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Optimisation on the Hydrolysis Process of Paper Waste Sludge to Produce Bacterial Cellulose through Fermentation

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This study investigates the sulfuric acid hydrolysis process to improve the efficiency of paper waste sludge (PWS) hydrolysis in a paper recycling plant. The Response Surface Methodology (RSM) was used to evaluate the influence of factors, including liquid-to-solid ratio, acid concentration, temperature, and time on the hydrolysis process. The optimal conditions for hydrolysis were determined to be a solid-to-liquid ratio of 1/30.15, an acid concentration of 8.17 wt%, at a temperature of 91.31 ℃, and a reaction time of 6.15 h, which yielded the highest sugar content of 11.12 $g.L^{-1}$ (with the hydrolysis efficiency of 61.4 %). The resulting sugar solution was then fermented by *Acetobacter xylinum* bacteria to produce bacterial cellulose (BC) layers. This study addresses the environmental organic pollution caused by PWS and transforms it into a more valuable form with high carbon content and purity, such as BC.

1. Introduction

The paper industry generates a by-product known as paper waste sludge (PWS), which comprises the solid waste residue resulting from pulping, bleaching, and wastewater treatment processes (Cherian and Siddiqua, 2019). PWS composed of cellulose, metals, lignin, hemicellulose, ash, and various organic and inorganic substances (Assis and Chirwa, 2021). Disposing of PWS can be challenging due to its high moisture content, low bulk density, and potential environmental impact (Turner et al., 2022). There are several ways to handle PWS, such as composting, which can turn it into compost that can be used as a soil amendment (Calvin, 2003). PWS can aslo produce biogas, a sustainable energy source that may be utilised for heating, electricity generation, or as fuel for vehicles. According to a study of Meyer and Edwards (2014), this method of waste management is both economical and effective in reducing greenhouse gas emissions. It has the disadvantage of using a lot of water and energy, which can lead to smells and air pollution. An alternate treatment method involves employing chemicals or microorganisms to dismantle and eliminate impurities, encompassing heavy metals and organic contaminants. This process transforms PWS into a novel fiber material termed bio-cellulose or bacterial cellulose (BC). Utilizing PWS for BC production offers a constructive departure from conventional practices reliant on cost-effective carbon sources. The conversion of PWS into BC introduces a prospective waste management and value augmentation avenue, given BC's diverse applications in domains like food packaging, pharmaceuticals, and textiles (Lin et al., 2013).
BC is synthesized through the metabolic activities of various bacteria, such as *Acetobacter, Achromobacter, Aerobacter, Agrobacterium, and Pseudomonas*, primarily utilizing carbohydrate sources like glucose (Chen et al., 2022). This study's premise is centered on the conversion of cellulose within PWS into a sugar solution via

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acid hydrolysis, followed by the fermentation of the resulting sugar solution to yield BC. In Vietnam, *Acetobacter xylinum* (*A. xylinum*), a species of bacteria of the *Acetobacter* genus, holds prominence for BC production, particularly when utilizing coconut water, colloquially termed "coconut jelly" (Nguyen, 2018). *A. xylinum* exhibits the capacity to efficiently generate BC from sugar solutions, characterized by cost-effectiveness, rapid growth kinetics, minimal degradation, and limited process variability throughout extended manufacturing durations (Nguyen, 2018). Benefiting from these advantageous attributes, the present study opts for the selection of the *A. xylinum* strain to effectuate the conversion of PWS into BC.

Hydrolysis is pivotal in PWS to BC conversion, disintegrating cellulose into sugars via acid prior to bacterial fermentation. PWS has been hydrolyzed using various acids, including sulfuric, nitric, and hydrochloric acids. Sulfuric acid hydrolysis, chosen for efficiency, cost, and scalability. The effectiveness of sulfuric acid hydrolysis is influenced by several variables, such as acid concentration, reaction temperature, time, and the solid-to-liquid ratio. By comprehending and adjusting these factors, the formation of undesirable byproducts may be minimised while maximising glucose output.The optimization makes the PWS a more economically viable source of BC. Response Surface Methodology (RSM) optimally supports the hydrolysis process, improving the efficiency of PWS to BC conversion.

