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BIO-ETHANOL PRODUCTION FROM SAWDUST OF HARD-WOOD, SOFTWOOD AND MIXED SAWDUST USING WILD YEAST FROM ROTTEN ORANGE AND BAKER'S YEAST.

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Key Words
Bioethanol production, Dark fermentation, Hardwood and Softwood, sawdust, Wild yeast.

ABSTRACT

Lignocellulosic biomass is likely to become the major key sources of renewable energy in the near future, to combat the impact of climate change, transition to sustainable energy is a necessity. Microbial inhibition during fermentation reaction and developing yeast strain that could withstand high ethanol concentration among other factors affecting ethanol yield are very important parameters for proper and wide range of research for economic consideration of bioenergy. This research work investigates bioethanol yield using sawdust of Mahogany, Ako and Mixed sawdust after acidic hydrolysis of Mahogany sawdust, Ako sawdust and mixed sawdust of different wood through Hydrogen Peroxide Acetic Acid (HPAC) pre-treatment process. Wild yeast isolated from rotten oranges and baker's yeast were used for the fermentation reaction. The result for fermentation reaction shows the kinetic parameter for *Saccharomyces Cerevisiae* ($\alpha = 0.9922, 0.9138\ and\ 0.8869, \beta$ =0.0051, 0.0046 and 0.0046 for mixed sawdust, Hardwood and Softwood respectively). While for wild yeast: ($\alpha = 0.9732, 0.6933\ and\ 1.2626, \beta$ =0.0019, 0.0017 and 0.0024 for mixed sawdust, Hardwood and Softwood respectively). This result shows that bioethanol formation during fermentation reaction is mixed-growth associated for both wild yeast and baker's yeast. Models were validated for bioethanol yield from lignocellulose biomass which could be used for optimization of the process.

INTRODUCTION

The world's energy needs are mainly reliant on non-renewable fossil fuel which are derivatives of crude oil, almost 90 % of the product are used for generation of energy and mobility among others [1] [2]. The problem of population explosion has led many developed and developing economies to increase their industrial activities, leading to rapid energy demands. It is certain to conclude that, fossil fuels which are the source of coal, natural gas and oil which are non-renewable energy with time will be scare as a result of depletion and inevitably exhausted, and over reliance on them at the expense of the climate for decades has led to a huge climatic change such as global warming due to emissions of greenhouse gas [3]. Therefore, it is important to explore every possibility of using

alternative source of energy, which are renewable, eco-friendly and even more efficient than fossil fuel, thus, bioethanol fermentation is key and integral [4]. Renewable energy such as biofuels is a perfect replacement for fossil fuels and a worth solution to economic challenges confronting the world and to the impact of climate change. Recently, more attention is devoted to biofuels production and its uses as the alternative which will help to reduce greenhouse gas emissions. The drastic rise in demand for ethanol use as a raw material for production of other chemicals and as a fuel for Automobiles, economic and domestic activities like energy source, preservatives, solvents for industrial processes, cleansing agents, and its unique role in fighting greenhouse gas emissions has led to the increase in demand and production, [5] [6].

Bioethanol is derived from lignocellulose biomass of tree trimmings, grasses, waste papers among others [7]. These feed stocks contain lignocellulose, which includes lignin, hemicelluloses and cellulose, which when broken down could release fermentable sugar for biofuel production.

Various treatment options are available for breaking down complex sugar into simple sugars; this can be done either through acidic hydrolysis at mild and high temperatures or through enzymatic hydrolysis which is another important treatment option [8] [9]. Our research interest explores cellulosic bioethanol production from sawdust of different wood origin. We will basically be focus on softwood sawdust of Ako tree and hardwood sawdust of Mahogany as the case study. Lignocellulose, the major component of the walls of plant cell, is mostly made up of, hemicellulose (about 20–40%), cellulose (about 40–60% of the total dry weight) and lignin (about 10–25%) [10].

