Production of Lactic Acid from Cassava Starch Hydrolysate using Immobilized Lactobacillus Casei in a Fibrous Bed Bioreactor

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***Abstract*—Lactic acid fermentation has been continuously investigated because of many industrial applications and almost all studies have been carried out with glucose or lactose but very few studies have been done using cassava hydrolysis. It would be a big advantage to use starch or pre-treated starch instead of other carbon sources from their economic point of view. Thus, the aim of this study was to demonstrate a fermentation process, which would economically produce lactic acid from inexpensive carbon source. This production was carried out in two phases. In the first, the spores of lactobacillus casei were inoculated to the growth medium. After immobilization of enough cells on carbon fibres, the medium was changed to production medium to enhance the production of lactic acid instead of biomass. Samples were taken at specific time interval and analyzed. It was found that a feed concentration of 25 g/l had the highest conversion of 97% at D = 0.47 hr^{-1} yielding maximum productivity of 11.40 g/lh of lactic acid, 8 hours after start up.**

***Keywords*—biomass, cassava, fermentation, hydrolysis, immobilization, inoculate, lactobacillus casei, spores.**

I. Introduction

Lactic acid, $C_3H_6O_3$, is an organic acid widely used in food, chemical and pharmaceutical industries, and can either be produced naturally or synthetically. It is interesting to note that this acid manufactured by either chemical synthesis or renewable carbohydrate fermentation is for commercial purpose. With the ever increased public concern and government regulations on greenhouse gas emissions and environmental pollution, lactic acid produced by environmentally friendly fermentation bioprocesses, using renewable biomass resources, is preferable to chemically synthesized one using non-renewable fuels like petroleum or natural gas.

The high cost associated with downstream processing of fermentation-derived lactic acid, due to carbohydrates-based impurities and cell breakdown products has greatly been reduced with the use of immobilized biocatalyst in biotechnology processes [1] – [5]. The world consumption of lactic acid stands enthused by its use in key industries such as cosmetics, biodegradable plastics and food additives, and other emerging possible applications. Hence, the world market for lactic acid is projected to reach 259,000 metric tons by the year 2012.

However, as world petroleum reserves are depleted, chemical and energy utilization is ever on the increase. Thus, the need for new sources of carbon and hydrogen is very imperative and pertinent to meet our chemical and energy requirements. Large quantities of biomass are available in most parts of the world and could be used as an energy mechanism or as raw material for chemicals manufacture. In Africa, for example, Nigeria is the world's largest cassava producer; lactic acid fermentation using cassava starch hydrolysate instead of other carbon sources would be a very big advantage from the economy point of view spurring rural industrial development in the continent.

Hence, production of lactic acid by fermentation of cassava starch hydrolysates in well-designed bioreactor offers key advantages over existing lactic acid production techniques. Some of these advantages are: significantly lower production cost, improved productivity achieved by high cell density fermentation via continuous, immobilized cell bioreactor and integrated in-situ product removal, a cell-free product stream obtained by setting a thin membrane layer between the immobilized cell gel-membrane and product stream, which prevents release of cells or cell debris into the product stream and a highly flexible system design, which can be readily integrated into existing and new options.

The aim of this study is to investigate the production of lactic acid from inexpensive carbon sources such as cassava starch hydrolysate via continuous fermentation of lactic acid bacteria in a fibrous bed bioreactor. The production of lactic acid was carried out in two phases. In the first phase, the spores of lactobacillus casei were inoculated to the growth medium that contains cotton fibers, glucose, yeast extract (nitrogen and mineral source). After immobilization of enough cells on fiber, the medium was changed to production medium (cassava starch hydrolysate, magnesium sulphate and ammonium sulphate) to enhance the production of lactic acid instead of biomass. The samples were taken at proper time interval and analyzed by UV-spectrophotometer.

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II. EXPERIMENTAL

A. Preparation of cassava starch hydrolysates

About 7 kg of freshly harvested cassava tubers were peeled and crushed. The resulting cassava mash (6.5 kg) was pressed in a sack to obtain about 2.5 kg of discharged liquid. This liquid was allowed to settle down for about 6 hours after which the clear solution was decanted leaving the residual raw starch behind.