The objective of this study was to convert PWS to BC under highly efficient hydrolysis conditions. The influence of factors on the hydrolysis process was investigated through one-factor experiment. RSM was used, which allows accurate assessment of the effects of factors and their interactions, and is a statistical method used to improve and optimise the hydrolysis of PWS with sulfuric acid. The fermentation process was also tested, with the investigation of the neutralizer before fermentation to obtain a sugar solution suitable for the growth and development of *A. xylinum* bacteria.

2. Materials and Methods

2.1 Material

PWS is collected from Khoi Nguyen recycled paper factory; the head office is located at Lot H14 - H15 - H16, D6 street, Minh Hung III Industrial Park, Minh Hung ward, Chon Thanh downtown, Binh Phuoc province.

The *A. xylinum* strain was provided by the University of Natural Sciences in Ho Chi Minh City. It was cultivated on agar and kept at 4 °C.

The chemicals such as acid Sulfuric acid, Sodium hydroxide, 3, 5-Dinitrosalicylic acid, and Potassium sodium tartrate were purchased from Sigma Aldrich.

2.2 HCl pretreatment

The pretreatment procedure employed in this study mixed PWS with HCl 1 M in a ratio 1:10 and put it in a shaker cabinet 24 h. After that, take it out and waste it with water until the pH equals 7, dried in the oven.

2.3 Investigate a single factor in the hydrolysis process

The objective of this survey was to evaluate the independent influence of different factors on the hydrolysis efficiency of sulfuric acid. The investigation will focus on keeping specific variables constant while changing other variables over a wide range. By doing so, the conditions that highly affect the hydrolysis yields can be concluded. The variables are investigated under the conditions shown in Table 1.

Table 1: Conditions of factors affecting the hydrolysis process

Experiment/Factor	RSL(w/w)	SAC (wt%)	Temperature (°C)	Time (h)
The influence of RSL	[1/10 – 1/40]	8	70	6
The influence of SAC	1/20	[2 - 14]	70	6
The influence of Temperature	1/20	8	[30 - 100]	6
The influence of Time	1/30	8	70	[3 - 12]

The hydrolysis of PWS was carried out with 0.5 g of pretreated sludge, in an Erlen flask with a magnetic stirrer, and then using sulfuric acid according to the concentrations, RSL, temperature and time shown in Table 1. Stir at 120 rpm.

2.4 Develop the model on the optimal condition for the hydrolysis process using Response Surface Methodology (RSM)

The experimental design was applied to evaluate the system efficiency using Design Expert V10 software. A factorial design was applied with four independent variables (RSL, SAC, temperature, and time) used in hydrolysis experiments. The levels of the variables (low, medium, high) were arranged as − 1, 0, and + 1 in Table 2. The experimental levels of each variable were determined according to the results of the preliminary studies and values in Section 3.2. Dependent variables (responses) were chosen as reducing sugar content and hydrolysis efficiency. To estimate a pure error sum of squares, 27 experiment sets were planned in the design center with three repetitions according to the Box-Behnken model (BBD). The target function, a function of independent variables, can be expressed with a quadratic polynomial model in Eq(1).

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} X_i X_j + \varepsilon \quad (1)$$

Where, Y is the estimated response, b_0 is the intercept parameter, b_i, b_{ii}, and b_{ij} are linear, quadratic, and interaction factor effects, X_i and X_j are independent variables, and ε is the error.
In evaluating the compatibility of the regression model, the coefficient of determination (R^2, R^2_{adj}) was used, and the statistical significance level was determined through variance analysis, where the F value and P-value were calculated. Based on a 95 % confidence level, model terms were selected or rejected depending on the probability value. To visualise the effects of independent variables, three-dimensional response surface graphs were generated using these values, illustrating both individual and interactive effects (Tan et al., 2021). After obtaining a reliable feedback model, Design Expert software will optimise hydrolysis conditions so that RSC and hydrolysis efficiency are highest. The experiment will be repeated under optimal conditions to compare the predicted value from the soft stool and the actual value.

