Advances in the Development of Bio-energies.
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Topic: Bioconversion of Lignocellulose wastes to products of Industrial Importance by Tropical Estuarine Bacteria

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- Why bacteria?, Why estuarine?
- Faster growth rate
- Better adapted to varied environmental conditions
- Easy to study/handle
- May be more amenable to genetic manipulation
- Estuarine bacteria have survived very hash repository Lagos lagoon

Background

- Global concern over fossil oil price increase and eventual depletion
- Environmental pollution concerns
- The search for “clean technologies” for biochemical production
- Bioconversion of renewable lignocellulosic biomass is gaining significant prominence
- Significant advances have been made in some developed nations. (Achinas and Euverink, 2016).
- **Over dependence on petroleum oil has led to the shutdown of refineries**
- **The contribution of renewable resources to bio based economy in Nigeria is currently negligible.**
- Biochemicals are imported into Nigeria; making the cost of industrial production very expensive.

Background Cont'd

- The challenges could be addressed through bioconversion of lignocellulose "wastes" into bio- based consumable

- **Emphasis is on the use of lignocellulose "wastes" rather than the use of food crops in order to minimize food-feed-fuel conflicts (Sun and Cheng, 2002).**

- **Lignocellulosic wastes are generated in large quantities in Nigeria and often pose environmental hazards.**

- They are indiscriminately disposed by burning (leading to respiratory disorders in humans)
- By dumping into water bodies creating anoxic condition resulting to death of aquatic animals and bad odour (Buraimoh *et al*., 2015).

- Moreover, this is a waste of resources which could be harnessed for biotechnological purposes (Suresh *et al*., 2013)

Sawdust disposed by dumping into the lagoon and burning at Ebute-metta, Lagos.

Aim

- Develop the potentials of tropical bacterial strains for bioremediation and generation of biochemicals with a view to establishing sustainable production plants in Nigeria.

- **This is set out through the following objectives:**
  - Isolation of lignocellulolytic bacterial strains from the Lagos lagoon
  - Investigating the ability of bacterial strains to utilize lignocellulose residues for growth
  - Preliminary Production of biochemicals of industrial importance) from renewable wastes for possible biotechnological applications without the need for saccharification with the goal to keep the cost of raw material low.

Justification

- The outcome of this project will promote the use of renewable bio-based consumables with subsequent reduction in the import bill
- generate increased research output, collaboration and employment opportunities; making multiple contributions to the fight to eradicate poverty
- bring about a reduction of greenhouse gas emissions (improved air quality) which usually results from the use of fossil fuel for oil-based consumables.
- "Wastes" which usually constitutes environmental hazards are removed from the environment (bioremediation).

MATERIALS AND METHODS

1. Sample collection	**(i) Fresh sawdust of *Uapaca heudelotii* from Okobaba saw mill** **(ii) Decomposing sawdust from Lagos lagoon (Okobaba axis)** **(iii) Lagoon water for physico - chemical analysis from lagoon front, University of Lagos**
2. Isolation of actinomycetes	(i) Streak-plate method using starch-casein agar for isolation of actinomycetes (28˚C for up to 7 days)
3. Screening of lignin degraders	(i) Ability of isolates to attack filter papers. (ii) Screening for the catabolism of lignin-related aromatic acids using bromothymol blue indicator(Nishimura *et al.,* 2006).
4. Identification of isolates	(i) Cultural and cellular morphology (ii) Biochemical characteristics (iii) Scanning and Transmission electron Microscopy (Bozola and Russell, 1999). (iv) I6S rRNA gene sequencing of isolates (Schuller *et al*., 2010).

MATERIALS AND METHODS CONT'D.

