Utilization of sugarcane bagasse and the generation of bioethanol by *Streptomyces coelicolor* strain COB KF977550 and *Streptomyces albus* strain DOB KF977551

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Introduction

Increase in fossil oil price and security of oil supply is a major concern worldwide. Also, increased energy demand and the global supply of fossil fuels contributes to the greenhouse gas (GHG) emission with an alarming negative impact on the environment and on human health (Hahn-Hägerdal *et al*., 2006). Lignocellulose, the most Abundant and renewable organic matter on earth can be used for the production and recovery of many value-added products, which make them attractive for the production of ethanol (Lau *et al*. 2010).

Currently, emphasis is on the use of lignocellulose "wastes" rather than the use of food crops such as corn and sugarcane to produce biofuels in order to minimize food-feed-fuel conflicts. The use of excessive high temperature for the pre-treatment of lignocellulose wastes ultimately lead to environmental degradation, while most of the chemical methods are expensive. Therefore, biological treatment method which involves the use of whole organisms such as fungi and bacteria or their enzymes in the pre-treatment of lignocellulose wastes appear to be a better choice because of its environmental friendliness. Although a number of reports are available on bioethanol production by fungi from lignocellulosic wastes, whereas those of bacteria are scarce from this part of the world

Aims

This study aimed at investigating the ability of wood-digesting *Streptomyces* strains isolated from a tropical estuarine ecosystem to produce bioethanol from a renewable agricultural waste (sugarcane bagasse) for possible biotechnological applications without the need for pre-treatment stage of saccharification with the goal to keep the cost of raw material low.

Significance of study

There is a strong global re-awakening for devising new, non-fossil based fuels that are generated in a sustainable way with minimum greenhouse gas production.

The production of ethanol from food plants, sugars or starch is expensive and could lead to food crisis. Lignocellulosic wastes are generated in large quantities through agricultural practices and forestry and often pose environmental pollution problems. Bioconversion offers a cheap and safe method of not only disposing the agricultural residues, but also have the potential to convert these "wastes" into value-added products such as bioethanol. Moreover, ethanol contains significant quantity of oxygen that helps complete combustion of fuel, hence reducing particulate emission that pose health hazard to living beings.

Materials and methods

Lignocellulose-utilizing bacterial strains were isolated from a tropical estuary in Lagos, Nigeria. They were identified based on cultural and biochemical characteristics, electron microscopy as well as 16S rDNA gene sequencing as previously described (Buraimoh *et al*., 2015). Growth of the test strains were performed under aerobic batch (submerged) fermentation. One ml of each organism was grown in separate Erlenmeyer flasks (250 ml) containing mineral salts medium (100 ml, pH 7.2) which was supplemented with 1.0 g (w/v) substrate (sugarcane bagasse) as sole carbon source. A flask containing substrate and medium but without organism serves as the control. The flasks were then incubated at 30 ^{o}C on a rotary shaker (150 rpm) for 21 days. Growth was evaluated at intervals (3 days) by the intensity of turbidity (O.D $_{600nm}$) in mineral salts medium, while the metabolic products were determined using GC-FID (Hewlett Packard (HP) 5890 series II ,California, USA) with an OV-3 glass column pack. Column temperature was 200 ^{o}C while the injector and detector temperatures were 200 ^{o}C and 270 ^{o}C respectively with N_2 carrier gas and H_2 at a flow rate of 22 ml/min and temperature/ramping rate of 5^{o}C/min.

Results and discussion

The results of the growth experiment showed that the Optical densities of *Streptomyces* strains COB and DOB increased from 0.10 to 1.41 and 0.16 to 1.23 respectively. Strain COB recorded the highest growth when the pH value of the medium was 6.66 while it was 6.48 for strain DOB (Fig**.**1A&B)**.** Figure 2 shows the gas chromatographs of the control (A) and the various products obtained in the growth media of strains COB and DOB respectively (B&C). Ethanol yields of strains COB and DOB were 43.08 and 28.80 gL^{-1} respectively (Table.1)**.** In addition to ethanol, varied acids of industrial importance were detected in the growth media of both strains

Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology. Lignocellulolytic microorganisms have attracted a great deal of interest as biomass degraders for large-scale applications due to their ability to produce large amounts of extracellular lignocellulolytic enzymes. Moreover, estuarine microorganisms, such as the ones used in this study are known to have the ability to survive extreme environmental conditions. We have selected a cheap and abundantly available lignocellulosic waste for bioethanol production. Seema *et al.* (2007) reported that wheat straw and rice straw pretreatment with *Aspergillus niger* and *Aspergillus awamori* yielded 2.5g L^{-1} and 2.2g L^{-1} of ethanol respectively, while 8.5g L^{-1} and 9.8g L^{-1} were recorded respectively for rice husk and bagasse when treated with *Aspergillus awamori* and *Pleurotus sajor-caju* . Cellulosic substrates were also used by Arthe *et al*. (2008) for bioethanol production by microbial extracellular enzymatic hydrolysis and fermentation where a yield of 8.9 L^{-1} was recorded. However, Saravanakumar and Kathiresan (2013) reported that sawdust hydrolysis by *Trichoderma* and yeast yielded 55.2 g L^{-1} of ethanol under the optimized temperature conditions of 36.5 °C. Their result is not too different from what obtain in this study. Undoubtedly, bacteria is worthy of exploration for the production of bioethanol considering their ease of cultivation, wider tolerance of temperature, pH and oxygen limitations as compared with fungi.

Summary of findings

1. Optical densities of *Streptomyces* strains COB and DOB increased from 0.10 to 1.41 and 0.16 to 1.23 respectively within 21 days of incubation.
2. Strains COB and DOB yielded 43.08 and 28.80 gL^{-1} of ethanol respectively.
3. In addition to ethanol, varied acids of industrial importance were detected in the growth media of both strains

Conclusions

Bioethanol generation from lignocellulosic wastes holds tremendous potential in terms of meeting energy needs, reduced cost, and providing environmental benefits if carefully harnessed. Furthermore, estuarine bacteria are vast and are known to possess robust enzymatic capabilities that could be fully exploited for possible biotechnological applications. Further work will focus on optimization, scale-up and kinetic modelling of the processes.

Acknowledgments

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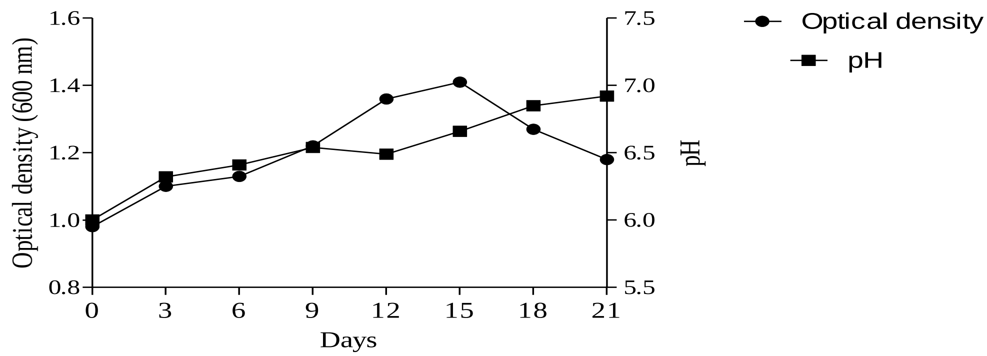

Fig 1A: Growth profiles of pure cultures of *Streptomyces coelicolor* strain COB KF977550