2.5 Synthesis of BC from sugar solution after hydrolysis by strain *A. xylinum*

In preparation for fermentation, *A. xylinum* bacteria were cultured using a suitable medium known as Hestrin–Schramm (HS) medium. The hydrolyzed solution containing sugar was first extracted and purified through filtration methods. The resulting liquid, which had a low pH, was then neutralised to create suitable conditions for bacterial growth. The neutralisation process involved adjusting the pH of the solution to a range of 6-7 using appropriate neutralising agents such as NaOH and $Ba(OH)_2$. Next, the cultured *A. xylinum* bacteria were introduced into the culture medium at a volume ratio of approximately 15 %. The fermentation process was conducted in aseptic conditions at a controlled temperature of 35 - 37 °C. After 14 days of culture, BC membranes were collected and subjected to washing.
The characteristics of BC membranes, such as thickness, dry weight, and wet weight, were measured to evaluate the efficiency of the fermentation process. These characteristics provide insights into the overall performance of the fermentation process.

2.6 Determine of reducing sugar content (RSC)

Reducing Sugar Content ($g.L^{-1}$) was measured by 3, 5-Dinitrosalicylic acid (DNS) Method, using a spectrophotometer (Agilent Cary 60, USA). A calibration curve was drawn with glucose at different concentrations (0.2 – 1.5 $mg.mL^{-1}$, R^2 = 0.9981) (Tan et al., 2021).

2.7 Calculation

The hydrolysis efficiency (η) depends on the actual RSC of theoretical cellulose calculated by Eq(2):

$$\eta = \frac{[RSC]}{m_{PWS}.\%m_{cellulose}.0.9}.100\ (\%) \quad (2)$$

Where: RSC is the amount of sugar obtained by DNS measurement, m_{PWS} is the dry mass of the PWS, $\%m_{Cellulose}$ is the mass percent of cellulose in the dry PWS, and 0.9 is the conversion factor between cellulose to glucose.
The BC conversion efficiency (E) was calculated based on the amount of dry BC obtained when *A. xylinum* converted the sugar present in the solution reached by hydrolysis PWS into BC according to Eq(3).

$$\mathrm{E} = \frac{m_{BC}}{m_{PWS}.\%m_{cellulose}}.100\ (\%) \quad (3)$$

Where: m_{BC} is the amount of dry BC obtained at the end of fermentation.

3. Results and Discussion

3.1 PWS composition changes before and after pretreatment

The fiber analysis method was employed to determine the composition of components in PWS, including cellulose, hemicellulose, soluble and insoluble lignin in acids, ash, and other components. Figure 1 displays the results of component analysis before and after pre-treatment.
Cellulose was identified as a major component in un-treated PWS, accounting for a high content of 59.26 % (Figure 1a). Acid-insoluble lignin and acid-soluble lignin were present at 5.04 % and 7.18 %. The inorganic component, ash, constituted 8.43 % of the PWS. Other components, which made up 20.09 %, had not been

specifically identified. Based on the production and wastewater treatment processes, it was predicted that these components may include polymers such as Poly Aluminum, used in the flocculation process, as well as proteins and other nutrients resulting from biological processes. The presence of fillers and impurities, such as $CaCO_3$, kaolite, TiO_2, SiO_2, and $Al_2(SO_4)_3$, associated with cellulose fibers, has been identified in the report by Sandeep Jain during the analysis of PWS composition (Jain, 2015).

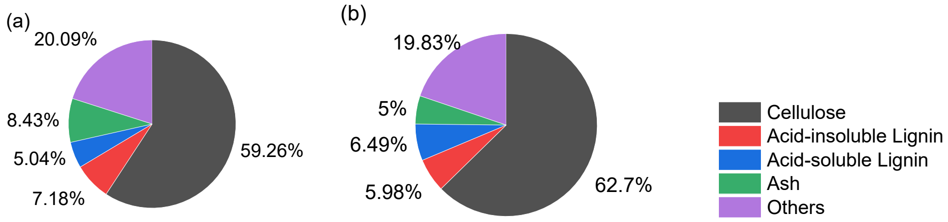

Figure 1: Composition of PWS (a) before and (b) after pretreatment

After PWS were treatment, the cellulose content in the PWS increased by 3.44 %, reaching 62.7 % (Figure 1b). The mass of the PWS material after pretreatment reduced by 10 %. This decrease can be attributed to the hydrolysing effect of HCl, it has removed impurities and an acid capable of breaks down cellulose. Hydrochloric aicds removed impurities and certain inorganic components like $CaCO_3$, kaolites, and polymers. After pretreatment, sulfuric acid can more easily access and hydrolyse cellulose. HCl also facilitates the removal of grease, dirt, and other dissolved substances present in the sludge. This remaining cellulose is more easily accessible and susceptible to hydrolysis.

