

Short Research Article

Fungi and Bacteria Strain Fermentation Comparison for Bioethanol Production from Waste Paper Using E.Coli and S.Cerevisiae Species.

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. Five laboratory tests were carried out in order to generate bio-ethanol from wastepaper. The wastepaper was dried in the oven before being cut into bits. It was then subjected to weak acid hydrolysis, fermentation, and distillation processes. A significant quantity of ethanol was detected at 20 ml/g (liquid to solid ratio) and after 2 hours. A cost-benefit study of ethanol production from wastepaper was carried out. The research revealed a paper to ethanol ratio.

Keywords: Wastepaper, Hydrolysis, Fermentation, Distillation and Bio-ethanol, Biological Activation.

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. (Solomon, B. D., et al., 2007). Non-renewable energy is defined as any energy supply that cannot be replaced during a human life span. (Stokes H. 2005). It takes thousands of years for the earth to form and live in a fixed amount. (Galbe, M. and Zacchi, G. 2007). They must be preserved before they are depleted. (L.C. Meher, D.V. Sagar, S.N. Naik. 2006 Balat H. 2010). Nuclear energy and fossil fuels are examples of nonrenewable energy supplies. (coal, oil, natural gas). Renewable energy is the umbrella word for these types of energy.

MATERIALS AND METHODS

The following instruments were employed: Autoclave, Centrifuge, Digital Balance, Digital PH Meter, Flasks of various sizes, and Graduated containers of various sizes, Scissors, incubator, and rotary evaporator.

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*).

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.

3.2. Methods

3.2.1. Sample Preparation

Wolkite University's waste office papers were gathered on-site. The wastepaper was dried in a tray drier. (60 °C for 48hr). 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 (M. Molina-Sabio, F. Rodriguez-Reinoso. 2004).

3.2.2. Dilute Acid Hydrolysis

Before fermentation, the carbohydrate polymers in lignocellulosic materials must be transformed to simple sugars via a process known as hydrolysis. (Madson, P. and Lococo, D. 2000). 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 (Miles, W. E. et al., eds. 1988). McMillan, J. 1994; Pulp and Paper 1988). 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.

Table 1. Experimental hydrolysis parameters

Liquid to solid ratio(gm/ml)	Extraction Content (mg/g)			
	Sugarcane	A4 paper	Fruit peel	Hard paper
1:20				
1:25				
1:30				
1:35				
1:40				
1:45				
1:50				
1:55				
1:60				
1:65				
1:70				
1:75				
1:80				
1:85				
1:90				
1:95				
1:100				

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 H₂SO₄ (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.

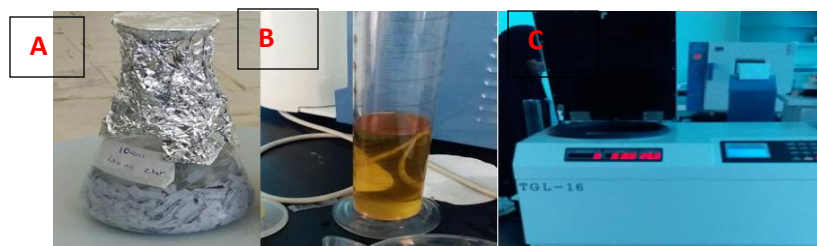


Figure 1: Acid hydrolysis (A), sample prepped for Dilute Acid Hydrolysis (B), after filtration, and centrifugation (C).

3.2.4. Fermentation

250ml 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.

Table 2. Experimental fermentation parameters

Time	Temperature	PH	Alcohol content because of E.Coli	Alcohol content because of E.Coli
78	36	6.5		
78	25	8		
36	36	8		
36	25	6.5		
78	36	6.5		
78	25	5		
78	36	6.5		
120	25	6.5		
78	47	8		
78	47	5		
78	36	6.5		
120	47	6.5		
78	36	6.5		
120	36	8		
120	36	5		
36	36	5		
36	47	6.5		

3.2.5. Distillation

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

3.2.6. 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)