The major obstacle in the hydrolysis reaction of cellulose from lignocellulose biomass to produce fermentable sugars lies in isolating it from lignin those bonds with it making it difficult to expose to hydrolysis agent [11]. It should be noted that raw material purity, competition in the fermenter, product inhibition and microorganism's intrinsic limits are the major parameters driving fermentation reaction process [12] [13]. Temperature, pH, aeration, substrate concentration, and nutrient availability all influence the fermentation process and metabolic pathway [14] [15].

In this study the sugar solution used was prepared through acidic hydrolysis of lignocellulosic biomass of different sawdust (softwood and hardwood) to yield fermentable sugar. Also, we will study the impact of cultured yeast (*Saccharomyces Cerevisiae)* concentration on the yield of bioethanol. This research work will also study the behaviors of wild yeast strain from rotten oranges on fermentation process.

2 Materials and Methods

In this study, lignocellulose biomass (sawdust) of particle size 2.00mm was used; Sample A: Hardwood sawdust (Mahogany), Sample B: Softwood sawdust (Ako), Sample C: Mixed Sawdust of Hardwood /Softwood, bio-digester, digital PH meter, High Powered Liquid Chromatography (HPLC), Spectrophotometer, wild yeast isolated from rotten Oranges.

2.1 Hydrolyzed Lignocellulose biomass fermentation Using *Saccharomyces Cerevisiae*

The pH of the hydrolyzed solution was adjusted to 4.5 which is conducive for *S. Cerevisiae*, using conical flask of 250ml the Hydrolyzed Lignocellulose biomass was transferred into the flask ready for fermentation reaction. The initial concentration of sugar from the hydrolysis broth was 70g/L. For an anaerobic condition, a solution of 1N lime was poured into another bottle and connected with a delivering tube. After which 3g/250mL of *S. Cerevisiae* was measured and mixed with warm water of 20ml. In order to activate the yeast strains the mixture was shaken vigorously. This was added to the 250ml mixture in the bottle and then closed tightly under anaerobic condition. The content was transferred to an autoclave for fermentation at a temperature of 25^0c and left for 96 hours to ferment, samples were withdrawn at an interval of 12 hours for Bioethanol analysis using Gas Chromatograph. These steps were re-

peated for temperature of 30^0c and 35^0c for Sample labeled as A, B, and C.

2.2 Hydrolyzed Lignocellulose biomass fermentation Using Wild Yeast Strain from Rotten Orange.

Rotten Orange fruits sample were picked at random from Oil Mill Market, Port Harcourt, Rivers State Nigeria and preferably rotten orange fruits were selected. Fruit sample 100g was taken in a sterile mortar and crushed to a fine paste by mixing with sterile water. Then mixture was kept for overnight at normal room temperature so that natural wild yeast present on fruit samples to grow, the sample was characterized for the presence of microbial flora.

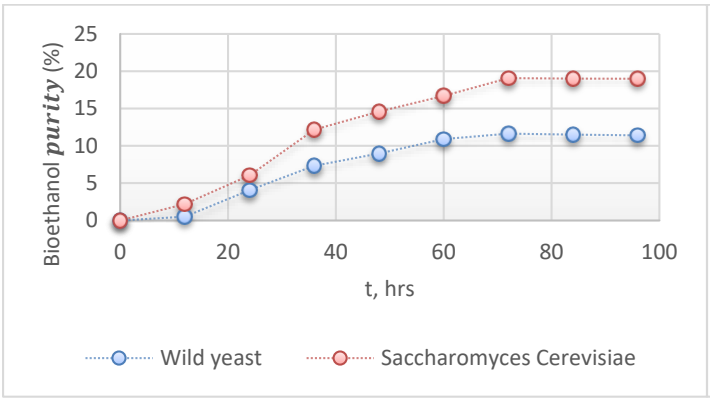
The pH of the mash solution was adjusted to 4.5 which is conducive for Wild yeast strain under study, 250ml of the mash solution was transferred into the conical flask. The initial concentration of sugar from the hydrolysis broth was adjusted to 70g/l. A tube was passed from the lime bottle into the mixture bottle for an anaerobic condition. After which 10g/250ml of Wild yeast strain was added to 20ml of warm water and to activate the yeast strain, the solution was vigorously shaken. This was added to the 250ml mixture in the bottle and then closed tightly. The whole mixture was transfer to Autoclave for fermentation at a temperature of 25^0c and left for 96 hours to ferment, samples were withdrawn at an interval of 12 hours for Bioethanol analysis, using Gas Chromatograph. This procedure was repeated for temperature of 30^0c and 35^0c for Sample A, B, and C.

