

Comparative Study for Bioethanol Production from Waste Paper Using *E. coli* and *S. cerevisiae* Specie

ABSTRACT

Biomass energy is a sustainable energy source derived from plant and animal material. Bio-ethanol, bio-methanol, and biodiesel are examples of biomass fuels. One of the most significant alternative energy sources that replaces fossil fuels is bio-ethanol. The goal of this study is to create bio-ethanol from waste workplace paper. It was then subjected to weak acid hydrolysis. For each row materials such as sugarcane, A4 paper, fruit peel and hard paper after those diluted acid hydrolysis process this analysis of study validate that (99.8,99.1 and 96.3) mg/g glucose content extracted from sugarcane, A4 paper, fruit peel and hard paper respectively with 1:85 (g/ml) of solid-liquid ratio. those extracted glucose content were also subjected to two different fermentation process by using *E. coli* and *S. centeriease* microbial strain followed by distillation for further processing the result analyzed by design expert software file version 13.0.5.0, study type response surface, design type Box-Bohnken and design model quadratics by three factors and levels with one response factors(alcohol content in %) then 17 runs were conducted for each microbial strain of experiments then the analysis attested or significant quantity of bio-ethanol was detected that 80.9% alcohol content obtained from 120 hr, 36 oc, 5 and 36 hr, 36 oc, 5 time temperature and PH value respectively by using *E. coli* and with other scenario by using *S. centriease* 97% of alcohol content obtained by 36hr, 47 oc and 6.5 time temperature and PH value respectively. Therefore obesely bioethanol is cost benefit energy source so any body should be apply in the field and home level users too.

Keywords: Wastepaper; hydrolysis; fermentation; distillation and bio-ethanol; filtration; centrifugation.

1. INTRODUCTION

The prosperity that must be transferred to an object in order to conduct work on or to the object is referred to as energy. There are two types of energy resources: sustainable and nonrenewable [1]. Non-renewable energy is defined as any energy supply that cannot be replaced during a human life span [2]. It takes thousands of years for the earth to form and live in a fixed number [3]. They must be preserved before they are depleted [4,5]. Nuclear energy and fossil fuels are examples of nonrenewable energy supplies (coal, oil, natural gas) [6-11]. Renewable energy is the umbrella word for these types of energy [12-16].

2. MATERIALS AND METHODS

2.1 Chemicals

For the experiment, the following chemicals were used: 5M sodium hydroxide (NaOH) solution, 98% sulfuric acid (H₂SO₄), distilled water, Potassium dichromate and dry quick yeast (*Saccharomyces cerevisiae*).

2.2 Sample Collection

Raw materials for this project 5kg of wolkite university staff office waste paper and wolkite gubre sub-city shopping goods packaging hard paper residue were gathered in an open plastic pot and dried form in Wolkite, Ethiopia.

2.3 Methods

2.3.1 Sample preparation

Wolkite University's waste office papers were gathered on-site. The wastepaper was dried in a tray drier (60 OC for 48 hr). The sample was cut into fragments with a scissor. In order to avoid

contact with the atmosphere, the cut material was stored in a desiccator until the next step of the experiment. Cutting the sample wastepaper into fragments increases the surface area of the sample, which improves contact between hemicelluloses and cellulose with dilute acid [17].

2.3.2 Dilute acid hydrolysis

Before fermentation, the carbohydrate polymers in lignocellulosic materials must be transformed to simple sugars via a process known as hydrolysis [18-22]. Despite the fact that there are numerous kinds of hydrolysis, dilute acid hydrolysis is a simple and productive process, with acid hydrolysis producing more alcohol than alkaline hydrolysis [23,24]. This procedure is carried out at high temperatures and pressures, with a reaction time of seconds or minutes, allowing for continuous processing. Table 1 shows the experimental conditions of the various tests performed, as well as the observed hydrolysis time and acid volume in each trial. Experiments were carried out using different

volume flasks to break down cellulose into glucose units. For that, 10 g of paper was soaked in different amounts of H_2SO_4 (5% weight): 200 and 300 mL [98% sulfuric acid (by volume to water) was diluted to 5% concentration]. The mixture was placed in an autoclave at 121 °C. The influence of time on the process was determined in the range of 60-180 min.

2.3.3 Fermentation

250 ml of hydrolyzed glucose unit material was supplemented with the yeast *Saccharomyces cerevisiae* and bacterial strain *E.coli*. All of the samples were fermented in an autoclave at 30 degrees Celsius for 72 hours.