B. Enzymatic hydrolysis of starch

About 0.5 kg of raw starch was dissolved in 1 litre of distilled water and heat treated in a jet cooker using a combination of injected steam and mechanical shear to gelatinize the starch at 80-85˚C. This process produced a viscous solution containing about 10% dry solids. A known quantity of this gelatinized starch solution was taken, and soluble enzymes (α-Amylase and Glucoamylase) were added. The reactions were carried out in stirred tanks at 45˚C, pH 4.5 for 72 hours. The resulting solution consists of 95 – 97% glucose with 3 – 5% higher disaccharides while the enzyme was removed by heat denaturation at higher temperature. Samples were collected at an interval of 2 hours to determine the total starch and reducing sugar level while maintaining the pH and temperature of the mixture. The iodine solution, Fehling's solution and Benedict's solution were preliminarily used for the determination. However, after stopping the reaction by heating the sample to about 105^{o}C, the extent of hydrolysis was then measured by the determination of the glucose formed using the Dubowski method [6] – [7].

C. Preparation of the culturing media

About 35 g of agar (in the powdered form) was weighed and dispersed into 500 ml of distilled water in a conical flask. This was allowed to soak for 10 minutes, swirled to mix and heated on hot plate for 5 minutes. A 7 ml of the mixture was pipetted into 15 bottles and 15 test tubes each. The small bottles were slightly heightened with their covers and the test tubes covered with cotton wools. These bottles and test tubes as well as the remaining mixture in conical flask were placed in the autoclave for sterilization at 121˚C for 15 minutes. After which the bottles and test tubes were placed on a tray in slant positions, in which the contents in the conical flask having cooled to 47˚C was poured into sterile Petri dishes. They were left in the open and allowed to solidify naturally for about 20 hours prior to inoculating of the microorganism.

D. Inoculation of the media with lactobacillus casei

A pure sample of lactobacillus casei, which had been kept in the refrigerator was brought out and allowed to acclimatize before inoculation on the prepared culture media. The inoculation was carried out near a Bunsen burner flame using sterile wire loop that had been heated red hot to eliminate any possible microbial contaminations, cooled for some seconds (3-4 seconds). The solidified agar medium was touched and covered lightly and quickly with sterile wire loop that had just touched the medium containing the inoculums, after it was sterilized again. This procedure was repeated for each medium in the small bottles, test tubes and Petri dishes. Then, all inoculated media were placed in the incubator at 33˚C for 72 hours.

E. Inoculation of the media with lactobacillus casei

The microbes were fully grown on the various media after 3 days. The spores of this lactobacillus casei were inoculated to the growth medium that contained cassava starch hydrolysate, NH_4SO_4 and $MgSO_4$.$7H_2O$ (or yeast extract – nitrogen and mineral source) using sterile wire loop to scoop them near the bunsen burner flame. Sterile distilled water was used for rinsing the media after scooping for complete washing off of the inoculums. This was then kept ready for immobilization.

F. Packing, preparation and immobilization of bioreactor

The cotton fiber was sterilized in an oven by heating at 121˚C for 6 hours 15 minutes. Then, the sterilized cotton fiber was packed into the clean glass column until the required height of 50 cm in the column was attained, giving a working volume of 800 cm^3. The glass reactor column packed with cotton fiber was made of borosilicate glass tube (4.5 cm inner diameter by 60 cm height). After sterilization, the packed column was connected to the peristaltic pump. The cotton fibre column was immobilized with the inoculums by pumping the entire growth medium after harvesting into the glass tube via the pump from the bottom**.** This was repeated to ensure that enough cells were immobilized. The concentrated lactobacillus casei suspension (200 g/l) was circulated through the reactor system at a dilution rate of 0.47 hr^{-1} for 10 hours in order to absorb the cell into the cotton fibre. During immobilization, at an interval of 1 hour, lactobacillus casei suspension was withdrawn from the column and its absorbance was measured by using Jenway 6061 calorimeter to quantify how much cells were attached to the fiber.

G. Treatment of cassava starch hydrolysate

The cassava starch hydrolysate, which is essentially glucose solution, was made into several concentrations by serial dilution and the concentrations determined as 150, 100 and 25 g/l from the standard glucose calibration curve. To each solution were added the required nutrients: 1 g $MgSO_4$. $7H_2O$ and 1 g of $(NH_4)_2SO_4$ as supplementary agents to reduce the inhibition effect of lactic acid in the inoculums (lactobacillus casei) during which production by enhancing the acid production product value of **S** was observed. These solutions were later sterilized in the autoclave for 15 minutes at 100˚C.