Figure 1: Inoculum samples for fermentation reaction

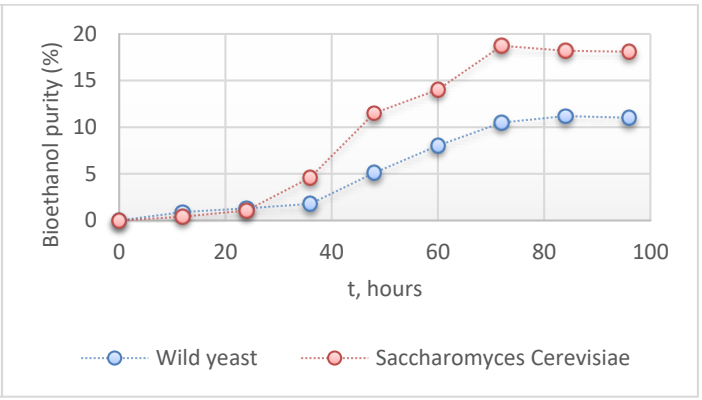
Different factors can affect the course of fermentation, influencing the ecology and adaptation of the micro-biota present [16]. The temperature is a variable that directly affects the growth rate of the microorganisms [17] [18]. Another significant variable is the fermentable sugar concentration. It is likely that the initial concentrations of glucose and fructose can selectively influence the species and strains of yeast present during fermentation. pH is another important variable for fermentation reaction, generally pH ranging from 2.75 to 4.25, is also considered an important factor for the survival and growth of yeasts [19] [20]. Hence, these factors were carefully studied in detailed as the study proceeds, especially the interactions between them and their influence on Saccharomyces Cerevisiae and wild yeast from rotten orange.

To help simplify this work, some basic initials were fixed from the beginning, initial sugar concentration of 70 g/l, *S. Cerevisiae* of 3.0g/250mL of broth and 10g/250mL of broth for wild yeast strain, while the studied was observed at pH of 4.5 all through the reaction duration at different temperatures. The outcomes from this research are depict on Figure 2 -4.

Figure 2 a-c shows the $C_2H_5OH\ purity\ (\%)$ for Mixed Sawdust at 25^oC, Softwood and hardwood respectively.



(a)



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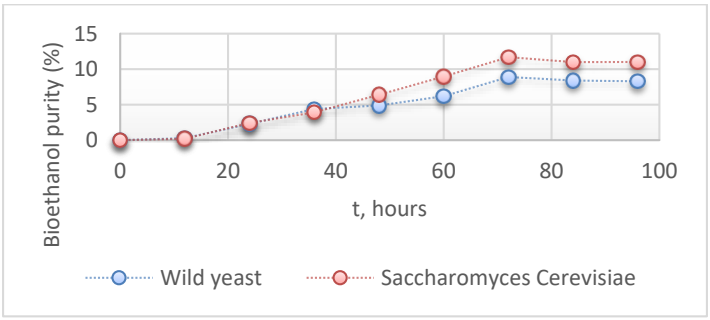
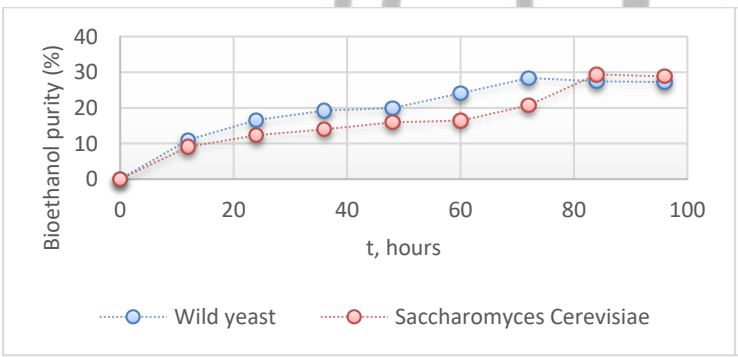
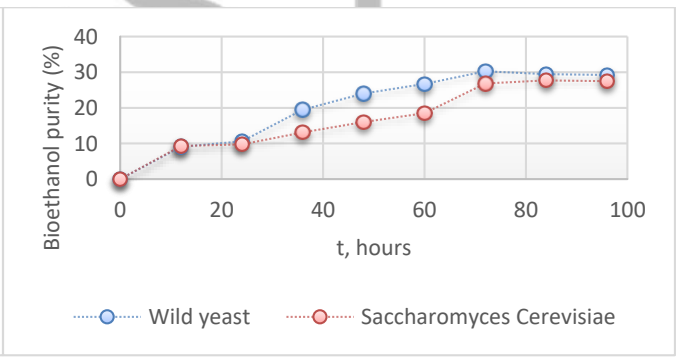