3.2 The effect of a single factor on hydrolysis

The hydrolysis of PWS using sulfuric acid was investigated, focusing on four factors: solid and liquid ratio (RSL), sulfuric acid concentration (SAC), temperature, and time. The aim was to evaluate the individual effects of these factors on hydrolysis efficiency, specifically measured by reducing sugar content (RSC) and overall hydrolysis efficiency. The results of the investigation are presented in Figures 2a, 2b, 2c, and 2d.

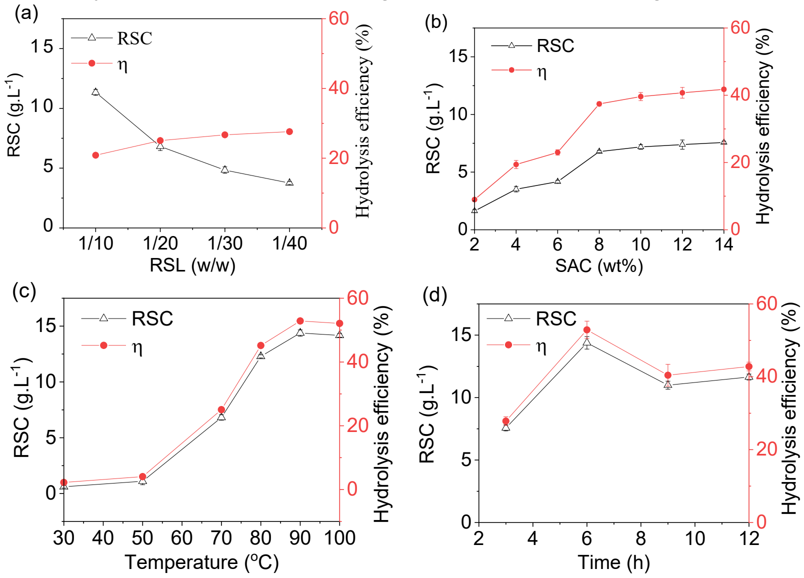

Figure 2: Effect of (a) RSL, (b) SAC, (c) Temperature, and (d) Time on sulfuric acid hydrolysis

RSL shows the quantity of solid PWS in solution; increasing the amount of solution can improve stirring and mass transfer efficiency. Increasing the liquid lowers sugar yield and does not improve equilibrium mass transfer efficiency. Figure 2a shows that the RSL used is from 1/20 to 1/40. The hydrolysis process is significantly influenced by temperature and concentration; as these two parameters increase, the hydrolysis process becomes more efficient. Higher H^+ concentration in the solution leads to more efficient cellulose hydrolysis. The temperature greater than 80 ℃ exhibits high hydrolysis efficiency, with an RSC was 12.29 $g.L^{-1}$ at 80 ℃ (Figure 2b), the hydrolysis efficiency has reached 45.12 %, nearly double temperature of 60 ℃. Increasing acid concentration is a method to rapidly increase H^+, but the hydrolysis efficiency does not significantly increase at concentrations of 10, 12 and 14 wt% (Figure 2c). Hydrolysis use high concentrations of acid will increase production costs as well as equipment corrosion, so the recommended concentration limit is less than 10 wt%. Using high temperatures and sudden increases in temperature and concentration can lead to local saccharification, as the acid reduces the amount of sugar obtained. The longer time allows the reaction to take place more completely. Figure 3d shows that RSC decreases with hydrolysis from 6 h to 12 h. So the value selected for the time element is from 3 h to 9 h. The experimental design matrix for the hydrolysis process included both coded and actual values for the variables, as outlined in Table 2.

Table 2: The coded and actual values of variables of the experimental design matrix for the hydrolysis process

Symbol	Factor	Unit	Coded variables		
			-1	0	1
A	RSL	w/w	1/20	1/30	1/40
B	SAC	wt%	6	8	10
C	Temperature	℃	80	90	100
D	Time	h	3	6	9

3.3 Optimisation of hydrolysis using RSM with Box-Behnken design

Figure 3 presents the RSM charts, exhibiting varying degrees of curvature based on the significance level of the interaction and quadratic terms. The quadratic response surface plot depicted different configurations, indicating the relationship between the factors and the response variable. Specific pairs of control variables demonstrate meaningful interactions in Figure 3. The pairs of RSL and SAC (Figure 3a and Figure 3c), and temperature and time (Figure 3b and Figure 3d), show significant interactions. This implies that simultaneously changing these factors will result in a more substantial shift in the response.