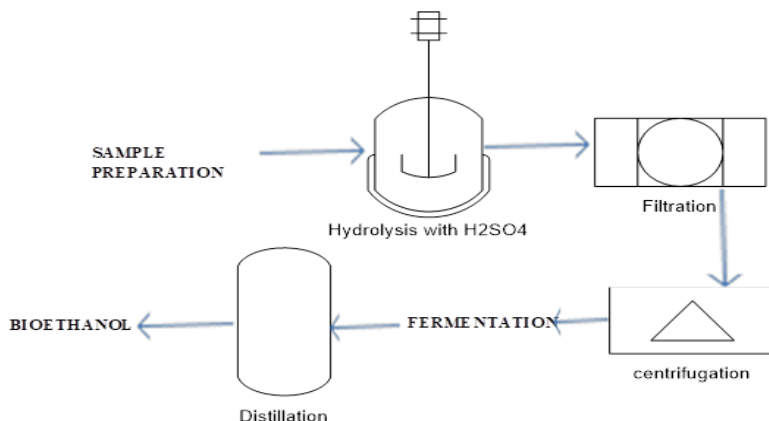


Figure 2: Process Flow Diagram for Bioethanol Production from Waste Paper

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 below shows the value of the hydrolysis (glucose unit) by used in this extraction.