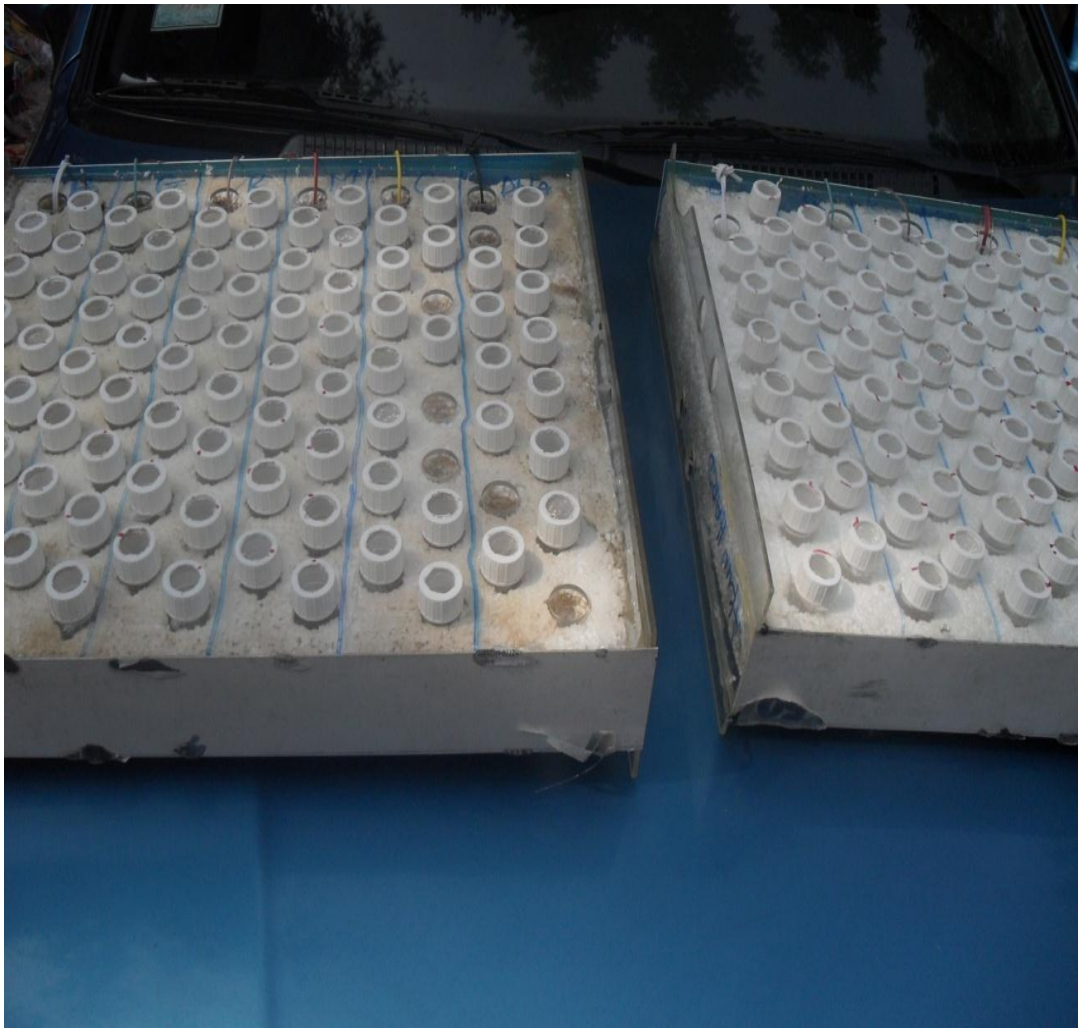
5. Microbial diversity of decomposing wood	1. Terminal restriction fragment length polymorphism (TRFLP) by capillary electrophoresis (Tiedje, 2002) 2. Illumina sequencing
6. *In situ* and laboratory studies. i. Physico - chemical analysis of lagoon water ii. Degradative potential of isolates on sawdust and selected lignocellulose wastes	 (i) Gravimetry (ii) Spectrophotometry (i)Weight loss method (Deschamps *et al*., 1981) (Gravimetry) (ii) Evaluation of Carbohydrate content using anthrone reagent.(Trevelyan *et al*., 1952) (iii) Evaluation of lignin in degraded wood residues (Van Zyl, 1978)
7. Degradative potential of isolates on lignin and lignin-related aromatic compounds	(i) Reduction in the concentration of lignin with time (Spectrophotometry) (ii) Growth measurement (Ball *et al*., 1989).

MATERIALS AND METHODS CONT'D.

8. Identification of metabolic products of degradation	**Use of HPLC, HPLC – MS, UV Spectrophotometry .**
9. Detection of major lignolytic and cellulolytic enzyme gene dusters (Cellulase, demethylase and 3,4-protocatechuate)	Genomic analysis using PCR (Pieter*, et al*.,2011; Chow *et al*.,1999 ; Buchan *et al*., 2000)
10. Enzyme activity assays	(i) Cellulase – Spectrophotometry (Somogyi,1952.; Nelson,1944) (ii) Peroxidase – Spectrophotometry (Mercer *et al., 1996)* (iii) Laccase – Spectrophotometry (Kizhekkedathu and Paru, 2005)
12. Protein analysis	(i) Lowry *et al*., 1951 (Spectrophotometry)
13. Optimization (Enzyme)	(i) Effect of pH (ii) Effect of substrate concentration (iii) Effect of temperature (iv) Effect metal ion (Somogyi, 1952; Nelson, 1944).

Satellite image of the Lagos Lagoon showing location of sample collection and *in situ* study site

Improvised boxes made from Perspex material and Styrofoam used for the *in situ* wood degradation studies (Floating raft experiment) (Area = 18000 cm^3)



Location of *in situ* Study site at the Lagos Lagoon

Table 1: Genotypic identities of lignocellulose-degrading bacterial isolates from amplified sequences of 16S rDNA fragment

Bacterial strain	Tentative Identity	GeneBank accession number	Closest relative	% identity	GeneBank accession number
AOB	*Streptomyces albogriseolus*	KF977548	*Streptomyces albogriseolus* ABRIIW EA1145	100	GQ925802.1
BOB	*Streptomyces aureus*	KF977549	*Streptomyces aureus* 3184	99	EF371429.1
COB	*Streptomyces coelicolor*	KF977550	*Streptomyces coelicolor* WBF-16	100	HQ848084.1
DOB	*Streptomyces albus*	KF977551	*Streptomyces albus* NBRC 13689	95	AB249915.1
EOB	*Streptomyces pseudogriseolus*	KF977552	*Streptomyces pseudogriseolus* S41	98	HQ850412.1
FOB	*Bacillus bataviensis*	KF977553	*Bacillus bataviensis* LMG 21832	98	AJ542507.1
NOB	*Bacillus megaterium*	KF977554	*Bacillus megaterium* L2S3	98	EU221414.1
OOB	*Bacillus* sp.	KF977555	*Bacillus* sp. LS29	97	FJ937891.1
ROB	*Paenibacillus* sp	KF977556	*Paenibacillus* sp. HQ-5	99.8	AM162315.1

Table 3: Weight losses and bioremoval of lignin and carbohydrates components of wood residues under *in situ* conditions.