The correlation between the independent variables and the system responses is represented by second-degree quadratic equations, as denoted by Eq(4) and Eq(5). These equations describe the relationship between the factors and the response variable, allowing for further analysis and optimisation.

$$RSC = 11.07 - 1.22A + 0.5835B + 0.5519C + 0.1786D - 2.36AB + 0.7573CD - 1.54A^2 - 2.2B^2 - 2.29C^2 - 2.01D^2 \quad (R^2 = 0.9157, R^2_{adj} = 0.8630) \tag{4}$$

$$\eta = 61.02 + 6.16A + 1.77B + 3.16C + 1.12D - 11.78AB + 4.18CD - 10.75A^2 - 12.58B^2 - 12.58C^2 - 16.17D^2 \quad (R^2 = 0.9237, R^2_{adj} = 0.8760) \tag{5}$$

Wher*e: A is RSL (w/w), B is SAC* (wt%), C is temperature (℃) and D is time (h).

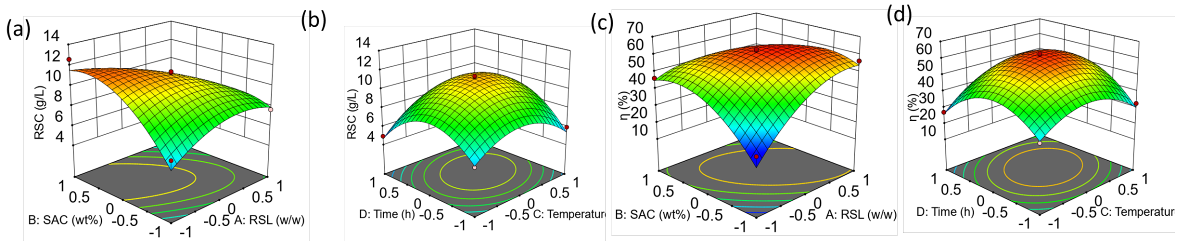

Figure 3: 3D plots surface of RSC (p-value <0.0001) as a function of (a) A: RSL and B: SAC, and (b) C: temperature and D: time and 3D plots surface of hydrolysis efficiency (p-value <0.0001) as a function of (c) A: RSL and B: SAC, and (d) C: temperature and D: time

Optimum conditions obtained with the help of the model are as follows: RSL of 1/30.15, SAC is 8.17 wt%, under 91.31 ℃ and hydrolysis duration of 6.15 h. Under these conditions, the RSC was estimated as 11.12 g.L^{-1}, with a hydrolysis efficiency was 61.4 %. Validation experiments conducted under optimum conditions, RSC was 10.98 $\pm$ 0.23 g.L^{-1}, and η was 60.64 $\pm$ 1.2 %. The low error in the experimental and predicted values indicates good agreement with the results predicted by the models and obtained from validation experiments. This optimal value was chosen for the hydrolysis of PWS for fermentation by *A. xylinum.*

3.4 Synthesis of BC from sugar solution after hydrolysis by strain *A. xylinum*

For the growth of *A. xylinum* in an acidic hydrolysing solution, the mixture was neutralised by different reagents to reach the desired pH, as shown in Figure 4. Using a mixture of NaOH and $Ba(OH)_2$ solution to neutralise the remaining H_2SO_4, it was possible to control the amount of glucose and Na_2SO_4 in the resulting sugar solution for the growth of *A. xylinum* bacteria. The BC obtained through this method was higher than other reagents (the dry mass of BC was 0.35 ± 0.02 g), corresponding to an overall conversion efficiency of PWS to BC of 52.01 ± 1.2 %.

When only $Ba(OH)_2$ was used to precipitate SO_4^{2-} ,the amount of BC obtained was significantly less than before (the dry mass of BC was 0.25 ± 0.02 g corresponding to an overall conversion efficiency of 37.15 ± 1.1 %). In the least favorable observation, neutralising the sugar solution after hydrolysis with NaOH did not produce BC because the soluble Na_2SO_4 salt in the solution was too high, suppressing the growth of *A.xylinum* bacteria.

