing industry. A full factorial design experiment (FFD) was conducted and a statistical model was developed for simulating the hydrolysis process. The most important factors were the hydrolysis temperature and the amount of H₂O₂ followed by the interaction of FeSO₄·7H₂O and temperature. At maximum levels of FeSO₄·7H₂O (1.00 g/L), H₂O₂ (0.51 g/L), and temperature (70.0 °C), a 99.5% hydrolysis yield was achieved, with a carbohydrate content of 28.65 g/L. Furthermore, analysis of hydrolysis kinetics demonstrated that an increased concentration of FeSO₄·7H₂O results in a decelerated rate of starch hydrolysis. Moreover, biodegradability tests were carried out to estimate the methane production potential from the produced hydrolysates. The specific methanogenic activity (SMA) was reached at 0.669 ± 0.014 g CH₄-COD g⁻¹ VSS day⁻¹ proving the effectiveness of the hydrolysis process and highlighting the potential of industrial starch for bioenergy production. A preliminary cost analysis showed that a small investment for utilizing the starch in an existing wastewater treatment facility of a potato processing company becomes profitable before the end of the 3rd year, obtaining a net present value

Keywords: starch hydrolysis; waste valorization; biomethane production; anaerobic digestion; potato processing industry; Fenton reagents

1. Introduction

The food processing and manufacturing stage of the food supply chain is responsible for generating a significant amount of waste on a global scale. In the European Union alone, approximately 30.5 Mtons of food waste were produced by food companies [1]. This waste not only causes economic losses to companies, but also environmental problems, since the production/extraction of the raw materials, their transport to the factory, and their processing, involved high environmental burdens. On top of that, food industries have to manage and treat the produced waste, adding extra costs and environmental impacts associated with their production processes. Nevertheless, food manufacturing waste due to the fact that is produced in large and homogeneous amounts offers substantial opportunities for recovery and production of added value materials, biofuels and biochemicals through various biochemical, physicochemical and thermal processing pathways [2].

Potato processing companies have attracted a lot of attention in recent years, as one ton of processed potato can lead to the production of around 0.16 tons of solid waste [3]. This waste is generated during the washing, frying, peeling and/or blanching of potato. High water consumption creates large amounts of wastewater with a high starch content and high chemical oxygen demand (COD) values of up to 10 g/L O₂ [4]. Starch has to be separated from the wastewater stream prior to it reaching the treatment unit, as due to its chemical characteristics, it can cause severe operational problems in the company's



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). wastewater treatment plant [5]. Furthermore, some reports indicate that the process of treating starch sludge in anaerobic digestion may result in the accumulation of volatile fatty acids (VFAs), leading to a decrease in pH levels [6]. Akbay et al. [7] investigated the production of biogas from starch sludge and its co-digestion with other organic waste. The authors observed that as the proportion of starch sludge in the mixture increased, the production of biogas decreased. Once the starch is recovered from the wastewater, it can be directly used as animal feed. However, the economic benefits are minimal. Instead, alternative utilization routes can be explored in order to maximize the added value of the starch by-product. This by-product from the potato processing industry can be initially hydrolyzed into easily biodegradable molecules, and then fed as a substrate into various bioprocesses. The high organic content of the hydrolysates can improve bioenergy production. Yokoi et al. [8] investigated the biohydrogen production from starchmanufacturing wastes using different cultures of microorganisms. The authors obtained a high hydrogen yield of 7.2 mol H_2 mol⁻¹ glucose when the medium contained sweet potato starch. In addition, Khongkliang et al. [9] studied the production of gaseous biofuels using anaerobic digestion from various concentrations and origins of starch. They achieved impressive biofuel yields ranging from 250.3 to 310.5 L-biomethane/kg COD and 48.2 to $81.5 \text{ L-H}_2/\text{kg}$ COD. Moreover, Lu et al. [10] who investigated the methane production in an up-flow anaerobic sludge blanket (UASB) reactor by feeding it treated starch wastewater, obtained a methane yield of 0.33 L CH₄/g COD_{removed} when the organic loading rate (OLR) and hydraulic retention time (HRT) were 4 g/L-day and 6 h, respectively. Furthermore, starchy wastes, usually after appropriate pretreatment, can be used for the production of various biochemicals such as bioethanol and biobutanol [11,12].

Starch is a biopolymer and consists of glucose homopolymers, amylose and amylopectin. It is a widely used material in the food industry as well as in industries that produce paper, textiles, bioethanol, bio-plastics and pharmaceutical products, since it is considered a cheap energy source [13]. Prior to most of its industrial applications, a pretreatment step is required due to its physicochemical characteristics, such as low solubility and digestibility, poor thermal properties and high viscosity [14]. It is reported that the various physicochemical properties depend on the botanical origin of the starch [15]. Among other starch feedstocks, potato starch displays unique properties due to: (1) its anionic character, which allows its combination with other materials promoting bioplastics production, (2) the amount of organically bound phosphorous that it contains [16] and (3) the large size of starch granules [17]. Different techniques have been proposed for its pretreatment based on physical, chemical or enzymatic conversions. All these techniques aim to break the polymer chains of starch down into smaller fragments [18]. While enzymatic hydrolysis is not considered an economical method [15], chemical degradation using strong acids can be hazardous and may inhibit the biological processes that follow. For this reason, alternative methods have been developed for starch modification, including thermal treatments and environmentally friendly chemicals such as ozone [19].

The oxidation process of starch, which involves the transformation of hydroxyl groups of starch, to carbonyl and carboxyl groups, has been thoroughly explained in previous studies [20]. It has also been mentioned that the structural properties of hydrolyzed starch depend on various parameters, including the structure of the starch granule, reaction time, temperature, and the type of oxidants used [21]. Some of the most commonly applied oxidants for the chemical modification of starch include sulfuric acid, sodium hypochlorite, ozone, periodate, permanganate, bromine, and hydrogen peroxide [13,18]. H₂O₂ is the most widely studied reagent among the options mentioned due to its affordability and environmentally friendly nature, as it breaks down into water and oxygen [22].