(c)

Figure 2: Ethanol yield for Mixed wood (a), Softwood (b), and Hardwood (c) Sawdust at 25^{o}C.



(a)



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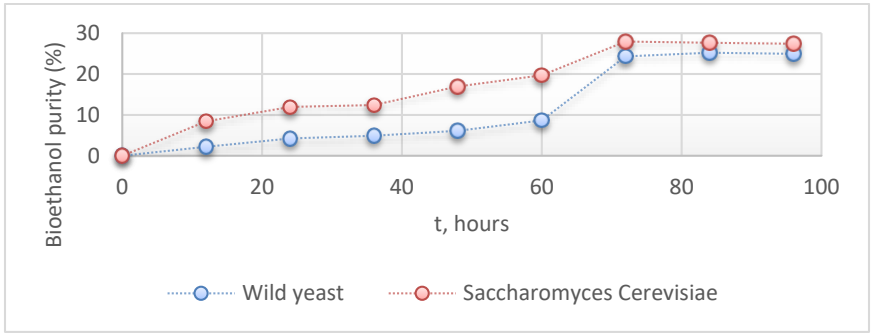
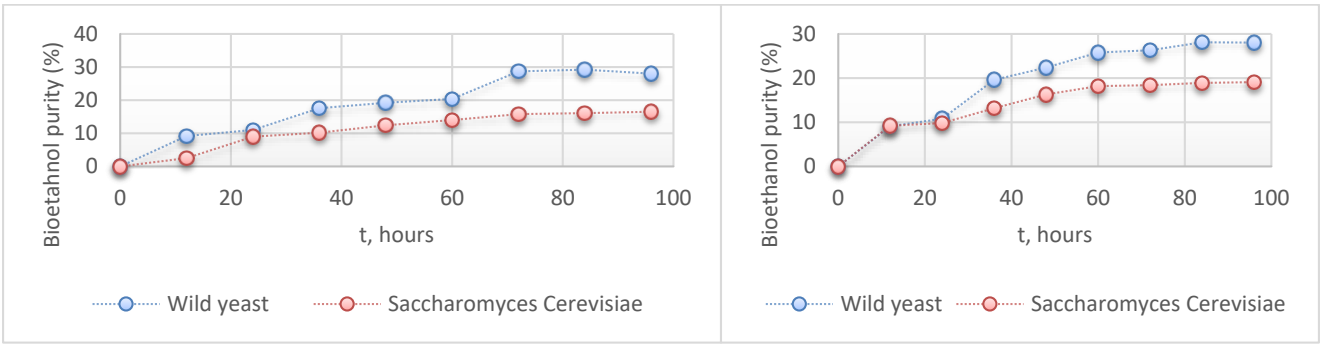