2.3.4 Distillation

All distillation tests were conducted at 85°C and a distillation time of 6 hours using a rotary evaporator to separate the bioethanol from the liquid mixture.

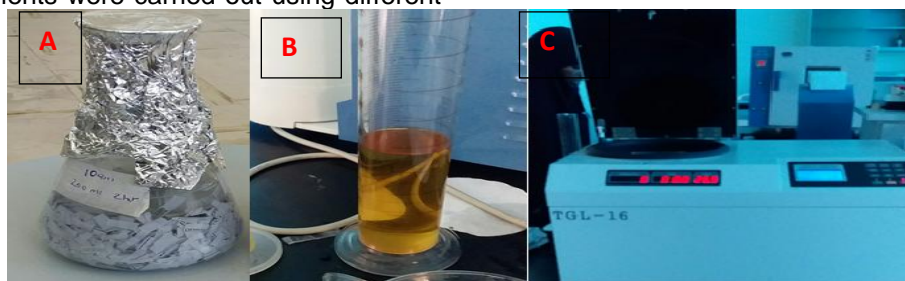


Fig. 1. Acid hydrolysis (A), sample prepped for Dilute Acid Hydrolysis (B), after filtration, and centrifugation(C)

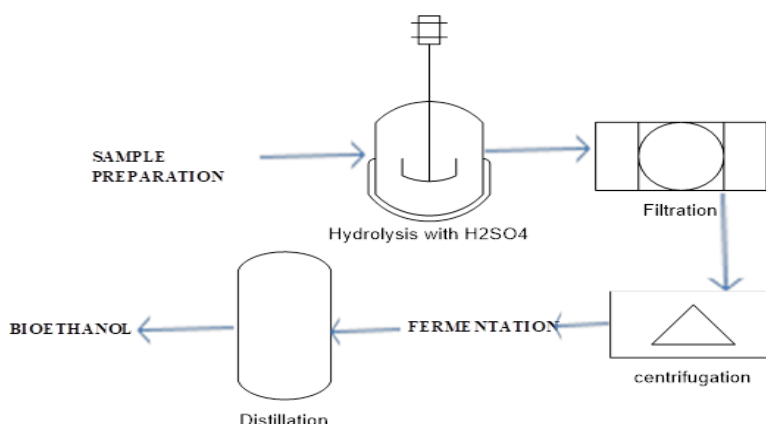


Fig. 2. Process flow diagram for bioethanol production from waste paper

2.3.5 Identification of Bioethanol

A pinch of potassium dichromate and a few drops of H_2SO_4 were applied to a 5 ml fermented sample. The presence of bio-ethanol

was suggested by a color from orange to green order change and alcohol content measurement, volatility, molecular component and IR-spectrophotometry test (functional and finger print analysis).

2.4 Material and Energy Balance

When dealing with chemical engineering, it is necessary to conduct material and energy balances on specific areas. Material balances are critical to process control, especially in the control of product yields [25-29]. The initial material balances of a new process are determined during the exploratory stages, improved during pilot plant trials when the process is planned and tested, checked out when the plant is commissioned, and then refined and kept as a control instrument as production continues. When there are changes in the procedure, the material balances must be recalculated. The rising expense of energy has prompted industries to look into ways to reduce energy consumption in processing. Energy ratios are employed in the investigation of certain experiment.

2.4.1 Material balance

2.4.2 Conservation of mass

Material balances are based on a conservation law which is stated generally in the form:

$$\begin{aligned} \text{Input} + \text{generation} - \text{output} - \\ \text{consumption} = \\ \text{Accumulation} \dots \dots \dots \text{eq} \end{aligned} \quad (1)$$

Where:

Input = enters through system boundaries
Generation = is producing within the system
Output = Exist through system boundaries
Consumption = is a soul within the system
Accumulation is built up within the system

The term that is added in a chemical process is known as generation and the term that is subtracted in a chemical process is known as consumption.

If there is some sort of chemical reaction entire the general equation for material balances,

$$\text{Input} + \text{generation} - \text{consumption} - \text{output} = \text{accumulation}$$

If there is no chemical reaction the process is at steady state (when there is no change) which means there is no generation and consumption. This implies accumulation is zero.