Organism	Weight loss		lignin degraded		(%) Carbohydrate utilization	
	%	g $d^{-1}cm^{-3}$	%	g $d^{-1}cm^{-3}$	%	g $d^{-1}cm^{-3}$
Streptomyces griseoflavus AOB KF977548	59	1.171×10^{-4}	58.7	1.28×10^{-5}	81	2.089×10^{-7}
Streptomyces aureus BOB KF977549	42.4	8.41×10^{-5}	77.48	1.69×10^{-5}	79	2.038×10^{-7}
Streptomyces coelicolor COB KF977550	55.2	1.095×10^{-4}	79.9	1.74×10^{-5}	80	2.063×10^{-7}
Streptomyces albus DOB KF977551	50.2	9.96×10^{-5}	81.7	1.78×10^{-5}	80	2.063×10^{-7}
Streptomyces pseudogriseolus EOB KF977552	28.3	5.62×10^{-5}	77.4	1.69×10^{-5}	69	1.780×10^{-7}
Bacillus bataviensis FOB KF977553	59.2	1.175×10^{-4}	75.02	1.64×10^{-5}	85	2.192×10^{-7}
Bacillus megaterium NOB KF977554	52.2	1.036×10^{-4}	82.52	1.80×10^{-5}	81	2.089×10^{-7}
Bacillus sp. OOB KF977555	46.3	9.19×10^{-5}	52.49	1.15×10^{-5}	58	1.496×10^{-7}
Paenibacillus sp. ROB KF977556	46.9	9.31×10^{-5}	52.33	1.14×10^{-5}	67	1.728×10^{-7}

Table 4: Summary of laboratory degradation studies of isolates on wood residues

Organism	Weight loss		lignin degraded		(%) carbohydrate utilization	
	%	g $d^{-1}cm^{-3}$	%	g $d^{-1}cm^{-3}$	%	g $d^{-1}cm^{-3}$
Streptomyces albogriseolus AOB KF977548	49.6	5.9×10^{-5}	47.51	1.037×10^{-6}	58	4.488×10^{-8}
Streptomyces aureus BOB KF977549	45.4	5.4×10^{-5}	44.98	9.817×10^{-7}	50	3.869×10^{-8}
Streptomyces coelicolor COB KF977550	46	5.48×10^{-5}	44.99	9.821×10^{-7}	48	3.714×10^{-8}
Streptomyces albus DOB KF977551	50.5	6.01×10^{-5}	44.99	9.821×10^{-7}	59	4.565×10^{-8}
Streptomyces pseudogriseolus EOB KF977552	49.7	5.92×10^{-5}	47.51	1.037×10^{-4}	50	3.869×10^{-8}
Bacillus bataviensis FOB KF977553	50.3	5.99×10^{-5}	50	1.091×10^{-6}	52	4.024×10^{-8}
Bacillus megaterium NOB KF977554	51.3	6.11×10^{-5}	52.49	1.146×10^{-6}	58	4.488×10^{-8}
Bacillus sp. OOB KF977555	25.95	5.19×10^{-3}	22.49	4.909×10^{-7}	28	2.167×10^{-8}
Paenibacillus sp. ROB KF977556	31.9	3.8×10^{-5}	22.49	4.909×10^{-7}	40	3.095×10^{-8}

Table 2: Physico-chemical properties of Lagos lagoon water at the *in situ* experimental site. (Jan – Dec 2011)

PARAMETER	RANGE	MEAN VALUES	LOCAL STANDARD	W.H.O STANDARD
Temperature (°C)	25 – 30	26.7500	< 40	< 40
pH	6.53 – 7.7	7.1325	6.0 – 9.0	6.0– 9.0
Electrical conductivity (μScm^{-1})	436.0 – 35600.0	11737.75	2000	2000
TDS (mg l^{-1})	162 – 17,700	4907.083	2000	2500
Acidity	8.0 – 48.0	21.50	N/A	–
Calcium Hardness (mg l^{-1})	52 – 2400	621.5	N/A	–
Chloride (mg l^{-1})	204 – 1600	763.5	600	< 1000
Dissolved Oxygen (mg l^{-1})	2.7 – 37.0	7.0333	8 –10	8 – 10
Nitrate (mg l^{-1})	1.25 – 100.2	17.331	20	< 50
Phosphate (mg l^{-1})	00.5 – 6.81	2.6716	5.0	–
Nitrite (mg l^{-1})	1.25 – 100.2	28.885	N/A	–
Salinity (‰)	0.36 – 24.60	8.5083	600	600
BOD_5 (mg l^{-1})	3.0 – 15.0	7.47	50	30–300
COD (mg l^{-1})	5.0 – 25.0	12.49	40	< 200
Copper ($\mu g\ g^{-1}$)	0.27 – 86.20	22.64	< 1.0	< 1.0
Lead ($\mu g\ g^{-1}$)	0.06 – 3.18	1.39	0.05	< 1.0
Zinc ($\mu g\ g^{-1}$)	3.84 – 136.50	59.86	1.00	-

N/A [a] = Not available ; – [b] = Varied between countries

Products released from degradation of sawdust by *Streptomyces pseudogriseolus* strain EOB with time usimg HPLC (mg/g)

Time (wks)	Ferulic acid	Vanillic acid	Arabinose	Ethanol	HMF	furfural	Xylitol	Glucose
Uninoculated control	0	0	0	0	0	0	0.15	0.67
Week 1	0.0204	0	0	0.55	0.03	0.03	0.26	0.56
Week 2	0.0207	0.015737	0	0.30	0.30	0	0.17	0.42
Week 3	0.0205	0	0	0.96	0.10	0	0.14	0.30
Week 4	0.0204	0	0.08	0.19	0.04	0	0.13	0.27
Week 5	0.0207	0	0.01	0.20	0.03	0	0.08	0.22

Summary of percentage degradation of lignin-related compounds by Actinomycetes during substrate specificity testing