In comparison with other native starches, potato starch has the best accessibility to oxidants due to the structure of its granules [23]. In starch oxidation, metal ions such as Cu^{2+} , Fe^{2+} and Zn^{2+} could act as catalysts. Pietrzyk et al. [24], found that using ferrous ions in acidic conditions during potato starch oxidation with H_2O_2 , can increase the amount of carboxyl and aldehyde groups and decrease the phosphorus and amylose levels. Chemical

oxidation using iron ions and hydrogen peroxide is an easy-to-apply catalytic oxidation process, which produces hydroxyl radicals with high oxidation potential that can hydrolyze starch into smaller fragments, called dextrins. The modified starch solution produced by the oxidation process displays improved rheological properties. Dang et al. [25], who investigated potato starch oxidation using an electro-Fenton system in the presence of ferrous ions, found that oxidized starch showed good thermal stability, which is important for starch-based biomaterials applications.

Summarizing, chemical oxidation of starch using Fenton reagents is an ideal pretreatment for bioenergy production through anaerobic digestion [26]. It is an environmentally friendly process, as no hazardous chemicals are involved that are usually associated with traditional chemical hydrolysis processes [27]. Instead, only ferrous sulfate and hydrogen peroxide can be used, highlighting the sustainable nature of the approach since they are added in appropriate amounts. After the reaction, various forms of iron oxides could be present in starch hydrolysates. It has been reported that iron oxides improve methane production during anaerobic digestion by promoting direct interspecies electron transfer (DIET) among anaerobic microorganisms [28]. In addition, the iron in the substrate can undergo a reaction with the sulfide produced during anaerobic digestion, leading to the formation of ferrous iron as either FeS or FeS₂. This reaction helps to mitigate the detrimental effects of sulfide on methanogenesis [29] and improves the stability of anaerobic granular sludge [30]. Although some studies have investigated the hydrolysis of starch using Fenton reagents, there has been no significant research on optimizing hydrolysis conditions to produce a hydrolysate that can effectively be utilized as a substrate for methane production. Moreover, while the utilization of Fenton reagents for oxidation is considered both environmentally friendly and highly efficient as a pretreatment method, it cannot be classified as a cost-effective process [31]. Therefore, it is essential to identify the optimal conditions so as to achieve the lowest possible hydrolysis cost while maximizing economic benefits through the production of biomethane.

This study examines an efficient and environmentally friendly method of starch hydrolysis, derived as a byproduct from a potato processing company, and investigates the effects of temperature, ferrous sulfate and hydrogen peroxide concentrations on starch oxidation. The conditions for the process were optimized using a full factorial design experiment (FFD) and the effectiveness of the starch hydrolysis was evaluated via specific methanogenic activity (SMA) tests. Furthermore, a kinetic study was conducted to estimate the kinetic constants of starch hydrolysis using Fenton reagents. Finally, a preliminary cost analysis was implemented to identify the economic benefits of this utilization pathway compared to the existing scenario.

2. Materials and Methods

2.1. Materials

Industrially derived starch was obtained from the potato processing company Tasty Foods SA, located in the northern suburbs area of Athens, Greece. Approximately 10 kg of starch were transferred to the Organic Chemical Technology Laboratory at NTUA and stored at 4 °C for further use. Anaerobic granular sludge used as inoculum for the methanogenic activity tests was collected from the UASB reactor operating at the wastewater treatment facilities of the company that provided us with the industrial starch. All chemicals were purchased from Merck unless it is stated otherwise.

2.2. Hydrolysis of Starch

In each experiment, 31 g of starch (dry weight) was suspended in 1 L of deionized H₂O and placed on a magnetic stirrer for stirring and heating. The initial portion of the starch was selected according to preliminary experiments. Once the temperature of the solution reached the desired value, which was subject to statistical optimization, Fenton's reagents, namely FeSO₄.7H₂O and H₂O₂ 50% (w/w), were added at various initial concentrations indicated by the experimental design. The addition of the oxidation chemicals to the

aqueous solution initiated the hydrolysis process, which lasted for three hours under continuous stirring at 200 rpm. The concentration of total organic carbon (TOC), as well as the total carbohydrate content in the soluble part of the solution, were chosen as the means to measure the efficiency of the starch hydrolysis. During the hydrolysis process, samples were collected every 20 min and centrifuged at 6000 rpm for 15 min. Liquid supernatants were stored in the fridge for further analysis, while residual solids were dried and weighed.

The efficiency of the hydrolysis process was measured using Equation (1):

% starch hydrolysis yield =
$$\frac{\text{TOC}_{\text{initial}} - \text{TOC}_{\text{final}}}{\text{TOC}_{\text{initial}}} 100\%$$
(1)

where $TOC_{initial}$ is the initial TOC, which refers to the TOC of the industrial starch before hydrolysis, and TOC_{final} refers to the liquid supernatant at the end of the hydrolysis process.

2.3. Experimental Design

The most important operational parameters, namely FeSO₄·7H₂O concentration, H₂O₂ concentration, and hydrolysis temperature, were subject to statistical optimization by performing a 2^3 FFD. The optimization parameter ranges were selected taking into account preliminary experiments (results not shown), the cost of Fenton reagents and previous studies on similar systems [5]. The levels and the value ranges used in the FFD are presented in Table 1. Every experimental trial lasted for 3 h. The eight trials of the FFD were complemented with four runs at the center point (Level 0) for statistical purposes. The output variables were the % starch hydrolysis yield, calculated by Equation (1), and the amount of total carbohydrates (g/L) in the suspension. Both of them were simulated by using Equation (2):

$$\Upsilon_{i} = \beta_{0} + \beta_{1}X_{1} + \beta_{2}X_{2} + \beta_{3}X_{3} + \beta_{1}\beta_{2}X_{1}X_{2} + \beta_{1}\beta_{3}X_{1}X_{3} + \beta_{2}\beta_{3}X_{2}X_{3} + \beta_{1}\beta_{2}\beta_{3}X_{1}X_{2}X_{3}$$
(2)

where Y_i corresponds to the predicted response ($Y_1 = \%$ hydrolysis yield, $Y_2 = g$ of total carbohydrates/L of starch solution), β_0 is the interception parameter, β_1 , β_2 and β_3 are the linear effect parameters, X_1 , X_2 and X_3 are the coded variables and $\beta_1\beta_2$, $\beta_1\beta_3$, $\beta_2\beta_3$ and $\beta_1\beta_2\beta_3$ are the linear interaction effect coefficients [32].