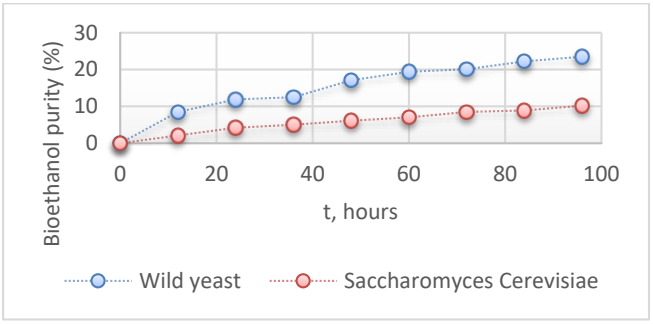
(c)

Figure 3: Ethanol yield for Mixed wood (a), Softwood (b), and Hardwood (c) Sawdust at 30^{o}C.



(a)

(b)



(c)

Figure 4: Ethanol yield for Mixed wood (a), Softwood (b), and Hardwood (c) Sawdust at 35^{o}C.

Generally, as shown from Figure 2(a, b, c) to 4(a, b, c), there are three distinguish regions as fermentation reaction proceeds. As a trend, the first region represents lag phase, the second region represents exponential phase and the third region represents degradation phase. It was observed that degradation of ethanol was as a result of formation of ethyl levulinate formed from the esterification of levulinic acid and ethanol, secondly due to the formation of ethoxy (furan-2-yl) methanol, which was due to the active presence of 5-hydroxy-methyl-furfural from the hydrolyzed mash.

At a 25°C of temperature and 30°C, *Saccharomyces Cerevisiae* experienced higher growth-related activity than Wild yeast. These were depicted on figure 2-4 for Mixed Sawdust, Hardwood Sawdust and Softwood Sawdust where the optimum ethanol yield for Saccharomyces Cerevisiae is 19.07%, 18.73%, 11.70%, 29.37%, 26.87% and 27.97% respectively after a reaction time of 72 hours. While for Wild yeast the optimum ethanol yield was 11.63%, 11.20%, 8.90%, 28.37%, 30.27% and 25.23% respectively at the same reaction duration, though a slide difference at a temperature of 30°C for Softwood Sawdust was observed. At this temperature, the optimum ethanol yield was 30.27% while that of *Saccharomyces Cerevisiae* is 26.87% after reaction duration of 72 hours.

However, at 30°C, Wild yeast experienced higher growth-related activity than *Saccharomyces Cerevisiae*. As shown in figure 2-4, the optimum ethanol yields for Mixed Sawdust, Hardwood Sawdust and Softwood Sawdust for Wild yeast are 29.20%, 28.10% and 23.50% respectively after 84 hours of reaction. While that of Saccharomyces Cerevisiae are 16.50%, 19.10 % and 10.20 % after a reaction time of 96 hours.

From the results, it is obvious that Wild yeast Strain tolerate higher temperature than *Saccharomyces Cerevisiae*. From figure 2-4 for mixed Sawdust, increase in temperature from $25^{o}C\ to\ 35^{o}C$, the ethanol yield for Wild yeast strain increased from 11.63% to 29.20%. While for *Saccharomyces Cerevisiae* ethanol yield decreased from 19.07% to 16.50%. Using mixed Sawdust as a standard, the optimum temperature for *Saccharomyces Cerevisiae* is $30^{o}c$ with bioethanol yield of 29.37%. While the optimum temperature for Wild yeasts strain is $35^{o}c$ with a yield of 29.20%.

2.3 Kinetic parameters for Fermentation Reaction

For the purpose of obtaining kinetic parameter for fermentation reaction, the outcomes from the experiment were processed for Medium A (mixed Sawdust), Medium B (hardwood sawdust) and Medium C (Softwood sawdust).

The data provided below are for medium A.