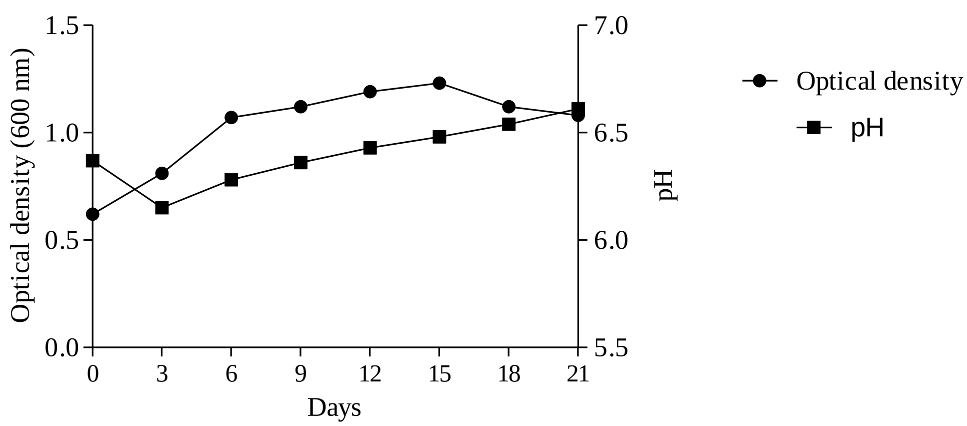

Fig. 1B: Growth profiles of pure cultures of *Streptomyces albus* strain DOB KF977551