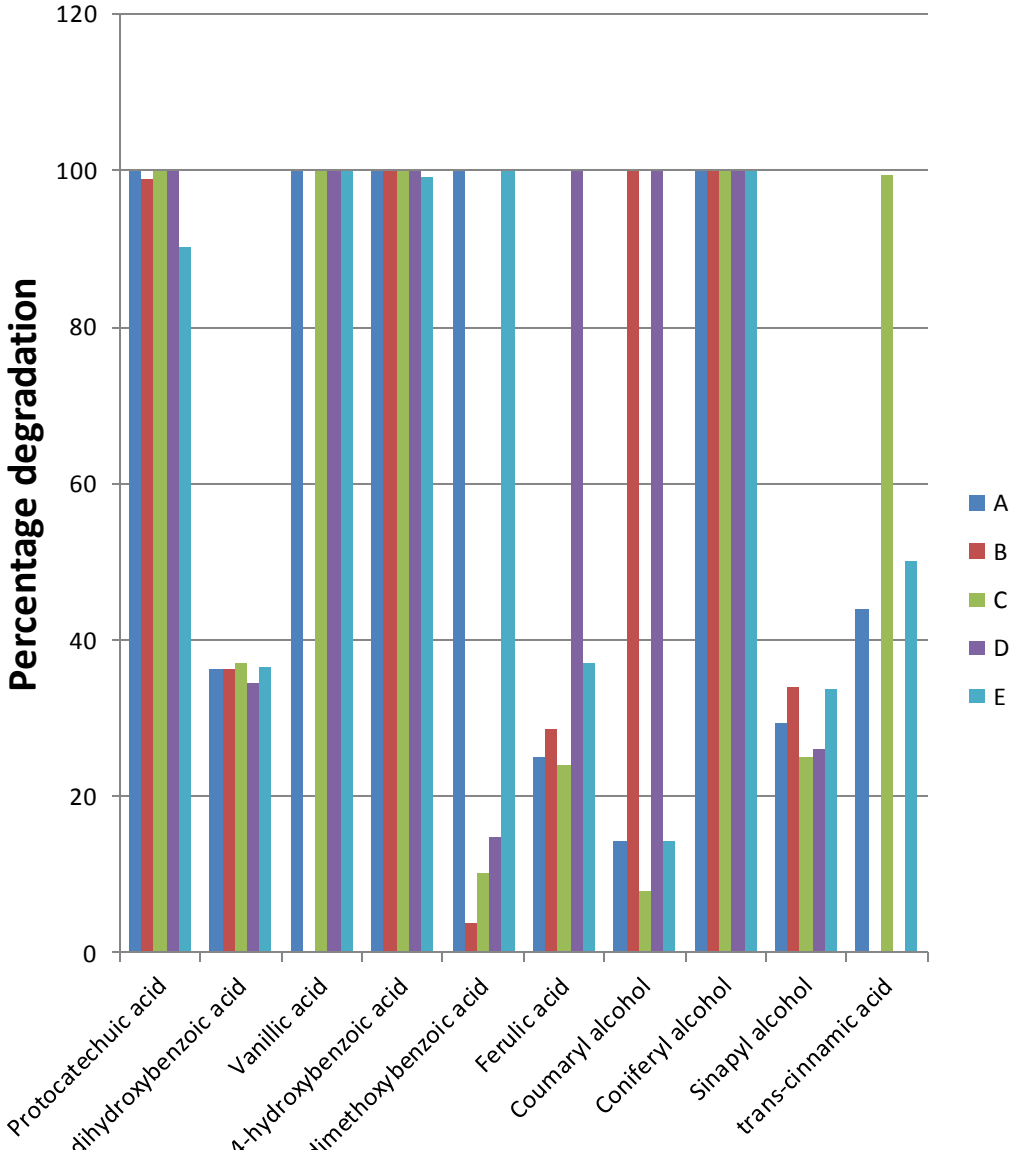
A-*Streptomyces albogriseolus* strain AOB

B-*Streptomyces aureus* strain BOB

C-*Streptomyces coelicolor* strain COB

D-*Streptomyces albus* strain DOB

E-*Streptomyces pseudogriseolus* strain EOB



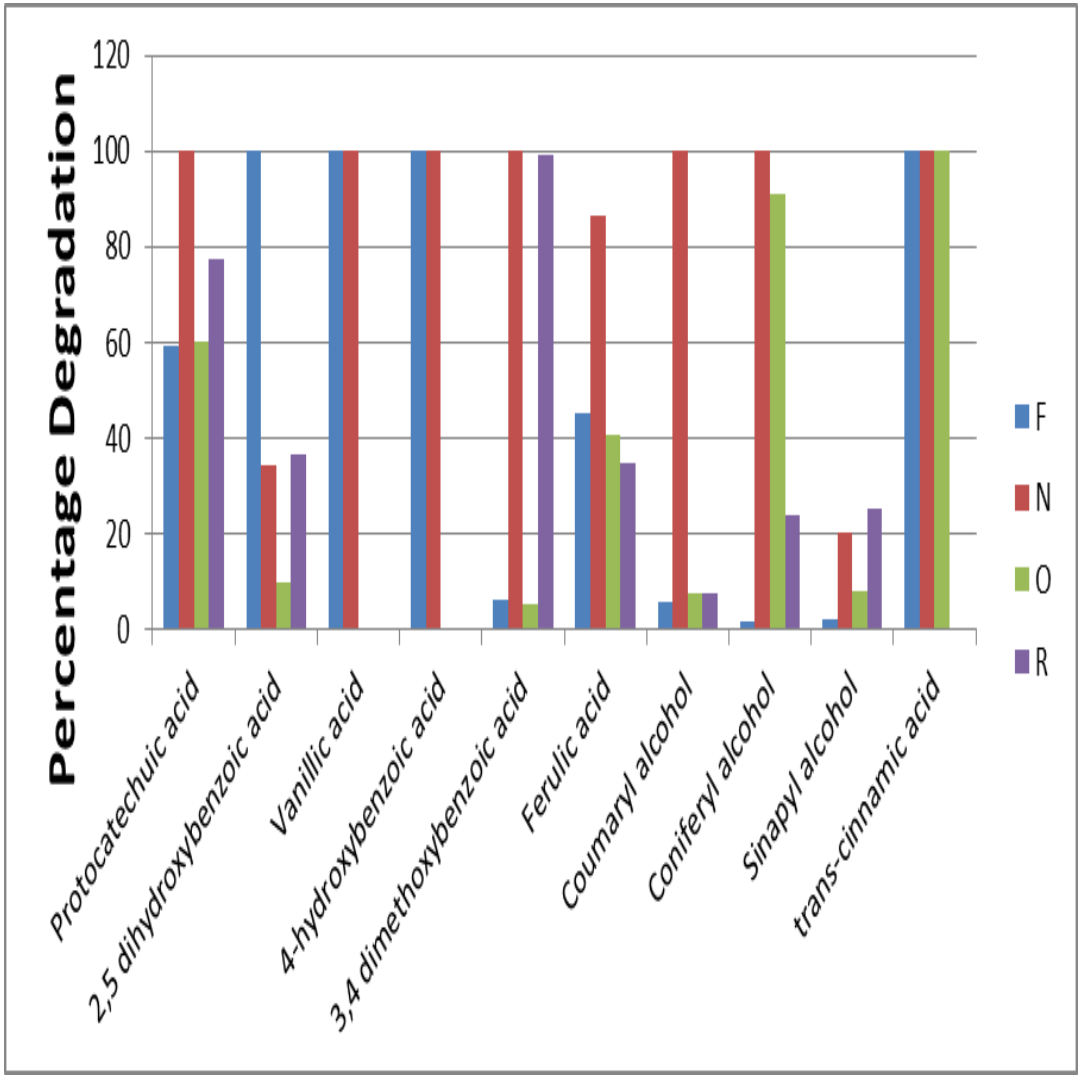
Percentage degradation of lignin-related compounds by *Bacillus* and *Paenibacillus* during substrate specificity testing.