$X_0 = 12\ g/L, X_{max.} = 17.9976\ g/L, \mu_{max} = 0.0830\ h^{-1}, \beta = 0.0051, \Delta t = 12\ h$

$X = 0.264X(\%)$

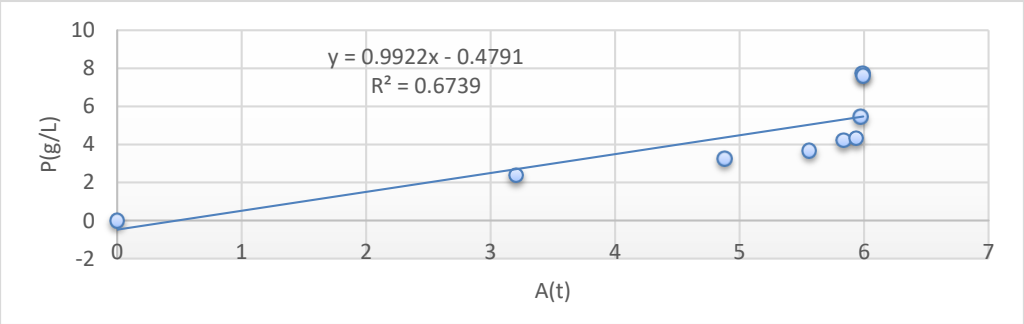


Figure 5: Optimum condition for Mixed Sawdust at 30^0 for *Saccharomyces Cerevisiae*.

The data provided below is for medium A.

$X_0 = 40\ g/L, X_{max.} = 46.9672\ g/L, \mu_{max} = 0.0833\ h^{-1}, \beta = 0.00195, \Delta t = 12\ h$

$X = 0.264X(\%)$

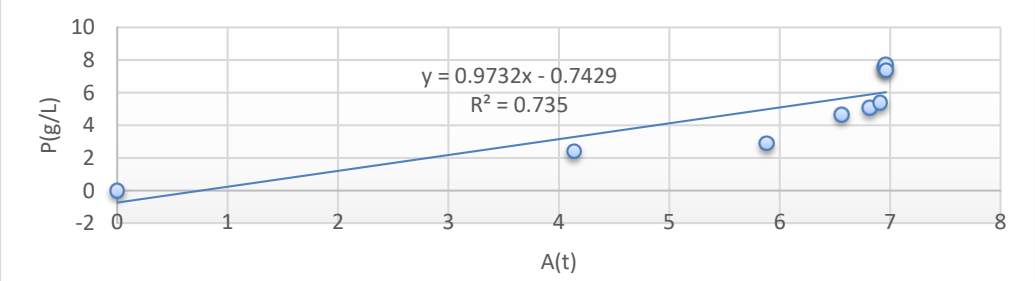


Figure 6: Optimum condition for Mixed Sawdust at 35^0 for Wild Yeast Strain

The data provided below are for medium B.

$X_0 = 12\ g/L, X_{max.} = 18.9972\ g/L, \mu_{max} = 0.0833\ h^{-1}, \beta = 0.0046, \Delta t = 12\ h$

$X = 0.264X(\%)$

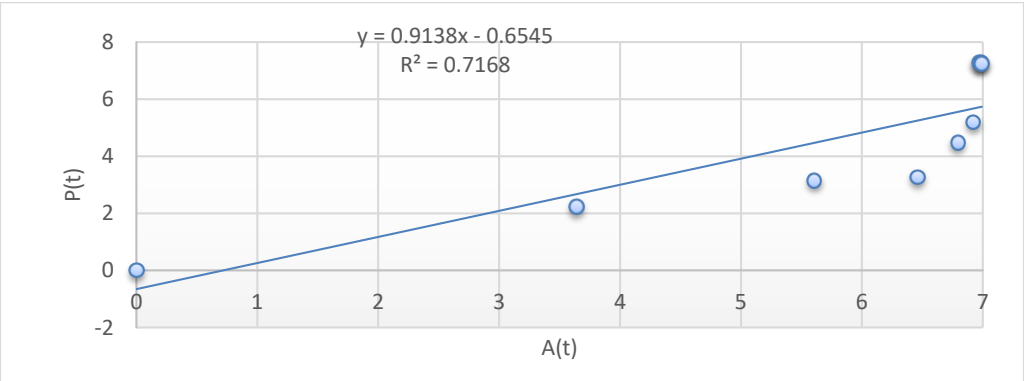


Figure 7: Optimum condition for Hardwood Sawdust at 30^0 for *Saccharomyces Cerevisiae*.