Table 3. 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

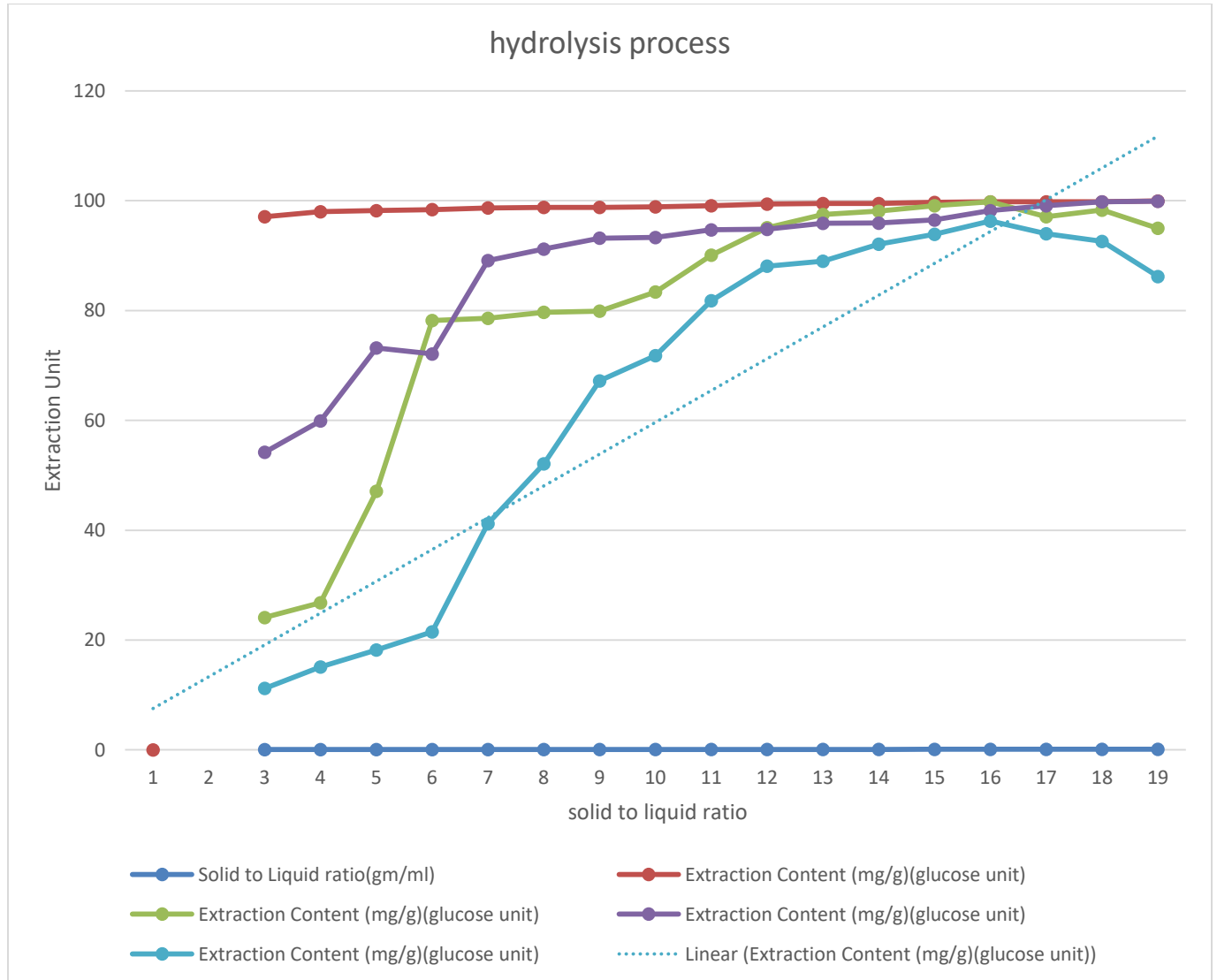


Figure 3. Influence of hydrolysis on glucose unit

Table 4. 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

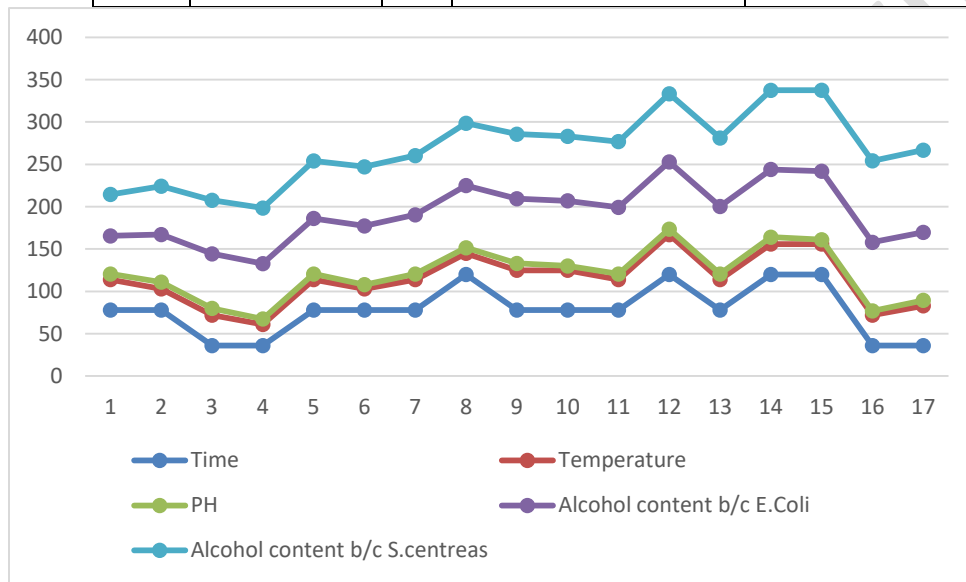


Fig 4. Alcohol content formulation variability among different parameters

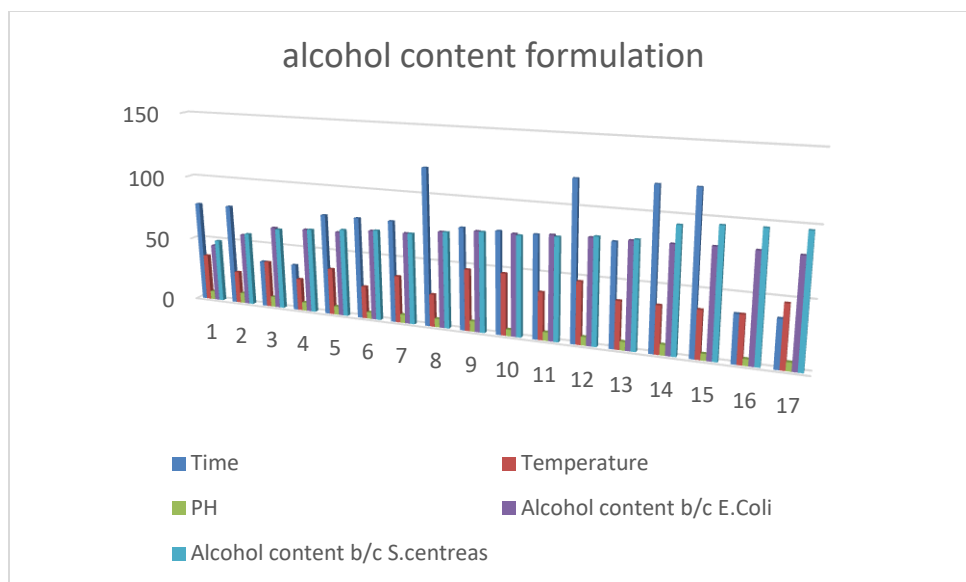


Fig 5. Bar graph showing influence of different parameters to alcohol

Table 5. Design model estimation

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

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. 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