F - *Bacillus bataviensis* strain FOB,

N - *Bacillus megaterium* strain NOB,

O- *Bacillus* sp. strain OOB,

R - *Paenibacillus* sp strain ROB



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Assessment of bacterial degradation of lignocellulosic residues (sawdust) in a tropical estuarine microcosm using improvised floating raft equipment

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ABSTRACT

In situ and laboratory studies were carried out to determine the ability of bacterial strains isolated from a tropical lagoon to degrade lignin and carbohydrate components of sawdust, with a view to abating the impact of sawdust pollution on these ecosystem. A floating raft system was designed and fabricated to carry out the *in situ* biodegradation studies over a period of 24 weeks. Nine bacterial strains identified by 16S rRNA gene sequencing as species of *Streptomyces*, *Bacillus* and *Paenibacillus* isolated from the lagoon were used as seed organisms. In the *in situ* study, 59.2% of sawdust was depleted at the rate of 1.075×10^{-6} g d^{-1} cm^{-3} by the bacterial isolates, whereas the lignin component of the sawdust decreased by up to 82.5% at the rate of 3.80×10^{-5} g d^{-1} cm^{-3}. The maximum decrease in carbohydrate content was 85% at the rate of 2.932×10^{-7} g d^{-1} cm^{-3}. In a similar experiment under laboratory conditions, total weight losses ranging from 26 to 51% in the wood residues were observed.