The data provided below are for medium B.

$X_0 = 40\ g/L, X_{max.} = 46.9972\ g/L, \mu_{max} = 0.0833\ h^{-1}, \beta = 0.0017, \Delta t = 12\ h$

$X = 0.264X(\%)$

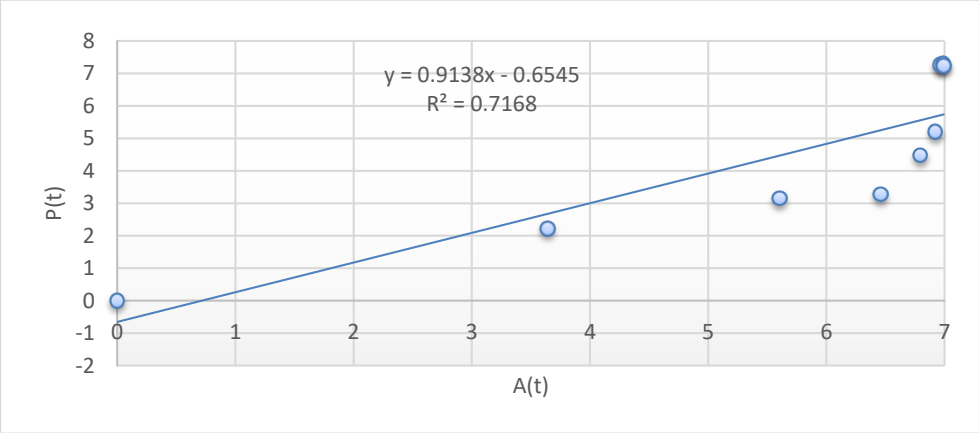


Figure 8: Optimum condition for Hardwood Sawdust at 30^0 for *Saccharomyces Cerevisiae*.

The data provided below are for medium B.
$X_0 = 40\ g/L, X_{max.} = 46.9972\ g/L, \mu_{max} = 0.0833\ h^{-1}, \beta = 0.0017, \Delta t = 12\ h$
$X = 0.264X(\%)$

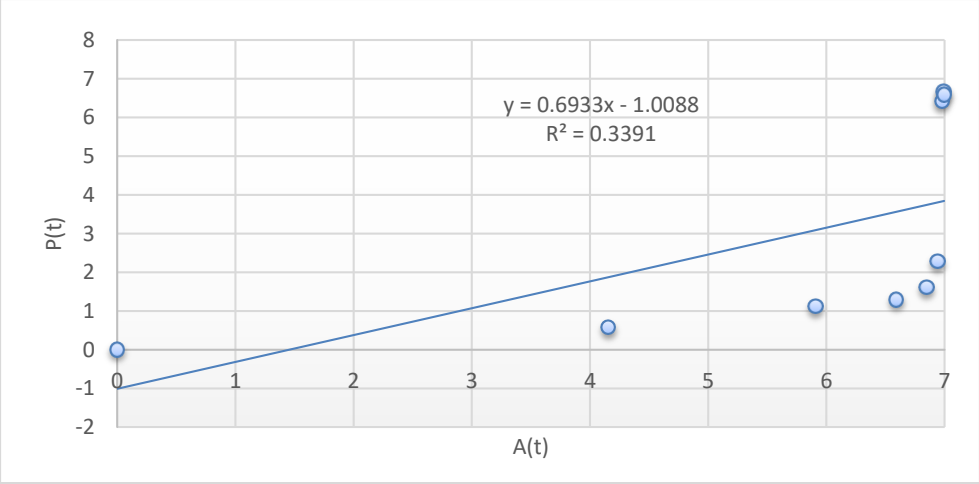


Figure 9: Optimum condition for Mixed Sawdust at 30^0 for Wild Yeast

The data provided below are for medium C.
$X_0 = 12\ g/L, X_{max.} = 18.9972\ g/L, \mu_{max} = 0.0833\ h^{-1}, \beta = 0.0046, \Delta t = 12\ h$
$X = 0.264X(\%)$