Table 1. Ranges of the three factors for the factorial experiment.

Level	FeSO ₄ ·7H ₂ O (g/L)	H ₂ O ₂ (g/L)	Temperature (°C)
-1	0.60	0.329	60
0	0.80	0.419	65
1	1.00	0.509	70

The optimization parameters were obtained by linear regression, minimizing the difference between predicted and experimental values. All 12 runs were performed randomly to reduce the systematic error. The coefficients in the FFD were estimated by Matlab software (R2014b).

2.4. Biomethane Production from Starch Hydrolysates

The produced hydrolysates were tested as feedstock in small anaerobic reactors for their ability to produce biomethane with the selected anaerobic sludge. For the biological degradation of the hydrolysates, five batch reactors based on Hungate's Serum Bottle Technique [33] were set up and used to determine the sludge activity and consequently the biodegradability of the hydrolyzed starch. Each reactor was a serum bottle sealed with a screw-cap, with a total volume of 500 mL. To capture the metabolic CO₂, the top of each serum bottle was connected to a calibrated cylinder filled with 1N NaOH solution and a phenanthroline indicator [33]. The reactor was inoculated with 100 mL of anaerobic granular sludge and filled up to 450 mL with water. Then, each reactor was charged with 50 mg of acetic acid three times to achieve the maximum activity of sludge [34]. After that,

an amount of starch hydrolysates was added to obtain 50 mg COD. Each reactor was placed in a water bath which was set at 35 °C and 200 rpm. Methane production was measured using the liquid displacement technique. A high-resolution camera connected to a computer was placed in front of the apparatus in order to record the amount of methane produced at regular time intervals. Four different hydrolysate solutions, generated from the FFD, as well as 50 mg COD of the initial starch, were used as substrates in the small anaerobic reactors experiment. All experiments were conducted in triplicate. Each experiment lasted for 4 days and the calculation of SMA was based on the slope of cumulative gas production versus time plots.

2.5. Analytical Procedures

Chemical analyses were performed in accordance with Standard Methods (1989) [35], as follows: (1) total solids, volatile solids and suspended solids, sections 2540 B., 2540 E. and 2540 D., respectively; (2) pH, section 4500-H+, and (3) chemical oxygen demand, section 5220 C. The initial TOC in solid starch and dissolved organic carbon in each starch solution were measured by a TOC analyzer (Shimadzu, SSM-5000A, Shimadzu, TOC-L), which oxidizes with ultraviolet/persulfate oxidation and analyzes the CO_2 with the non-dispersive infrared analysis (NDIR) method. In addition, the amount of total carbohydrates was measured according to the Anthrone test method [36] using a UV–Vis spectrophotometer (Jasco V-530) and compared with a glucose calibration curve.

2.6. Cost Analysis

The cost analysis was performed for a hypothetical potato processing company, which produces 1000 tons of starch annually as by-product of operating 8000 h/year. These assumptions lead to a starch production rate of 125 kg/h. The plant already possesses the facilities to treat its liquid waste generated from the use of water throughout its processing stages. These facilities contain, initially, an anaerobic digestion process followed by an aerobic treatment [37]. It was assumed that one ton of starch produced as a by-product in a potato processing company requires 13.2 tons of potatoes and generates approximately 49.3 tons of liquid waste. The generation of liquid waste as well as the mass balances of a potato processing plant have been described previously in [37]. Detailed cost calculations for capital expenditure (CAPEX), operational expenditure (OPEX) and profitability indicators are demonstrated below as well as in SI.

2.6.1. Calculation of CAPEX

The cost of equipment was calculated by using the empirical Equation (3) for closed vessels mounted with agitation and mantle [38].

$$C_{p}^{o}(\$@2007) = \exp(10.211 + 0.51\ln(V))$$
(3)

where C_p^o is the free-on-broad (fob) purchase cost of the hydrolysis reactor, which is a closed vessel with agitation and mantle, and *V* is the total volume of the reactor calculated using Equation (4) with a safety factor of 1.20.

$$V = Q\left(\frac{m^3}{h}\right) HRT(h) 1.20$$
(4)

where Q is the volumetric flowrate and HRT is the hydraulic retention time. Both of them are derived from the selected experimental runs.

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The updating to today's prices was conducted by using the Chemical Engineering Plant Cost Index (CEPCI) as indicated in Equation (5):

$$C_{p}(\$@2020) = \frac{CEPCI_{2020}}{CEPCI_{2007}} C_{p}^{o} \$@2007$$
(5)

where 2007 was the year C_p^o was calculated, while CEPCI₂₀₀₇ and CEPCI₂₀₂₀ are equal to 525.4 and 596.2, respectively.

Finally, the Fixed Capital Investment (FCI) was calculated by using the Langmuir factor equal to 5 [39].

2.6.2. Calculation of OPEX

The Total Production Cost (TPC) was calculated by using Equation (6) [39].

TPC (€/y) = 1.23 (
$$C_{RM} + C_{UT} + C_{WT}$$
) + 2.73 C_{OL} + 0.18FCI (6)

where C_{RM} is the cost of Raw Materials

 C_{UT} is the cost of utilities C_{WT} is the cost of waste treatment C_{OL} is the cost of labor

2.6.3. Profitability Indicators

The investment for introducing a hydrolysis reactor into an existing wastewater facility of a potato industry was evaluated by calculating two profitability indicators net present value (NPV) and payback period (PBP) by using Equations (7) and (8), respectively [40].