Peroxidase Activity for *Streptomyces* species

KEY

A-*Streptomyces*

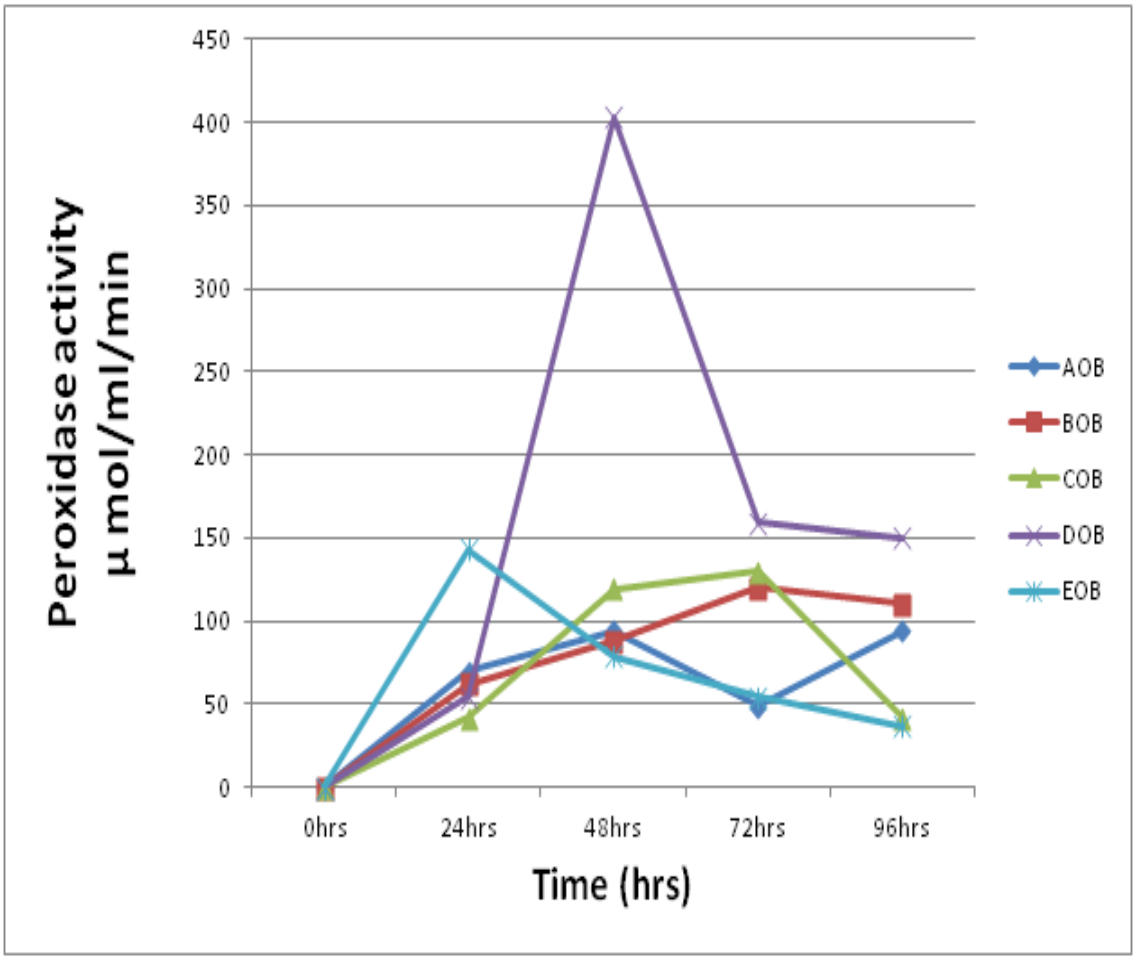
*albogriseolus*strainAOB

B-*Streptomyces aureus*strain BOB

C-*Streptomyces coelicolor*strain COB

D-*Streptomyces albus*strain DOB

E-*Streptomyces pseudogriseolus* strain EOB



Peroxidase activity for *Bacillus* spp.

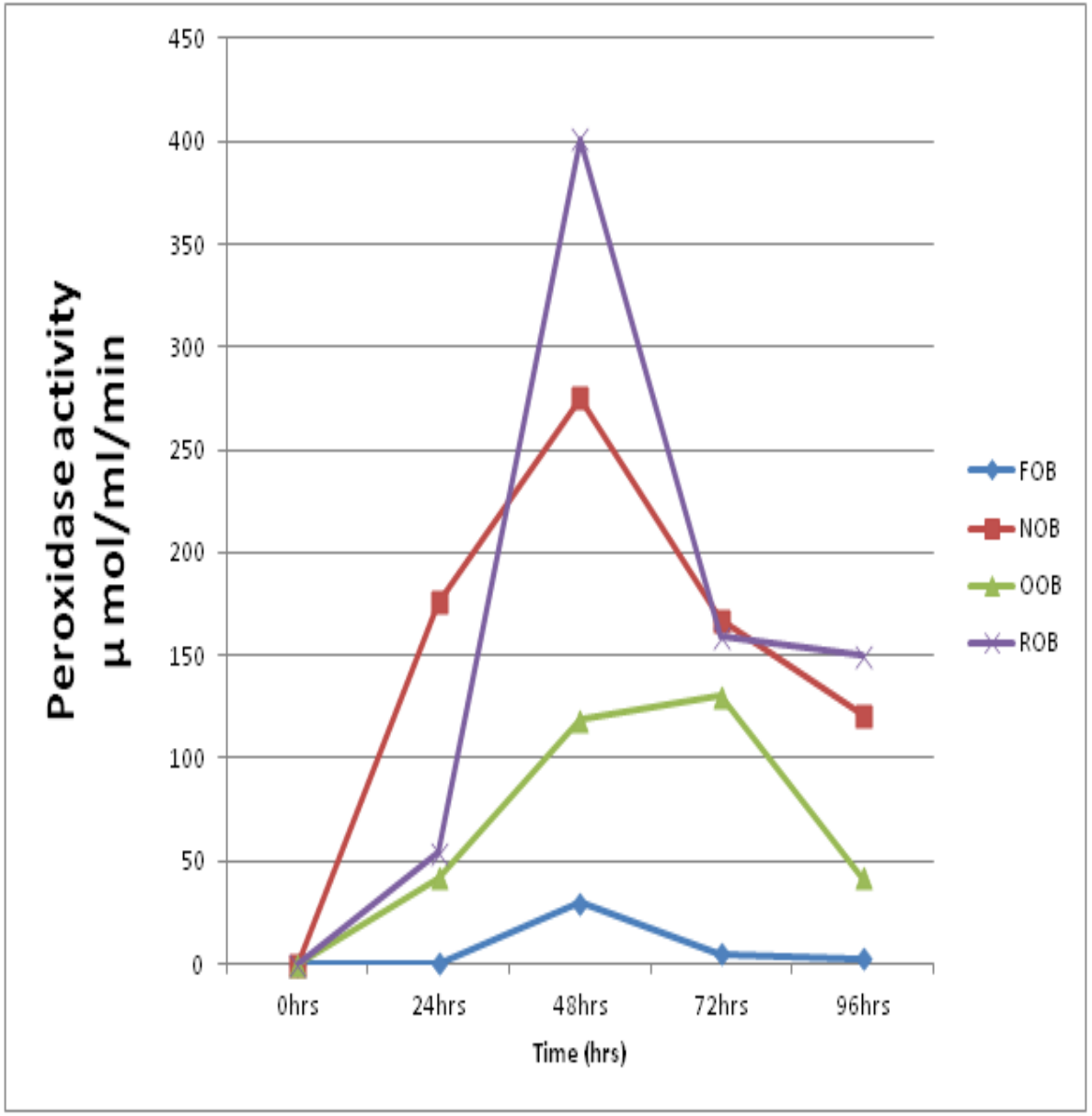
KEY

F- *Bacillus bataviensis* strain FOB

N-*Bacillus megaterium* strain NOB

O - *Bacillus* sp. strain OOB

R - *Paenibacillus* sp. strain ROB



Identification of Protocatechuate 3,4-dioxygenase gene

	Closest Relative	**Gene bank accession No**	**% ID**	**Description**
Streptomyces albogriseolus strain AOB	*Streptomyces* sp. 2065	AF-10938662	88	Protocatechuic acid catabolic gene cluster
Streptomyces aureus strain BOB	*Streptomyces coelicolor* $A_3(2)$	AL-939128.1	88	Protocatechuic acid catabolic gene cluster
Streptomyces albus strain DOB	*Streptomyces* sp 2065	AF-1093866	88	Protocatechuic acid catabolic gene cluster
Streptomyces pseudogriseolus strain EOB	*Streptomyces* sp 2065	AF-1093866	100	Protocatechuic acid catabolic gene cluster