Accumulations are time rates of change of the amount of the entities within the boundary. For example, in the absence of sources and sinks, an accumulation occurs when the input and output rates are different.

$$\text{Input} - \text{output} = 0, \text{ i.e. input} = \text{output} [\text{at steady state} - \text{accumulation is zero}]$$

Except in nuclear processes, mass is neither generated nor consumed; but if a chemical reaction takes place a particular chemical species may be formed or consumed in the process.

2.5 Energy Balance

As with mass, energy can be considered to be separately conserved in all but nuclear processes.

The conservation of energy, however, differs from that of mass in that energy can be generated (or consumed) in a chemical process.

2.5.1 Conservation of energy

As for material a general equation can be written for the conservation of energy:

$$\text{Accumulation} = \text{Energy in} + \text{generation} - \text{consumption} - \text{Energy out}$$

An energy balance can be written for any process step. Chemical reaction will evolve energy (exothermic) or consume energy (endothermic). For steady-state processes the accumulation of both mass and energy will be zero.

So for unit mass of material:

$$\begin{aligned} U_1 + P_1 V_1 + 0.5 U_{12} + Z_1 g + Q = U_2 + \\ P_2 V_2 + 0.5 U_{22} + Z_2 g + \\ W \dots \dots \dots \text{eq} (2) \end{aligned}$$

It is convenient and useful, to take the terms U and PV together; defining the term enthalpy, usually symbol H, as:

$$H = U + PV \dots \text{eq} (3)$$

In chemical processes, the kinetic and potential energy terms are usually small compared with

the heat and work terms, and can normally be neglected. So if the kinetic and potential energy terms are neglected equation 2 simplifies to;

$$H_2 - H_1 = Q - W \dots \dots \dots \text{eq(4)}$$

For many processes the work term will be zero, or negligibly small, and equation 2 reduces to the simple heat balance equation:

$$H_2 - H_1 = Q \dots \dots \dots \text{eq (5)}$$

Where heat is generated in the system; for example, in a chemical reactor:

$$Q = Q_p + Q_s \dots \dots \dots \text{eq(6)}$$

Where:

Q_s = heat generated in the system
 Q_p = process heat added to the system to maintain required system temperature.

If heat is evolved (exothermic processes) Q_s is taken as positive, and if heat is absorbed (endothermic processes) it is taken as negative

2.5.2 Computer aid analysis

Table 1. Design model estimation

File Version	13.0.5.0	Subtype	Randomized
Study Type	Response Surface	Runs	17.00
Design Type	Box-Behnken	Blocks	No Blocks
Design Model	Quadratic		

3. RESULTS AND DISCUSSION

The goal of this study is to create bio-ethanol from waste office paper through acid hydrolysis

and later fermentation. The Table 2 shows the value of the hydrolysis (glucose unit) by used in this extraction.

Table 2. Values of hydrolysis parameters and the corresponding result of the research

Solid to Liquid ratio(gm/ml)	Extraction Content (mg/g)(glucose unit)			
	Sugarcane	paper A4	Fruit peels	Hard paper
1:20	97.1	24.1	54.21	11.21
1:25	98	26.8	59.91	15.1
1:30	98.2	47.1	73.2	18.2
1:35	98.4	78.2	72.1	21.5
1:40	98.7	78.6	89.12	41.2
1:45	98.8	79.7	91.2	52.1
1:50	98.8	79.91	93.2	67.2
1:55	98.9	83.4	93.31	71.8
1:60	99.1	90.1	94.7	81.8
1:65	99.4	95.1	94.8	88.1
1:70	99.5	97.5	95.9	89
1:75	99.5	98.1	95.93	92.1
1:80	99.7	99.1	96.5	93.9
1:85	99.8	99.8	98.2	96.3
1:90	99.81	97.1	99.1	94
1:95	99.8	98.3	99.8	92.6
1:100	99.95	95	99.91	86.2