NPV = -FCI + Pr
$$\frac{((1+i)^{n}-1)}{(i(1+i)^{n})}$$
 (7)

$$PBP = \frac{Pr}{FCI}$$
(8)

where: Pr is the annual net profit (€/y), i is the interest rate equal to 10% and *n* is the period of the investment set at 15 years.

As revenues, the reduction in the plant's utilities costs due to the decrease in natural gas consumption was considered. The latter is being substituted by the biogas produced from the starch hydrolysates fed into the anaerobic digester. The production of biomethane from 1 ton of starch was taken from the experimental results. Moreover, we have considered that 1 m^3 of biomethane provides 10 kW of energy heat [41]. The profitability of the new investment is then compared with an existing utilization pathway, which uses the starch as animal feed at a price of 90 \notin /ton [42].

3. Results and Discussion

3.1. Hydrolysis Yield Results

The TOC of the industrial starch was equal to 0.441 g/g starch, and hence, the total TOC in the initial starch suspension prior to hydrolysis was 13.67 g/L. The results of the 2^3 FFD and model predictions are shown in Table 2 where X_1 , X_2 , and X_3 correspond to the input parameters FeSO₄·7H₂O, H₂O₂, and temperature, respectively. The starch hydrolysis yields range between 22.4 and 99.5%. The highest starch hydrolysis yield is 99.5% in the 8th run, where all the parameters are at the upper level. High efficiency also occurred in the 4th run (89.5%) were all parameters are at +1 level apart from FeSO₄·7H₂O concentration, which is at the -1 level. The lowest efficiency takes place in the 1st run (27.2%), where all parameters are at the low level. It is observed that the pH values after the hydrolysis process remained low in all the experiments. The optimum pH should not be higher than four, in order to avoid the production of iron as $Fe(OH)_3$ or decomposition of H_2O_2 into O_2 and H_2O [43]. Moreover, a pH value lower than two could decrease Fe²⁺ regeneration [44]. The main effect plots of low and high levels of $FeSO_4$ ·7 H_2O_7 H₂O₂ and temperature are shown in Figure 1, while Figure 2 illustrates the interaction plots for the starch hydrolysis yield %. In Figure 1, the slope of each plot indicates the importance of each factor on the response variable. It is observed that temperature and hydrogen peroxide have a positive effect on starch hydrolysis, while the effect of ferrous sulfate is less important. Crossed

lines in the interaction plots indicate the significance of the interaction among the factors. It can be seen that the most significant interactions are those between ferrous sulfate and hydrogen peroxide.

Table 2. Experimental and predicted results of starch hydrolysis yield % and total carbohydrates of the 2^3 full factorial design.

Run	X ₁	X ₂	X ₃	pH after Oxidation	Experimental Hydrolysis Yield %	Predicted Hydrolysis Yield %	Experimental Total Carbohydrates (g/L)	Predicted Total Carbohydrates (g/L)
1	$^{-1}$	-1	-1	2.82 ± 0.02	27.19 ± 0.61	28.55	4.30 ± 0.12	5.34
2	$^{-1}$	$^{-1}$	1	3.02 ± 0.02	71.32 ± 0.73	72.68	12.23 ± 0.41	13.27
3	-1	1	$^{-1}$	2.90 ± 0.05	58.86 ± 0.38	60.22	8.36 ± 0.32	9.40
4	$^{-1}$	1	1	2.66 ± 0.03	89.46 ± 0.87	90.82	24.96 ± 0.31	26.00
5	1	$^{-1}$	$^{-1}$	2.83 ± 0.01	30.43 ± 0.25	31.79	9.46 ± 0.27	10.50
6	1	$^{-1}$	1	2.68 ± 0.02	80.90 ± 0.53	82.26	20.87 ± 0.48	21.91
7	1	1	$^{-1}$	2.99 ± 0.06	45.81 ± 0.26	47.17	14.12 ± 0.11	15.16
8	1	1	1	2.59 ± 0.02	99.50 ± 0.77	100.86	28.65 ± 0.47	29.69
9	0	0	0	2.75 ± 0.03	65.84 ± 0.34	64.29	17.83 ± 0.21	16.41
10	0	0	0	2.98 ± 0.02	67.92 ± 0.48	64.29	18.99 ± 0.34	16.41
11	0	0	0	2.78 ± 0.01	66.56 ± 0.31	64.29	18.72 ± 0.29	16.41
12	0	0	0	2.76 ± 0.01	67.73 ± 0.28	64.29	18.45 ± 0.46	16.41



Figure 1. Main effects of the starch hydrolysis yield %.



Figure 2. Interaction effect plots for the starch hydrolysis yield %.

According to regression analysis for the starch hydrolysis yield, Equation (2) becomes:

$Y_1 = 64.292 + 1.226 X_1 + 10.474 X_2 + 22.361 X_3 - 1.979 X_1 X_2 + 3.679 X_1 X_3 - 1.289 X_2 X_3 + 2.094 X_1 X_2 X_2 + 2.094 X_1 X_2 X_2 + 2.094 X_1 X_2 + 2.094 X_2 X_3 + 2.094 X_3 +$

where X_1 , X_2 , X_3 correspond to FeSO₄·7H₂O, H₂O₂ and temperature, respectively.

The significance of the model was indicated by the F-test and the *p*-value of the model, which is much less than 0.05. In Table 3 the parameter values are illustrated together with their t-ratio values and the ANOVA results. The importance of the parameters was assessed by the student's t-distribution. Analysis of variance shows that temperature and concentration of hydrogen peroxide are the most significant factors for starch hydrolysis, followed by the interaction of ferrous sulfate concentration and temperature. Temperature seems to play an important positive role on starch hydrolysis. Several studies suggest the heat pretreatment of starch at a temperature below the gelatinization temperature of the starch [45,46]. Pietrzyk et al. [47] who investigated the oxidation of potato starch with hydrogen peroxide found out that the presence of a metal catalyst during the oxidation process can produce a starch with high water solubility at 80 °C. Figure 3 illustrates the correlation between the experimental and predicted values of the hydrolysis yield % where R^2 is 0.991 and confirms the strong predictive capability of the model.