5.1. Material Balance

5.1.1. Conservation Of Mass

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

$$\text{In put} + \text{generation} - \text{output} - \text{consumption} = \text{Accumulation} \dots \dots \dots \text{eq(1)}$$

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.

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

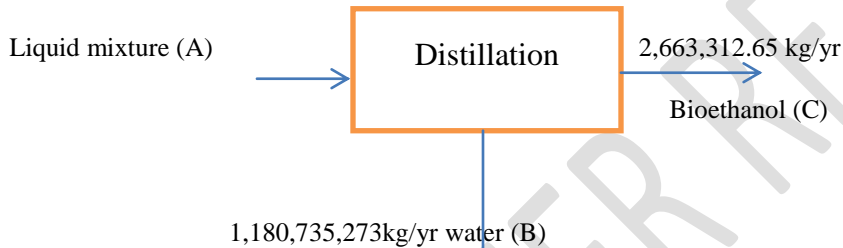
Except in nuclear processes, mass is neither generated nor consumed; but if a chemical reaction takes place a particular chemical species may be formed or consumed in the process.

5.1.2. Formulation

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



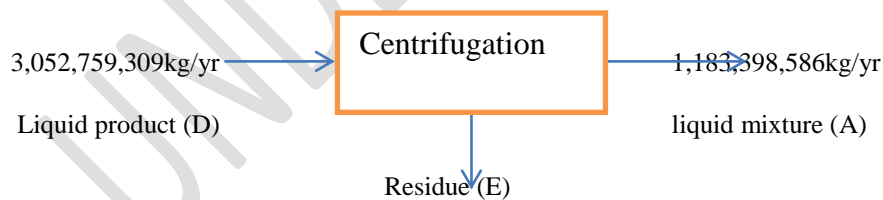
- If there is no chemical reaction the steady-state balance reduces to