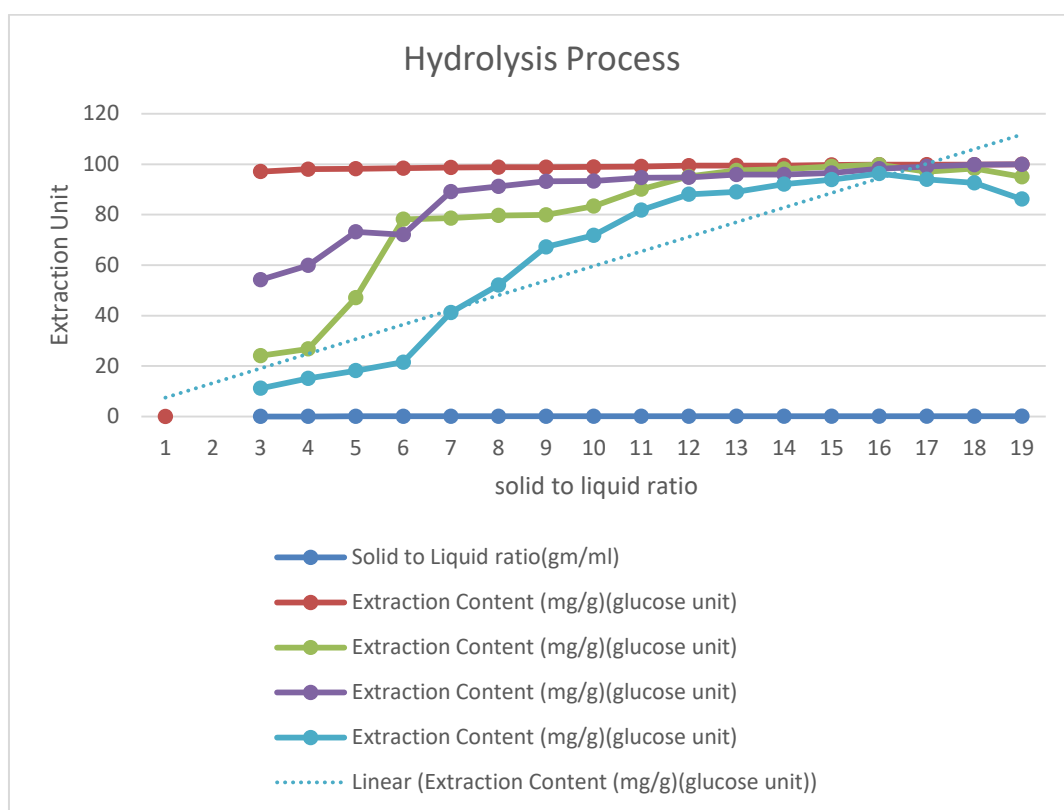


Fig. 3. Influence of hydrolysis on glucose unit

Table 3. Influence of temperature and pH on alcohol content

Time	Temperature	PH	Alcohol content b/c E.Coli	Alcohol content b/c E.Coli
78	36	6.5	45	49
78	25	8	56.1	57.1
36	36	8	64.3	63.3
36	25	6.5	65.3	65.6
78	36	6.5	65.67	67.89
78	25	5	69.3	69.78
78	36	6.5	69.89	69.9
120	25	6.5	73.5	73.5
78	47	8	76.4	76.2
78	47	5	76.98	76.11
78	36	6.5	78.7	77.7
120	47	6.5	79.4	80.4
78	36	6.5	79.8	80.8
120	36	8	80	93.4
120	36	5	80.9	95.6
36	36	5	80.9	96.3
36	47	6.5	80.23	97