Table 3. ANOVA results and parameter values of the 2³ FFD for the starch hydrolysis yield.

Source	DF	Sum of Squares	Mean Square	F Ratio	p > F
Model	7	5077.776	725.397	61.369	< 0.0007
Error	4	47.281	11.820		
Lack Of Fit	1	44.362	14.415	45.612	0.0066
Pure Error	3	2.918	2.624		
Total Error	4	47.281	0.973		
Parameter Estimates	Estimate	Std Error	t Ratio	Prob> t	
β0	64.292	0.992	64.78	< 0.0001	
β1	1.226	1.216	1.01	0.3701	
β2	10.474	1.216	8.62	0.0010	
β3	22.361	1.216	18.40	< 0.0001	
β1β2	-1.979	1.216	-1.63	0.1789	
β1β3	3.679	1.216	3.03	0.0389	
β2β3	-1.289	1.216	-1.06	0.3488	
β1β2β3	2.094	1.216	1.72	0.1601	



Figure 3. Experimental versus predicted values for starch hydrolysis yield %.

The response surface plots between the output variable (% hydrolysis yield) and the three optimization parameters are shown in Figure 4. Figure 4A illustrates the interactions of X_1 and X_2 , Figure 4B illustrates the interactions of X_1 and X_3 and Figure 4C illustrates the interactions of X_2 and X_3 . From Figure 4, it becomes obvious that high levels of optimization parameters lead to high hydrolysis yields. In all the response surface plots, the third parameter was kept constant at level 1. The developed model can be used as a groundwork to build more advanced models based on machine learning. These models are able to be steadily fed with new input/output data and continuously evolved to accurately predict the targeted responses. This is particularly significant as there is a growing interest in using artificial intelligence technologies within circular bioeconomy and biorefinery systems [48].



Figure 4. Response surface plots of the starch hydrolysis yield % with respect to (**A**) X_1 and X_2 (**B**) X_1 and X_3 and (**C**) X_2 and X_3 .

3.2. Production of Total Carbohydrates during the Hydrolysis Process

After the hydrolysis process, various types of carbohydrates are contained in the starch solution, which can be directly used in many microbial processes such as anaerobic digestion. The analysis of total carbohydrates is sufficient to control starch hydrolysis, since after hydrolysis, in addition to sugar monomers, dextrins or oligomers are also considered as oxidation products. Therefore, apart from the hydrolysis yield, we have also measured as an output variable the total carbohydrates in g/L and results (both experimental and model predictions) are shown in Table 2.

Total carbohydrates in all runs vary from 4.00 to 28.65 g/L. The highest amount of carbohydrates was measured at the end of the 8th run, where $FeSO_4$ ·7H₂O and H₂O₂ concentrations are 1 g/L and 0.509 g/L, respectively, while temperature is equal to 70 °C. On the contrary, low amounts of carbohydrates (4.3 g/L) are observed in the 1st run where all the parameters are at their low -1 level. The main effect plots for total carbohydrates in starch solution are shown in Figure 5. It is observed that all the parameters positively affect the response. The interaction plots for total carbohydrates are presented in Figure 6 where the most vital interaction is between hydrogen peroxide and temperature which, however, remains in low interaction.



Figure 5. Main effects for total carbohydrates.



Figure 6. Interaction effect plots for total carbohydrates.

All parameters after statistical analysis are illustrated in Table 4. According to the F-test, the F (7,4) was higher than the tabulated value of 0.05 level of significance, so the model is significant. Figure 7 shows the experimental and predicted values of total

carbohydrates where R² is 0.951, while the prediction expression after regression analysis for total carbohydrates becomes:

 $Y_2 = 16.412 + 2.906X_1 + 3.654X_2 + 6.309X_3 - 0.544X_1X_2 + 0.176X_1X_3 + 1.474X_2X_3 - 0.694X_1X_2X_3 + 0.176X_1X_3 + 0.176$

Source	DF	Sum of Squares	Mean Square	F Ratio	<i>p</i> > F
Model	7	516.611	73.802	10.997	0.018
Error	4	26.844	6.711		
Lack of Fit	1	26.104	26.104	105.846	0.002
Pure Error	3	0.740	0.247		
Total Error	4	26.844			
Parameter Estimates	Estimate	Std Error	t Ratio	Prob> t	
β0	16.4120	0.748	21.950	< 0.0001	
β1	2.9063	0.916	3.170	0.0338	
β2	3.6538	0.916	3.990	0.0163	
β3	6.3088	0.916	6.890	0.0023	
β1β2	-0.5440	0.916	-0.590	0.5847	
β1β3	0.1760	0.916	0.190	0.8568	
β2β3	1.4738	0.916	1.610	0.1829	
β1β2β3	-0.6940	0.916	-0.760	0.4910	

Table 4. Analysis of variance for total carbohydrates.



Figure 7. Predicted and experimental values for total carbohydrates.

3.3. Kinetic Study on Starch Hydrolysis with Fenton Oxidation

It is very important to investigate how each of the three factors affect the kinetics of starch hydrolysis. Gaining knowledge about the point at which the reaction is complete, or high hydrolysis yield is achieved, is crucial for avoiding wasting time, resources and utilities in industry. After the FFD, a kinetic study was conducted to calculate the kinetic constants of starch hydrolysis. Figure 8 illustrates the increase of the TOC (g/L) in starch solution during the hydrolysis process for each of the eight runs of the factorial experiment as well as for the average TOC of the four center points. It becomes obvious that after 120 min, the TOC production rate decreases significantly in all runs. The maximum hydrolysis yield % is observed at the 8th run where FeSO₄·7H₂O, H₂O₂ and temperature were 1.00 g/L, 0.509 g/L and 70 °C, respectively. However, during the 4th run, where hydrolysis yield % was 89.46% and oxidation conditions were 0.60 g-FeSO₄·7H₂O/L, 0.509 g-H₂O₂/L and

70 °C, the oxidation process was practically over at 60 min. The kinetic study confirms that temperature is the most important factor for starch hydrolysis, since in the experimental trials where temperature was at the high level of 70 °C, the TOC production rate was elevated. Specifically, during the first 30 min, more than 42% of the initial starch was hydrolyzed when the temperature was at its high level (runs 2, 4, 6 and 8), while the hydrolysis yield was lower than 20% in the runs 1, 5 and 7 at the same hydrolysis time. In the 3rd run, even if the temperature was at its low level, hydrolysis yield was 1.86 times higher than 20%. This is mainly due to the high concentration of H_2O_2 that also plays an important role in the hydrolysis process.