Identification of demethylase gene clusters

Bacterial Strain	Closest Relative	Gene bank Accession Number	% ID	Description
Streptomyces albogriseolus strain AOB	*Streptomyces coelicolor* $A_3(2)$	AL-939111.1	80	-
Streptomyces aureus strain BOB	*Streptomyces griseoflavus*	ZP-07313543.1	93	Branched chain amino acid binding protein
Streptomyces coelicolor strain COB	*Streptomyces griseoflavus*	ZP-07313543.1	96	Branched chain amino acid protein
Bacillus megaterium strain NOB	*Acidovorax* sp. Kks102.	CP-003872.1	85	Signal transduction histidine kinase
Bacillus sp. strain OOB	*Rhodococcus equi* 1035	ZP – 11107696.1	56	Acetyl-coA dehydrogenase

Identification of cellulase gene clusters

Bacterial strain	Closest relative	Gene bank accession No	% ID	Description	Search engine
Streptomyces albogriseolus strain AOB	*Streptomyces griseoflavus*	ZP-07314033.1	86	Polyketide cyclase/dehydrase super family	BLAST X
Streptomyces aureus strain BOB	*Streptomyces griseoflavus*	NZ-GG657758.1	83	Polyketide cyclase/dehydrase	BLAST
Streptomyces coelicolor strain COB	*Rhodococcus equi* ATCC33707	ZP-08153042.1	99	LYSR family transcriptional regulator	BLAST X
Streptomyces albus strain DOB	*Streptomyces albus*	ZP-06590404.1	98	Glutamine synthetase super family	BLAST X
Streptomyces pseudogriseolus strain EOB	*Streptomyces ghanaensis*	NZ-D5999641.1	79	NS	BLAST
Bacillus megaterium strain NOB	*Rhodococcus equi* ATCC 33707	ZP-073114933.1	83	Polyketide cyclase/dehydrase super family	BLAST X
Bacillus sp. strain OOB	*Branchiostoma floridae*	XP_002600744.1	43	Hypothetical protein	BLAST

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KRAFT LIGNIN DEGRADATION BY AUTOCHTONOUS *STREPTOMYCES* STRAINS ISOLATED FROM A TROPICAL LAGOON ECOSYSTEM

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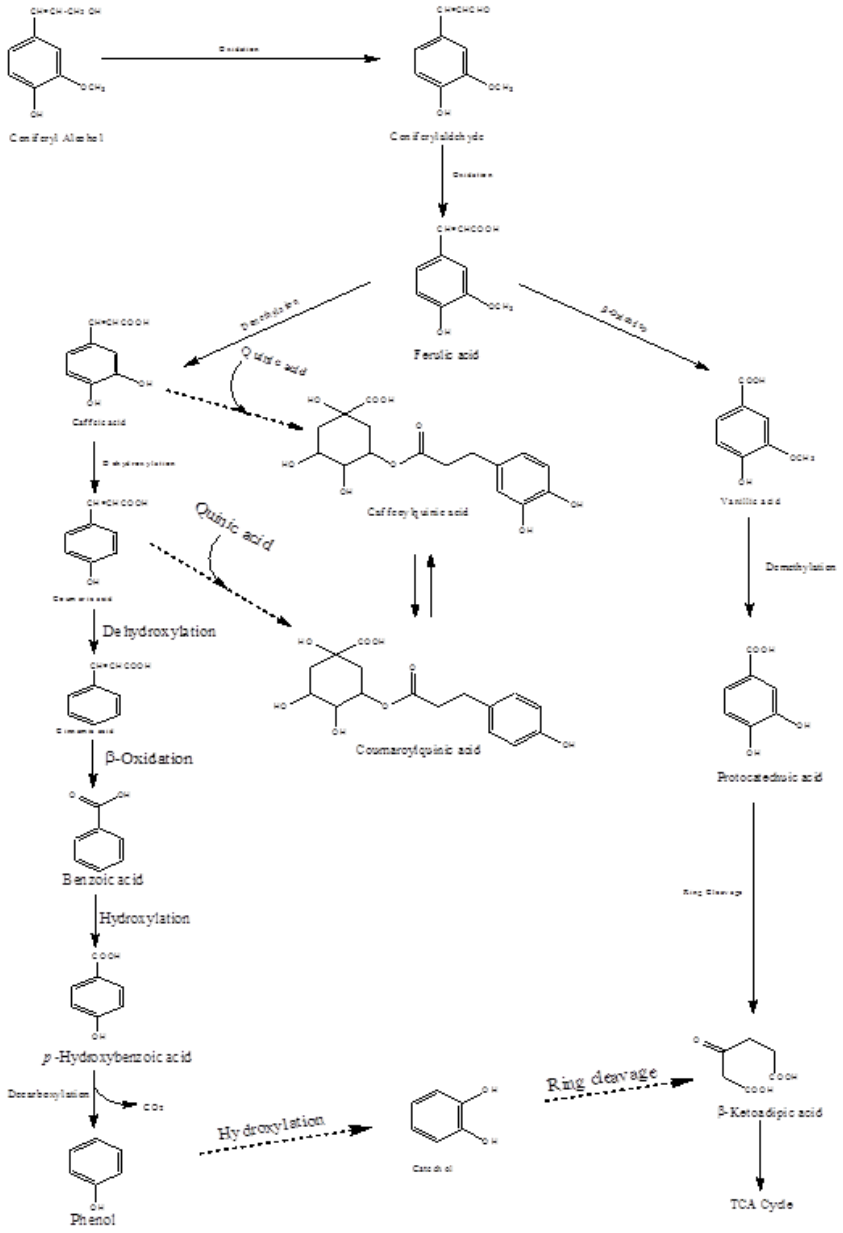
ABSTRACT