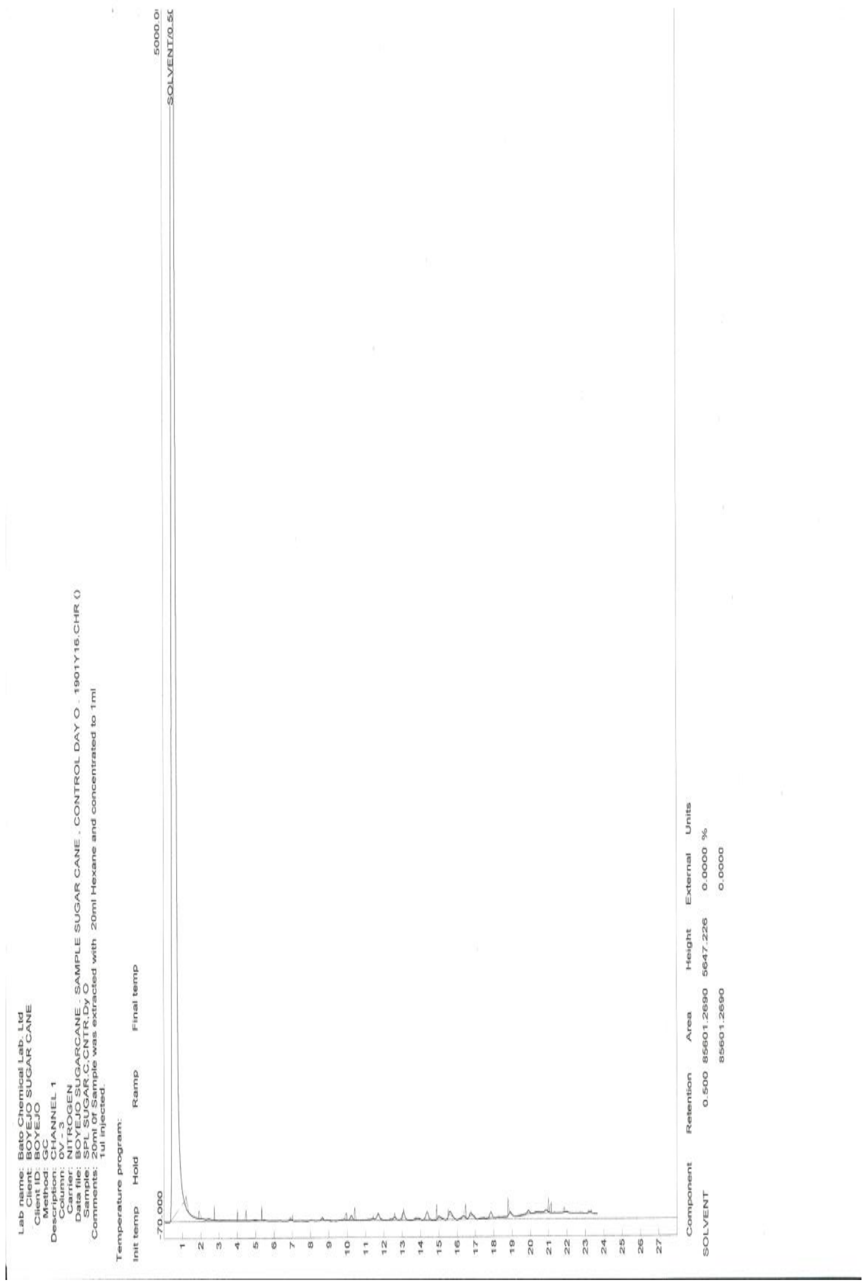

Fig.2A: Gas chromatographic profiles for Ethanol generation (control)

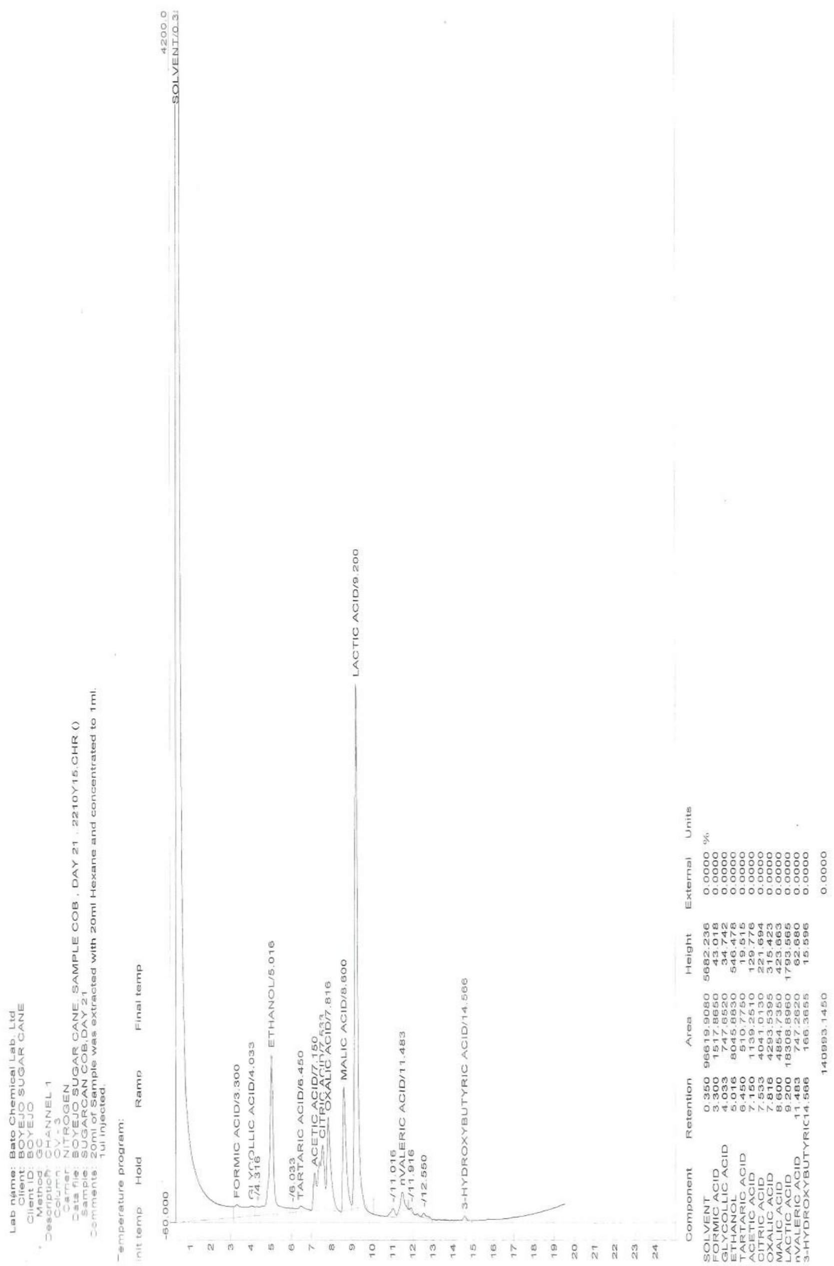

Fig. 2B: Gas chromatographic profiles for Ethanol generation and production of diverse biochemicals from the medium containing sugarcane baggase as the sole carbon and energy source during aerated batch culture of *Streptomyces ceolicolor* strain COB by day 21