Figure 8. TOC increase during the hydrolysis process for the FFD runs.

Comparing the runs where the only difference was the addition of H_2O_2 , it is clear that a higher amount of hydrogen peroxide leads to faster starch hydrolysis. For instance, during the 1st run where all factors were at a low level, the hydrolysis yield reached a low value of 14.3% in the first half an hour, while at the same time the 3rd run (all factors at low level except H_2O_2) had a substantially higher yield equal to 37.1%. In addition, the hydrolysis yield of the 4th run (high level X_2 and X_3 and low level X_1) reached 68.3% in the first 30 min, while the yield of the 2nd run (low level X_1 and X_2 and high level X_3) was 41.8% at the same time period. Similar observations were also collected from runs 5 (high level X_1 and low level X_2 and X_3) and 7 (high level X_1 and X_2 and low level X_3). During the latter, 19.5% of the initial starch was hydrolyzed during the first half an hour, in contrast with the first one where the respective yield reached a minimum value of 9.2%.

The comparison between the runs shows that their only difference was the amount of ferrous sulfate (1st and 5th run, 2nd and 6th run, 3rd and 7th run, 4th and 8th run), which reveals that the high ferrous sulfate concentration led to a slower starch hydrolysis, as it is shown in Figure 8. These results are not only confirmed from the hydrolysis yield during the initial part of the process, but also from the last 60 min (120 to 180 min). During this time period, the increase in hydrolysis yield was varying from 1.6 to 3.9% in the runs where ferrous sulfate concentration was at the low level, indicating that the hydrolysis process had almost finished in the first 120 min. An exception is Run 2, where the yield of hydrolysis is 6.2% during 120–180 min. In contrast, the increase in hydrolysis yield in the runs with high ferrous sulfate concentration (5th, 6th, 7th and 8th run), was varying from 5.7 to 8.2% at the respective time (120–180 min), indicating that the hydrolysis was not over during the first 2 h of the process.

After applying several types of kinetic equations to experimental results using the least square method, the second order kinetic model (Equation (9)) provided the best fit.

$$\frac{dC_t}{dt} = k(C_e - C_t)^2 \tag{9}$$

where k is the hydrolysis rate constant (L/g min), C_e is the maximum TOC concentration (g/L) in the soluble starch solution and C_t is the TOC concentration (g/L) at time t.

The linearization of Equation (9) leads to Equation (10):

$$\frac{t}{c_{t}} = \frac{1}{kc_{e}^{2}} + \frac{1}{c_{e}}t$$
(10)

In Figure 9, the correlation of t/C_t with time is shown to have a linear behavior in all runs, with R² varying from 0.894 to 0.997, confirming the predictive capability of the second kinetic order model. In Table 5, the hydrolysis rate constants of the 2nd order equation together with C_e and R² are illustrated. It becomes obvious that the kinetic rate constant is affected by the conditions of oxidation with Fenton reagents, and more particularly, when ferrous sulfate concentration is at the low level the reaction rate is higher (Runs 1 to 4). This could be explained by the fact that the high amount of ferrous sulfate can cause premature polymerization of the starch surface due to the formation of Fe-starch complexes that prevent hydroxyl radicals entering starch molecules [25,49].



Figure 9. Relationship between t/C_t with time for the hydrolysis experimental design.

Table 5. Hydrolysis rate constants obtained from the 2nd kinetic order equation.

Runs	C _e (g/L)	k (L/g min)	R ²
1	4.246	$9.162 \ 10^{-3}$	0.974
2	10.040	$8.621 \ 10^{-3}$	0.991
3	7.831	$9.719 \ 10^{-3}$	0.991
4	12.804	$9.298 \ 10^{-3}$	0.997
5	4.721	$4.197 \ 10^{-3}$	0.894
6	12.531	$2.757 \ 10^{-3}$	0.961
7	7.179	$3.583 \ 10^{-3}$	0.944
8	14.993	$2.856 \ 10^{-3}$	0.978
Center point	9.597	$4.831 \ 10^{-3}$	0.967

3.4. Biodegradability Tests

Anaerobic treatment of starch solution was carried out to examine the effect of Fenton reagents on biodegradability of starch through methanogenic activity of anaerobic sludge. The latter is defined as methane production rate k_{max}/g -VSS or g CH₄-COD g⁻¹ VSS day⁻¹. The granular sludge used as an inoculum had total suspended solids (TSS) levels of 117.40 ± 0.98 g/L, volatile suspended solids (VSS) levels of 40.04 ± 0.24 g/L, and a moisture content of $88.26 \pm 0.10\%$. Considering that the 8th run exhibited the highest hydrolysis efficiency, it may be the sole candidate to be tested for methane production. However, it is crucial to investigate if biodegradability of starch solution is affected by the efficiency of hydrolysis. For this reason, the 1st and 9th runs, with the lowest and an intermediate hydrolysis yield%, respectively, were also examined in biodegradability tests. Moreover, the 5th run, where the hydrolysis yield % was low and the iron concentration was the only variable of high level which was tested, not only to confirm the results from the 1st, but also suggest further investigating how the excess of iron could affect the methane production. Subsequently, batch reactors were fed with 50 mg COD of the four pretreated solutions from FFD and the initial starch. While the sludge portion was the same in all reactors (100 mL), the amount of substrate in each reactor varied based on their COD concentration. Consequently, the reactors fed with the 5th, 8th, 9th, and 1st run, and native starch had different inoculum/substrate ratios based on volume: 7.4, 8.3, 27.2, 18.0, and 10.0, respectively. High inoculum to substrate ratios were applied to reduce digestion time. In a study by Scaglione et al. [50], who examined the anaerobic biodegradability of various organic substrates in short-term batch tests, an inoculum sludge ranging from 100 to 300 mL was used, while the substrate volume varied from 10 to 20 mL.