H. Biochemical reaction procedure for lactic acid production

After immobilization of enough cells on fibre, the growth medium was replaced with the sterilized treated cassava hydrolysate to enhance the production of lactic acid instead of biomass. The bio-reaction commenced in the column by passing solution containing various concentrations of glucose solution 150, 100 and 25 g/l through the bioreactor in a continuous fashion for the production of Lactic acid with the aid of the peristaltic pump maintained at 6.25 ml/min volumetric flow rate and dilution rate of 0.47 hr^{-1}. The bio-reaction was completed after 8 hours each; however, batch samples were prepared making a solution of the sterile treated cassava starch hydrolysate of the same concentrations with that of the continuous process. To each 250 ml of the concentrations, 25 ml of inoculated growth

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medium was added. These were then placed in the mechanical shaker with water in equipment maintained at 37˚C. At regular intervals, the lactic acid and glucose concentrations were examined. At the end of the fermentation, both processes results were compared.

III. RESULTS AND DISCUSSION

This study focuses on the production of lactic acid from locally prepared and treated cassava starch hydrolysate (substrate) using Nigerian cultured and grown lactobacillus casei as homofermentative bacteria in an immobilized fibrous bed bioreactor. The fermentation parameters of the batch production were well monitored to obtain the corresponding maximum lactic acid productivity for all the various glucose concentrations at varying dilution rate. This production was carried out at different dilution rates D=0.47, 0.60, 0.62, 0.75 and 0.80 hr^{-1} for varying glucose concentrations in the cassava starch hydrolysate S_0=25, 100 and 150 g/l. All laboratory experiments were conducted in triplicate and data presented are the average of the three values. The operating temperature and pH of initial hydrolysate medium were maintained at 35^{o}C and 6 respectively under ambient condition.

TABLE I
PRODUCTION CONCENTRATION, PRODUCTIVITY AND YIELD FOR DIFFERENT INITIAL GLUCOSE CONCENTRATION AND CONSTANT DILUTION RATE OF 0.75, 0.60 AND 0.80 HR^{-1}

D = 0.75 hr^{-1}				
S_O (g/l)	S (g/l)	P (g/l)	Q_P (g/lh)	$Y_{P/S}$ (g/g)
8.47	0.09	8.31	6.23	0.99
21.0	8.38	10.42	7.82	0.83
25.0	10.45	12.22	9.17	0.84
28.0	11.01	15.99	11.99	0.94
150.0	59.51	90.49	67.87	0.99
D = 0.60 hr^{-1}				
S_O (g/l)	S (g/l)	P (g/l)	Q_P (g/lh)	$Y_{P/S}$ (g/g)
8.47	0.07	8.29	4.97	0.98
21.0	8.0	12.01	7.21	0.92
25.0	9.01	14.01	8.41	0.88
28.0	9.92	17.09	10.25	0.95
150.0	38.71	91.49	54.89	0.82
D = 0.80 hr^{-1}				
S_O (g/l)	S (g/l)	P (g/l)	Q_P (g/lh)	$Y_{P/S}$ (g/g)
8.47	0.11	7.31	5.85	0.87
21.0	8.39	10.27	8.22	0.81
25.0	12.09	10.96	8.77	0.85
28.0	16.49	10.78	8.62	0.93
150.0	68.71	80.06	64.05	0.98

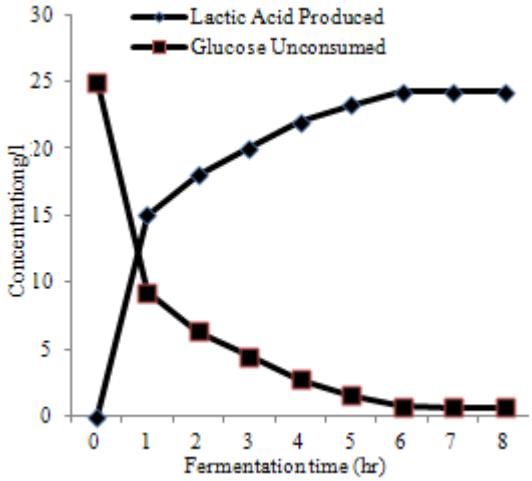

Fig. 1. Performance of the immobilized cell reactor for 25.0 g/l of cassava starch hydrolysate at dilution rate of 0.47 hr^{-1}