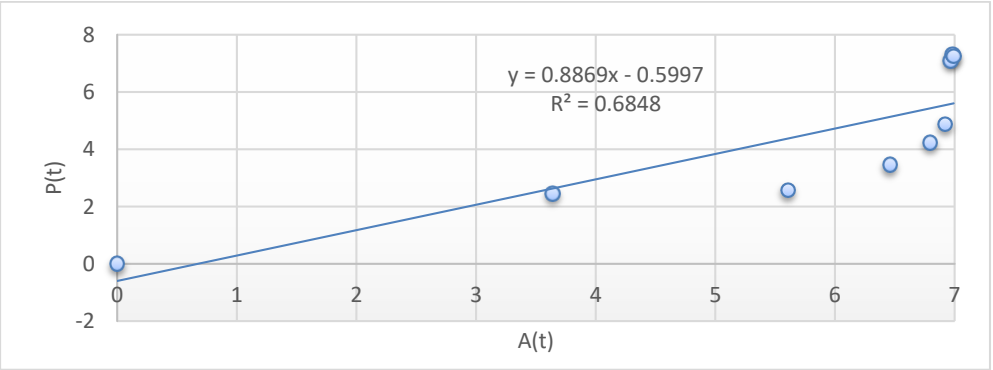


Figure 10: Optimum condition for Softwood Sawdust at 30^0 for *Saccharomyces Cerevisiae*.

2.4 Discussion of Kinetic parameters for Fermentation Reaction
As earlier stated, $\alpha\ and\ \beta$ are the product formation constants that vary with the fermentation condition. If $\alpha \neq 0\ and\ \beta = 0$, then

Product formation is growth associated, if $\alpha \neq 0 \; and \; \beta \neq 0$, then Product formation is mixed-growth associated, if $\alpha = 0 \; and \; \beta \neq 0$, then Product formation is non-growth associated.

For *Saccharomyces Cerevisiae*; for Medium A (mixed sawdust) $\alpha = 0.9922 \; and \; \beta = 0.0051$, for Medium B (Hardwood) $\alpha = 0.9138 \; and \; \beta = 0.0046$ and for Medium C (Softwood) $\alpha = 0.8869 \; and \; \beta = 0.0046$.

For Wild yeast; for Medium A (mixed sawdust) $\alpha = 0.9732 \; and \; \beta = 0.0019$, for Medium B (Hardwood) $\alpha = 0.6933 \; and \; \beta = 0.0017$ and for Medium C (Softwood) $\alpha = 1.2626 \; and \; \beta = 0.0024$.

Since $\alpha \neq 0 \; and \; \beta \neq 0$ for both *Saccharomyces Cerevisiae* and Wild yeast, in conclusion the fermentation reaction for bioethanol formation is mixed-growth associated. Therefore, the fermentation reaction is influenced by bioethanol concentration, sugar concentration and yeast growth among other factors.

Conclusion

After careful analysis of the results of this research, the following points and conclusions were drawn:

1. The fermentation reaction condition of pH 4.5 and reaction temperature of 30^oC gave the optimum bioethanol yield for both Wild yeast and *Saccharomyces Cerevisiae*.
2. The optimum bioethanol yield for *Saccharomyces Cerevisiae* at 30^oC was 29.37% for mixed sawdust, 27.73% for Softwood and 27.67% for Hardwood.
3. The optimum bioethanol yield for Wild yeast was 30.27% for Softwood, 28.37% for mixed sawdust and 25.23% for Hardwood sawdust.
4. Wild yeast strain withstood higher temperature condition than Saccharomyces Cerevisiae as can be seen at reaction temperature of 35^oC: For Wild yeast the optimum yield was 30.27% after reaction time of 72 hours, while for *Saccharomyces Cerevisiae* the optimum yield was 19.10% after reaction time of 96 hours.
5. The fermentation reaction kinetic parameters $\alpha \; and \; \beta$ which were greater than zero indicate that bioethanol formation was mixed-growth associated for both Wild yeast and *Saccharomyces Cerevisiae.*

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