Methane production was measured at different time intervals and the maximum slope of accumulated methane produced was used for SMA calculation. Since it is assumed that the third feed with acetic acid had increased the sludge methanogenic activity, the normalized values were calculated as a quotient of the quantity of methane produced from the hydrolyzed starch feed divided by the volume of methane produced from acetic acid feed in the same reactor.

Table 6 shows the methane production rate and the SMA calculated as $g CH_4$ -COD g^{-1} VSS day⁻¹, after feeding with hydrolyzed starch solutions as well as native starch. An increase in sludge activity is observed when the optimal conditions for hydrolysis are used. The highest SMA is observed after feeding on the 8th run (all the parameters were at the high level) where a hydrolysis yield of 99.5% was achieved. It has been reported that the SMA after feeding with liquid sugar wastewater could reach 0.9 g CH_4 -COD g⁻¹ VSS day $^{-1}$, while SMA after feeding with maize starch wastewater and potato processing wastewater was equal to 0.11 and 0.13 g CH₄-COD g^{-1} VSS day⁻¹, respectively [51]. On the contrary, the methanogenic activity was low when the 1st run was used as a substrate. During hydrolysis of the latter, all the parameters except the temperature are at the low level and the hydrolysis yield reaches 27.2%. The low methanogenic activity observed after feeding with native starch and hydrolysate from the first run indicates that the substrate is not readily available for methane production. Prior to methanogenesis, anaerobic digestion steps such as hydrolysis and acidogenesis occur, causing a delay in the activity of methanogenic bacteria. The total amount of methane produced after feeding with substrates from the 9th, 8th and 5th runs approaches that of acetic acid at a rate of more than 86%, with the maximum being the 8th run with 92.9%. These substrates have a higher amount of iron compared to that of the 1st run and native starch. According to Yu et al. [52], who studied the effect of trace elements on methane production in an anaerobic reactor treating starch wastewater, found that in contrast to other trace elements, high doses of iron can stimulate the SMA. The iron added during oxidation has a positive effect on anaerobic digestion since it affects microbial activity, methanogenic performance and sludge granulation [53]. In addition, Table 6 shows that when native starch is used as a substrate, the amount of methane produced as well as the SMA are significantly low. The chemical hydrolysis of the starch has broken down the macromolecules into smaller compounds,

which are easily metabolized from anaerobic bacteria, contrary to the untreated starch which hinders the methane production. In accordance with Lu et al. [10], the methanogenic activity tests that were fed with starch yielded a low SMA, but when glucose was used as substrate the SMA greatly increased. The methane yield from hydrolysates in Runs 5, 8, 1, and 9 can be conveniently obtained from Table 6 and is equal to 0.326 ± 0.018 , 0.368 ± 0.022 , 0.268 ± 0.011 , and 0.354 ± 0.021 L-biomethane/g-COD, respectively. These values were found to exceed the reported values in the literature for starch wastewaters mentioned above [8]. In contrast, when native starch was used as feed, the methane yield was significantly lower and equal to 0.184 ± 0.078 L-biomethane/g-COD. The methane production graphs (see Supplementary Material Figures S1-S5) showed that when acetic acid was used as feed, 50% of methane production was observed in the first 20–30 min in all bioreactors, indicating the high methanogenic activity of the anaerobic sludge. This sludge was already activated and even adapted to potato wastewaters since it was retrieved from the wastewater facilities of the same company where native starch was derived. In addition, as can be seen in Table 6, the total amount of methane produced after the 3rd feed with acetic acid slightly exceeded the theoretical maximum amount (350 mL-CH₄/g COD at STP) due to the consecutive feeds. When granular anaerobic sludge is used as inoculum, it has been reported that background methane is likely to be observed. This can occur due to the breakdown of residual substrate, which remains within the granules and/or the natural decomposition of microbial cells [54]. Furthermore, after feeding the hydrolyzed starch, 50% of methane production was observed in a shorter period of time than that of native starch. Specifically, the 50% of methane production from the 1st, 5th, 8th and 9th runs was observed at 14, 26, 15 and 31 h, respectively, contrary to the untreated starch where 50% of methane was produced at 38 h.

Table 6. Methane production and SMA from different substrates.

Substrate	Maximum Specific Rate (mL CH ₄ /min)	${ m SMA}~({ m g~CH_4-COD}~{ m g}^{-1}~{ m VSS~Day}^{-1})$	mL CH ₄	mL CH ₄ (Acetic Acid as Feed)	Normalization %
Run 5th	0.375 ± 0.061	0.388 ± 0.063	16.3 ± 0.81	18.95	86.02
Run 8th	0.650 ± 0.139	0.669 ± 0.014	18.4 ± 1.21	19.80	92.93
Run 9th	0.416 ± 0.074	0.428 ± 0.076	17.7 ± 1.04	20.10	88.06
Run 1st	0.078 ± 0.016	0.080 ± 0.016	13.4 ± 0.53	18.80	71.28
Native starch	0.075 ± 0.004	0.077 ± 0.004	9.2 ± 0.40	19.05	48.29

Every experiment lasted for 4 days, since the methane production rate after this time period was negligible. Considering that methane production, in most cases, stopped within 90 h, a test period of 4 days was considered sufficient for SMA tests. The duration of the tests is influenced by factors such as the composition and COD concentration of the substrate, the operating conditions of the reactor, and the activity of anaerobic bacteria [55]. However, several studies have reported that the duration of SMA tests typically ranges from 2 to 7 days [50,56]. Ince et al. [55] investigated the SMA of anaerobic sludge taken from various treatment plants using acetate as a substrate. The authors observed that the anaerobic sludge derived from a bakery-yeast plant ceased producing methane after 28 h when it was fed with 2 g/L acetate. Furthermore, the specific methanogenic activity of the sludge obtained from a UASB reactor at an alcohol distillery was observed to decline to zero within a time period of less than 60 h when exposed to feedings of 1, 2, 3, and 4 g of acetate/L. Finally, it is worth mentioning that the maximum rate of methane production after feeding the hydrolyzed starch solution was observed at the start of the anaerobic digestion processes. The latter finding indicates the excellent biodegradability of the starch hydrolysates, the significance of the hydrolysis process and the potential of utilizing this industrial-derived by-product for energy generation through biomethane production.