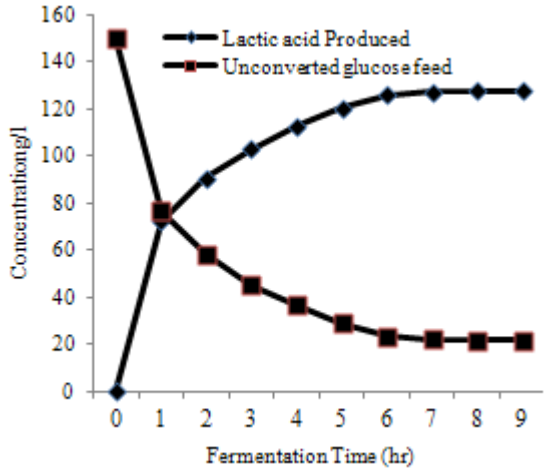

Fig. 2. Performance of the immobilized cell reactor for substrate feed of 150 g/l at dilution rate of 0.47 hr^{-1}

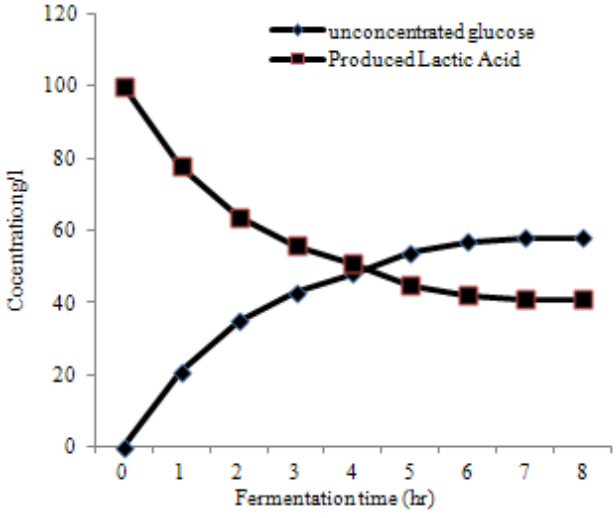

Fig. 3. Performance of immobilized cell reactor for a feed of 100 g/l at a dilution rate of 0.624 hr^{-1}

The substrate and product concentrations from the immobilized cell bioreactor studies are represented as a function of fermentation time (hr) in Figs 1 – 3**.** It can be observed that the product concentration increases and the substrate concentration decreases with increase in fermentation time in all the Figs. The rate of lactic acid production is seen to go through a maximum of 11.40 and

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59.97 g/l.hr at the optimum dilution rate of 0.47 hr^{-1} for feed substrate of 25 and 150 g/l concentrations respectively. However, at an increased dilution rate of 0.62 hr^{-1}, a lower productivity and substrate conversion was observed for substrate concentration of 100 g/l. It is noticed that there was no significant increase in lactic acid productivity beyond the dilution rate of 0.8 hr^{-1} in this system; a dilution rate above this value may result in cell de-immobilization or wash-out. Fig. 1 shows the concentration profile in the fibrous bed reactor 8 hours after start up. The flow rate for this run was 375 ml/hr. The immobilized cell reactor converted all the glucose into lactic acid to yield 24.3 g/l in the effluent for a feed of 25 g/l concentration. Moreover, for both the continuous and batch fermentation processes, a pH range of 3.7 – 4.2 was observed which indicates the acidic medium of the product at 35˚C.

It is noted that the batch process sample has a higher conversion of glucose to lactic acid than the continuous process but lower productivity as shown in Tables 1 & 2. It can also be seen that with increase in the dilution rate, the effect of free cells on lactic acid production decrease quickly. In addition, considering the inhibition of the microorganism, lactobacillus casei; in the presence of lactic acid, the addition of $MgSO_4.7H_20$ and $(NH_4)_2SO_4$ salts to the feed substrate has enhanced the conversion of glucose to lactic acid.

IV. CONCLUSION

Lactic acid was produced from inexpensive carbon source which is cassava starch hydrolysate using immobilized lactobacillus casei in a fibrous bed bioreactor. The lactic acid production increased with glucose concentration and 100% conversion could not be achieved as a result of lactic acid inhibition. However, the presence of certain additives such as ammonium sulphate and manganese sulphate significantly increased the volumetric product productivity and minimized lactic acid inhibitive effect. For three trial runs various concentrations of the hydrolysates in the immobilized cell reactor at varying dilution rates. The lowest feed concentration of 25 g/l had the highest conversion of 97% at a dilution rate D = 0.47 h^{-1} to yield a maximum volumetric lactic acid of 11.40 g/lh at 8 hours after start up.

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