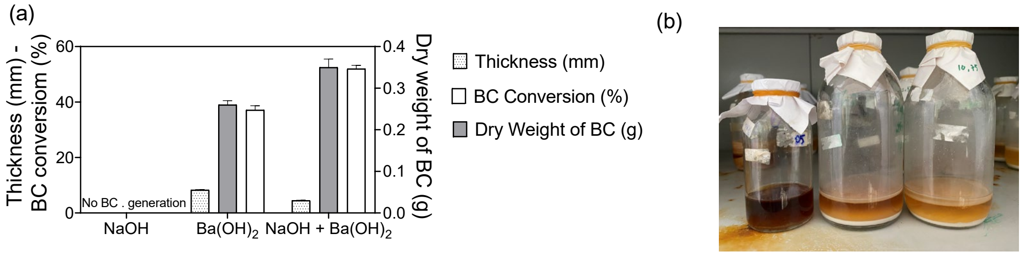

Figure 4: (a) Thickness, dry weight of BC and BC conversion efficiency and (b) image of BC after 14 days of fermentation

4. Conclusions

The results of this study confirmed the significant influence of RSL, SAC, temperature, and time on sulfuric acid's hydrolysis of PWS. The effects of these parameters on RSC and hydrolysis efficiency were explored using the RSM approach and the Box-Behnken model. The suggested hydrolysis conditions should be followed for optimum outcomes, with an estimated RSC of 11.12 $g.L^{-1}$ and a hydrolysis efficiency of 61.4 %. The optimal conditions are achieved at RSL of 1/30.15, SAC of 8.17 wt%, the temperature at 91.31 °C, and hydrolysis duration of 6.15 h.

After hydrolysis under the abovementioned conditions, the sugar solution was fermented using *A. xylinum* for 14 days to produce BC. For the fermentation process, neutralising with either $Ba(OH)_2$ or a mixture of NaOH and $Ba(OH)_2$ is necessary. Neutralisation with NaOH combined with $Ba(OH)_2$ gives better results when obtaining dry BC of 0.35 ± 0.02 g and BC conversion of 52.01 ± 1.2 %.

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References

Assis E.I., Chirwa E.M., 2021, Physicochemical Characteristics of Different Pulp and Paper Mill Waste Streams for Hydrothermal Conversion, Chemical Engineering Transactions, 86, 607-612.

Calvin C., 2003, Paper Mill Waste Mixed with Compost and Other Ingredients as Container Nursery Substrates, Compost Science & Utilization, 11(1), 16-26.

Cherian C, Siddiqua S., 2019, Pulp and Paper Mill Fly Ash: A Review. Sustainability, 11(16), 4394.

Chen C., Ding W., Zhang H., Zhang L., Huang Y., Fan, Mengmeng F., Jiazhi Y., Sun D., 2022, Bacterial cellulose-based biomaterials: From fabrication to application, Carbohydrate Polymers, 278, 118995.

Jain S., 2015, Utilization of waste paper sludge in construction industry, Indian Institute of Technology, Delhi, India.

Lin S. P., Loira Calvar I., Catchmark J. M., Liu J. R., Demirci A., Cheng K. C., 2013, Biosynthesis, production and applications of bacterial cellulose, Cellulose, 20(5), 2191-2219.

Meyer T., Edwards E. A., 2014, Anaerobic digestion of pulp and paper mill wastewater and sludge, Water research, 65, 321-349.

Nguyen X. T., 2018, Study of some Properties of Curcumin Load 3D-nano-Cellulose Networks Produced by Acerobacter xylinum, TNU Journal of Science and Technology, 184(8), 83 -88.

Paranjpe A., Saxena S., Jain P., 2023, A Review on Performance Improvement of Anaerobic Digestion Using Co-Digestion of Food Waste and Sewage Sludge, Journal of Environmental Management, 338, 117733.

Tan N.T.L., Dam Q.P., Mai T.P., Nguyen D.Q., 2021, The Combination of Acidic and Alkaline Pretreatment for a Lignocellulose Material in Simultaneous Saccharification and Fermentation (SSF) Process, Chemical Engineering Transactions, 89, 43-48.

Turner T., Wheeler R., Oliver, I. W., 2022, Evaluating land application of pulp and paper mill sludge: A review, Journal of Environmental Management, 317, 115439.