3.5. Plain Cost Analysis of Starch Hydrolysis and Biomethane Production

The industrial starch generated as a by-product in a potato processing plant showed that it may become a critical source of energy in the form of biomethane after its pretreatment. At this stage, it is crucial to implement an initial cost analysis on the optimum starch hydrolysis conditions and the respective methane production from the hydrolyzed starch to estimate the economic viability of a potential investment. The profitability of the new investment is then compared with an existing utilization pathway, which is the use of starch as animal feed at a price of $90 \notin$ /ton. Detailed calculations for CAPEX, OPEX and profitability indicators are presented in the SI.

Since there is an existing installation for treating the liquid waste coming from the potato processing plant, the CAPEX of the new investment is related only to the hydrolysis reactor. The generated starch is mixed with the required amount of Fenton reagents and liquid waste to dilute the raw material and generate the conditions of Run 8. The hydraulic retention time was set at 1 h and, therefore, we require a reaction tank of 5.04 m³ that receives 125.0 kg of starch/h diluted with the plant's liquid waste to reach a volumetric flow of 4.03 m³/h. The FCI is estimated at EUR 306,202.53. The potato processing plant consumes large amounts of water for washing the processed potatoes in the various unit operations (potato washing, peeling, sorting, slicing, washing slices) as well as from washing the equipment at the end of each day. Thus, these facilities generate, daily, vast amounts of liquid waste [5].

Regarding the OPEX, the cost of Fenton reagents amounts to EUR 40,048.4/year by considering a price of FeSO₄·7H₂O and H₂O₂ equal to EUR 0.40/kg and EUR 1.65/kg, respectively [57]. The consumption of Fenton reagents was based on the hydrolysis in Run 8, which obtained a hydrolysis yield of more than 72% for an HRT of 1 h and a methane potential rate of 18.4 mL/min. For the utilities cost, we accounted for the thermal heating of the starch solution at from 25 °C to 70 °C and the required electricity for mixing. The required thermal heating was calculated at 211.0 kW per h, assuming that 1 m³ requires 1000 kcal to increase 1 °C. This thermal heating corresponds to a cost of EUR 253,185.8/year, since the cost of 1 kWh was taken as equal to EUR 0.15 [58]. The electricity cost of mixing was estimated at EUR 6021.5/y taking into account the price of 1 kWh of electricity equal to EUR 0.168 [59] and that 1 kW is required to agitate 1 m³ of liquid [60]. Based on the above calculations, the TPC was estimated at EUR 423,200.84/year.

According to the experimental results, from one ton of hydrolyzed starch (conditions of Run 8) 368 m³ of biomethane can be produced. The aforementioned yield, together with the plant's capacity, corresponds to revenues of EUR 552,000/year. Figure 10 illustrates the cumulative cash flows for the two scenarios. After the 8th year, the investment in the hydrolysis reactor becomes more attractive than the base case scenario. The NPV (n = 15 years and i = 10%) and PBP for the starch hydrolysis investment were calculated at EUR 505,785.4 and 2.4 years, respectively, while for the base case scenario the NPV is EUR 367,731.9, 37.5% lower than the starch hydrolysis scenario. Therefore, methane production from starch hydrolysates is not only feasible but also economically viable for a potato processing company. The proposed valorization route paves the way for the industry's energy independence and reducing the risk of any economic instability that may be caused by fluctuations in the price of natural gas that Europe, as well as other parts of the world, have experienced in the last two years.



Figure 10. The cumulative cash flows for the hydrolysis and the base case scenarios.

4. Conclusions

Chemical oxidation with Fenton's reagents is an efficient process for starch hydrolysis. The highest hydrolysis yield (99.5%) and total amount of carbohydrates (28.65 g/L) were observed at the high level (+1) of the FFD. A statistical model was constructed for simulating the hydrolysis process, revealing high correlation between predicted and experimental values ($R^2 > 0.99$). The most important factors were found to be the hydrolysis temperature and the amount of hydrogen peroxide, followed by the interaction of ferrous sulfate and temperature which was also statistically significant. Moreover, hydrolysis kinetic study was modeled by a second order kinetic model and the kinetic rate constants were estimated, revealing that high ferrous sulfate concentration leads to slower starch hydrolysis. Specific methanogenic activity tests showed that after chemical oxidation with Fenton reagents, the starch hydrolysates are easily biodegradable and capable of methane production. Finally, a preliminary cost analysis revealed that a small investment can become profitable before the end of the third year, leading to an NPV 37.5% higher than the NPV of the base case scenario.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/su152014860/s1, Figure S1: Cumulative methane production from 50 mg COD of 9th run; Figure S2: Cumulative methane production from 50 mg COD of 8th run; Figure S3: Cumulative methane production from 50 mg COD of 1st run; Figure S4: Cumulative methane production from 50 mg COD of 5th run; Figure S5 Cumulative methane production from 50 mg COD of native starch; Figure S6: Linearization of 1st order kinetic model equation for the 12 runs of the FFD experiment; Figure S7: Linearization of Michaelis—Menten model equation for the runs of the FFD experiment; Equations (S1)–(S8): Cost Calculations; Equations (S9)–(S12): Kinetic equations on starch hydrolysis; Table S1: Hydrolysis rate constants obtained from the 1st kinetic order equation; Table S2: Hydrolysis rate constants obtained from the Michaelis—Menten model equation. References [39,57–62] are cited in the Supplementary Materials.

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