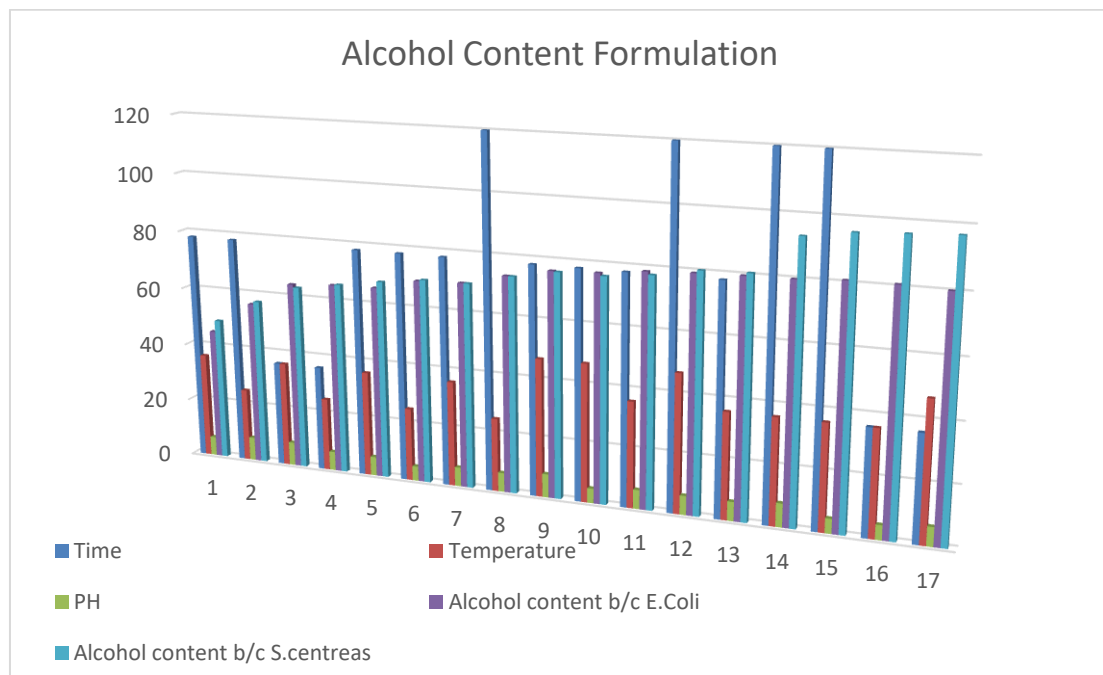


Fig. 4. Bar graph showing influence of different parameters to alcohol

3.1 Material and Energy Balance

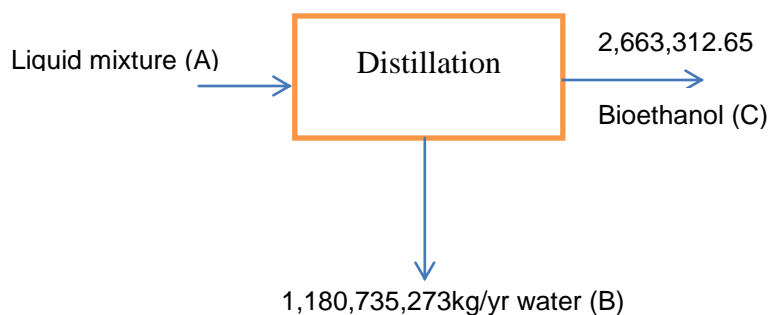
3.1.1 Material balance

The total material balance is written as: Material in = Material out Or $M_i = M_o$ (i = input; o = output). The mass balance calculation starts with wastepaper inputs. The wastepaper to ethanol

conversion process can be divided into five basic steps [Sample Preparation (drying), acid hydrolysis, Filtration, centrifugation and Distillation].

Assume Production capacity of 3,000,000 gal/yr of ethanol with plant operation of 300 days/yr.

I. Distillation



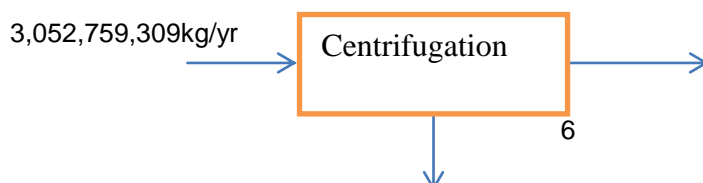
➤ If there is no chemical reaction the steady-state balance reduces to

Material in= Material out

$$A = B + C = 1,180,735,273 \text{ kg/yr} + 2,663,312.65 \text{ kg/yr} = 1,183,398,586 \text{ kg/yr}$$

$$A = 1,183,398,586 \text{ kg/yr}$$

II. Centrifugation



Liquid product (D) 1,183,398,586kg/yr
liquid mixture (A)

Residue (E)

Overall material balance

- no chemical reaction so, the steady-state balance reduces to: material in= Material out

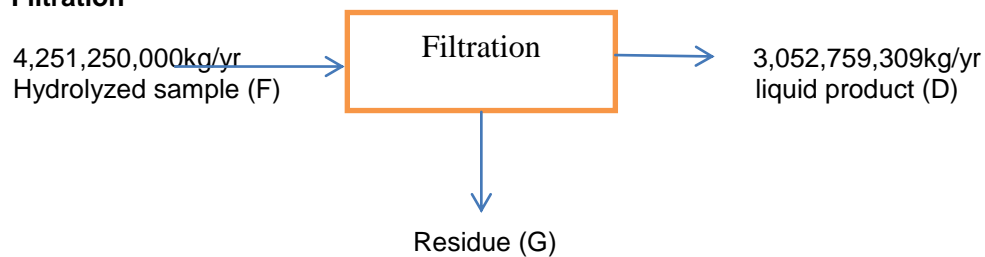
$$D = E + A$$

$$E = A - D$$

$$E = 3,052,759,309\text{kg/yr} - 1,183,398,586\text{kg/yr} = 1,869,360,723\text{kg/yr}$$

$$E = 1,869,360,723\text{kg/yr}$$

III. Filtration



Overall material balance

- no chemical reaction so, the steady-state balance reduces to: material in= Material out