$$\text{Material in} = \text{Material out}$$

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

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

II. Centrifugation



Overall material balance

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

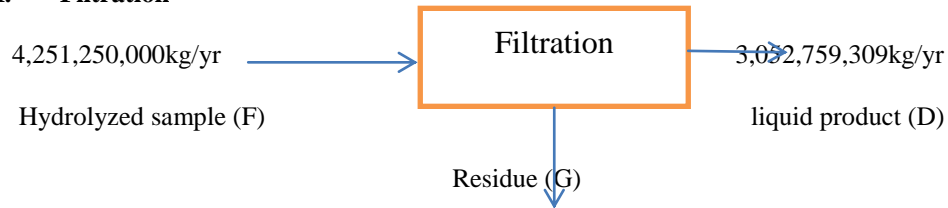
$$D = E + A$$

$$E = A - D$$

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

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

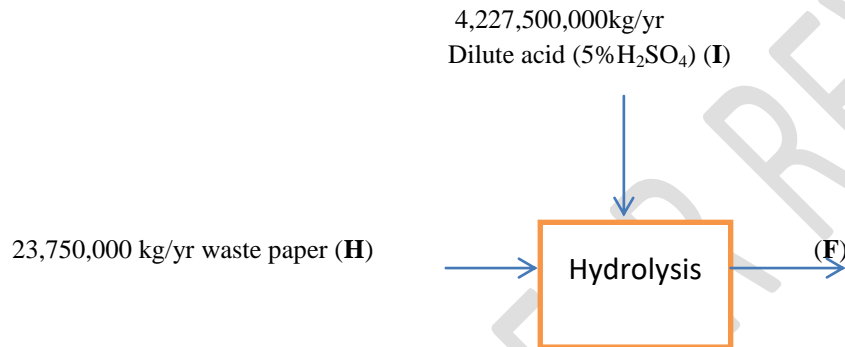
III. Filtration



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=1,198,490,691kg/yr

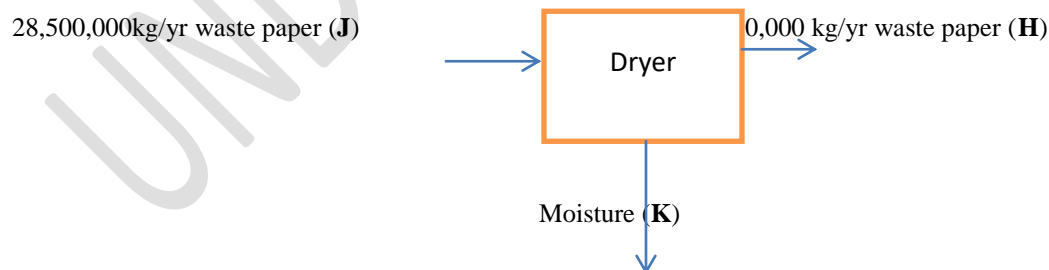
IV. Acid hydrolysis



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=4,251,250,000kg/yr

V. Dryer



Overall material balance

- no chemical reaction so, the steady-state balance reduces to: material in= Material out
 $J = K + H$
 $K = J - H = 28,500,000\text{kg/yr} - 23,750,000\text{ kg/yr} = 4,750,000\text{kg/yr}$

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

5.2. 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.

5.2.2. Conservation Of Energy

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

Accumulation = Energy in + generation — consumption — 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:

$$U_1 + P_1V_1 + 0.5 U_{12} + Z_1 g + Q = U_2 + P_2V_2 + 0.5 U_{22} + Z_2 g + W \dots \dots \dots \text{eq(2)}$$

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:

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

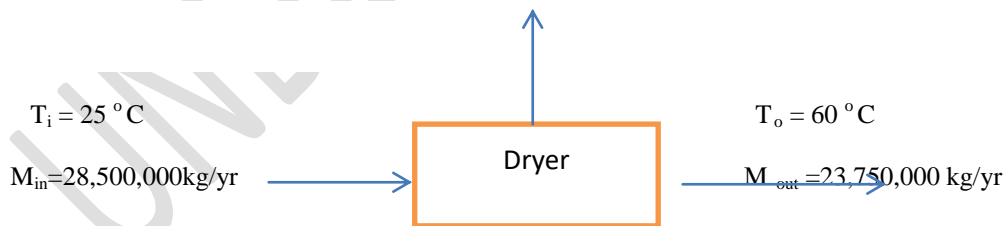
Where: Q_s = heat generated in the system

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

I. Dryer

Water = 4,750,000 kg/yr



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

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

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

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

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

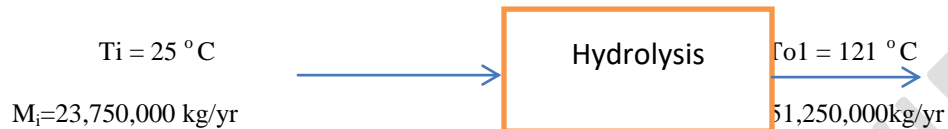
$$Q = 1.0241 * 10^{10} \text{ KJ/yr}$$

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

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

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

II. Acid hydrolysis



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

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

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

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

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

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

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

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

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

III. Distillation



$$M = 1,180,735,273 \text{ kg/yr}$$

$$\Delta T = 65 \text{ °C} = 338 \text{ K}$$

$$C_p = 4.18 \text{ J/g} * \text{K}$$

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

$$Q = 1,180,735,273 \text{ kg/yr} * 4.18 \text{ J/g} * \text{K} * 338 \text{ K}$$

$$Q = 1.67 * 10^{12} \text{ KJ/Kg}$$

$$P = Q * T = 1.67 * 10^{12} \text{ KJ/Kg} * (1 \text{ yr} / 300 \text{ day}) * (1 \text{ day} / 16 \text{ hr}) * (1 \text{ hr} / 3600 \text{ s}) = 96,539 \text{ KW}$$

$$E=P*T = 96,539\text{KW} *16\text{hr}= 1,544,620 \text{ KWh}$$

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

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. 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.

Declarations

Ethics approval and consent to participate

Not applicable to this manuscript.

Consent for publication

Not applicable to this manuscript.

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