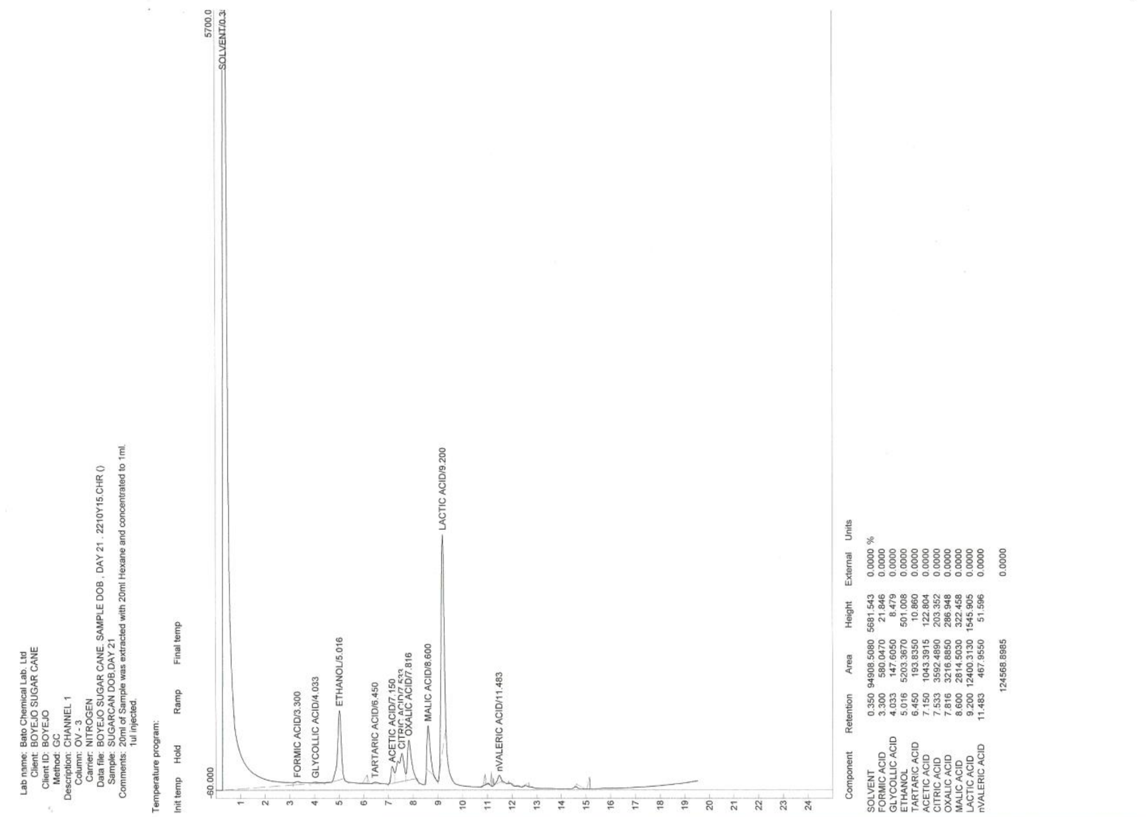

Fig.2C: Gas chromatographic profiles for Ethanol generation and production of diverse biochemicals from MSM containing sugarcane bagasse during aerated batch culture of *Streptomyces albus* strain DOB by day 21.

Table 1: Generation of ethanol with time by strains COB and DOB in MSM containing sugarcane bagasse as sole carbon and energy source

	Quantity of	Ethanol	g/L
Day	COB	DOB	Control (No isolate)
0	0	0	0
3	0.08	0.06	0
6	0.15	0.12	0
9	10.86	8.72	0
12	15.02	9.70	0
15	21.72	12.94	0
18	38.62	18.62	0
21	43.08	28.80	0