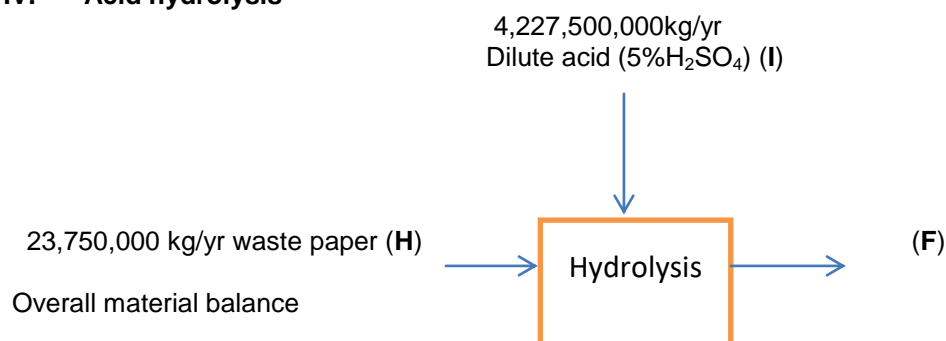
$$F = D + G$$

$$G = F - D$$

$$G = 4,251,250,000\text{kg/yr} - 3,052,759,309\text{kg/yr} = 1,198,490,691\text{kg/yr}$$

$$G = 1,198,490,691\text{kg/yr}$$

IV. Acid hydrolysis



Overall material balance

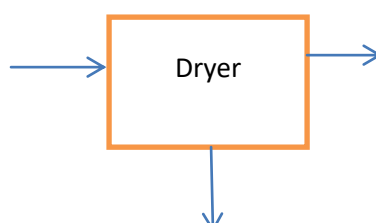
- no chemical reaction so, the steady-state balance reduces to: material in= Material out

$$H + I = F$$

$$F = 23,750,000\text{ kg/yr} + 4,227,500,000\text{kg/yr} = 4,251,250,000\text{kg/yr}$$

$$F = 4,251,250,000\text{kg/yr}$$

V. Dryer



28,500,000kg/yr waste paper (**J**)

23,750,000 kg/yr waste paper (**H**)

Moisture (**K**)

Overall material balance

- no chemical reaction so, the steady-state balance reduces to: material in= Material out

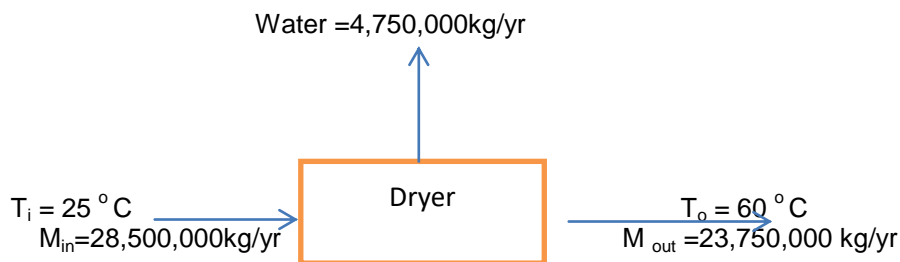
$$J = K + H$$

$$K = J - H = 28,500,000 \text{ kg/yr} - 23,750,000 \text{ kg/yr} = 4,750,000 \text{ kg/yr}$$

$$K = 4,750,000 \text{ kg/yr}$$

3.1.2 Conservation of energy

I. Dryer



$$M = 23,750,000 \text{ kg/yr}$$

$$\Delta T = 35 \text{ °C} = 308 \text{ K}$$

$$C_p = 1.4 \text{ J/g} \cdot \text{K}$$

$$Q = M \cdot C_p \cdot \Delta T$$

$$Q = 23,750,000 \text{ kg/yr} \cdot 308 \text{ K} \cdot 1.4 \text{ J/g} \cdot \text{K}$$

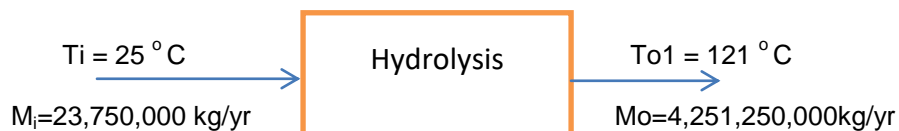
$$Q = 1.0241 \cdot 10^{10} \text{ KJ/yr}$$

$$P = Q \cdot T = 1.0241 \cdot 10^{10} \text{ KJ/yr} \cdot (1 \text{ yr}/300 \text{ day}) \cdot (1 \text{ day}/16 \text{ hr}) \cdot (1 \text{ hr}/3600 \text{ s}) = P = 592.65 \text{ KW}$$

$$E = P \cdot T = 592.65 \text{ KW} \cdot 16 \text{ hr} = 9,482.4 \text{ KWh}$$

The quantity of energy required for the dryer per year is: 9,482.4 KWh.

II. Acid hydrolysis



$$M = 4,251,250,000 \text{ kg/yr}$$

$$\Delta T = 96 \text{ °C} = 369 \text{ K}$$

$$C_p = 2.79 \text{ J/g} \cdot \text{K}$$

$$Q = M \cdot C_p \cdot \Delta T$$

$$Q = 4,251,250,000 \text{ kg/yr} \cdot 2.79 \text{ kJ/kg} \cdot \text{K} \cdot 369 \text{ K}$$

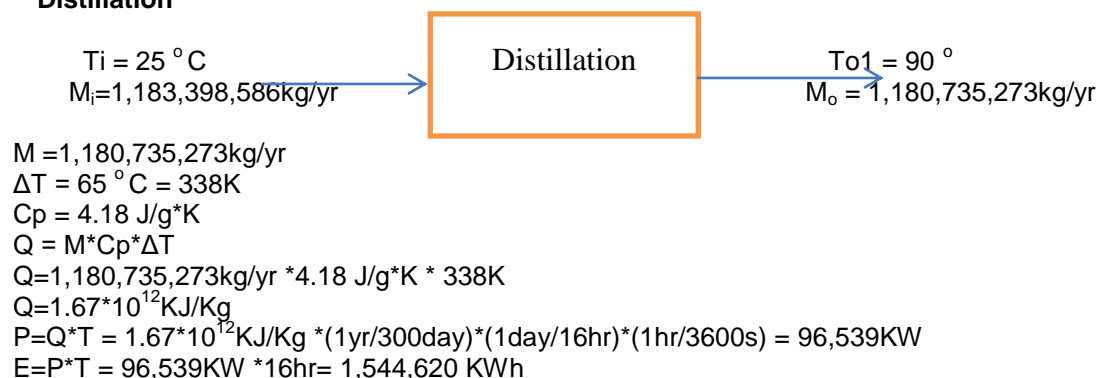
$$Q = 4.37 \cdot 10^{12} \text{ KJ/Kg}$$

$$P = Q \cdot T = 4.37 \cdot 10^{12} \text{ KJ/Kg} \cdot (1 \text{ yr}/300 \text{ day}) \cdot (1 \text{ day}/16 \text{ hr}) \cdot (1 \text{ hr}/3600 \text{ s}) = 253,281 \text{ KW}$$

$$E = P \cdot T = 253,281 \text{ KW} \cdot 16 \text{ hr} = 4,052,504 \text{ KWh}$$

The quantity of energy required for the hydrolysis per year is: 4,052,504 KWh

III. Distillation



The quantity of energy required for the distillation per year is: 1,544,620 KWh

4. CONCLUSION

Waste paper is a suitable raw material for bio-ethanol production, making a significant annual profit with a payback time of less than three years and paper have to different type of production based customer required such as A4 paper ,cards(hard paper). Within this category perspective A4 paper were more glucose content for bioethanol production related to sugarcane substrate as characterization control This technology can be used as an alternative solution for sustainable waste management and material/energy recovery.

AVAILABILITY OF DATA AND MATERIALS

We declare that the data and materials used in this manuscript can be made available as per the editorial policy of the journal.

DISCLAIMER

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The preprint document is available in this link: https://assets.researchsquare.com/files/rs-1009779/v1_covered.pdf?c=1637250143 [As per journal policy, preprint /repository article can be published as a journal article, provided it is not published in any other journal]

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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