Kraft lignin contributes to the toxicity of the pulping plant effluent and is known to resist microbial treatment.The lignin component must be removed from lignocellulose biomass to enhance the release of fermentable sugars for the production of biofuel and other value-added end products. Lignin-degrading bacteria provide an advantage due to their ease of isolation,wider tolerance of environmental conditions and genetic manipulations compared with their fungal counterparts. There is no documented evidence on the degradation of kraft lignin by bacteria in the tropical estuarine ecological niche in Nigeria. Bacterial growth and assessment of kraft-

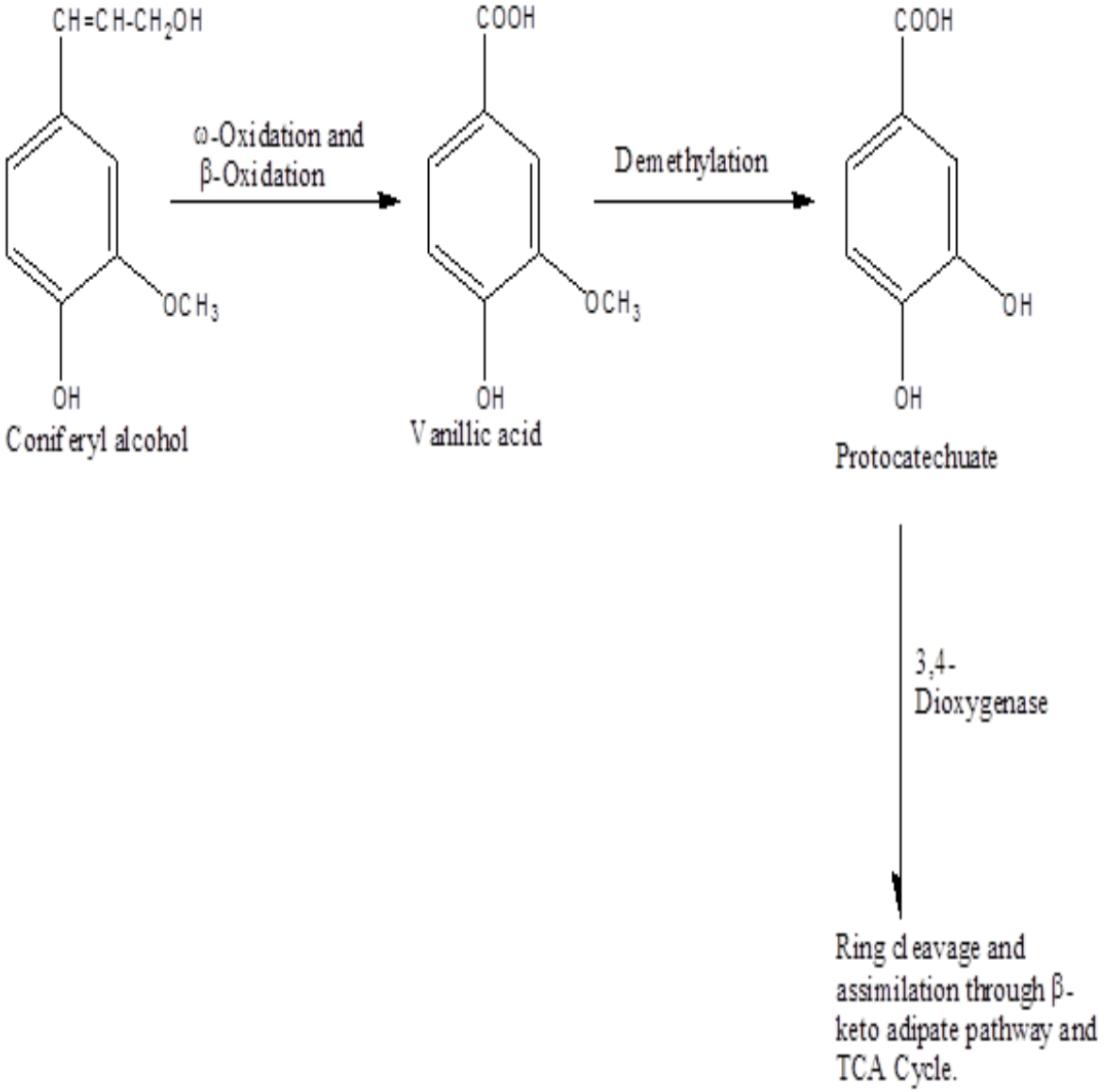
Identification of metabolic products of degradation from coniferyl alcohol using HPLC-MS, electrospray ionization and UV spectrophotometry

Compound	RT (min)	ESI^-	ESI^+	MW(g)	UV (nm)
Coniferyl alcohol	15.30		215/213	180	262, Ca. 300sh
Ferulic Acid	17.53	193		194	322, 286sh,232
Coniferylaldehyde	18.062	177		178	322, 323 sh 233
Caffeoylquinic acid	22.895	353		354	325,223 286sh
Caffeoylquinic aldehyde	23.195	337		338	345,229 Ca 285 sh
5-0-ρ-Coumarylquinic acid	23.195	337		338	345,229 Ca 285 sh
Coumaric acid	18.062	162		164	340, Ca 300 sh 236
Caffeic acid	17.52	178		180	322,286 sh, 233
Benzoic acid	15.74	121		122	262, sh 300
5-0-p-Cinnamic acid	22.89	323	322	322	325, 287 sh
p-Hydroxybenzoic acid	20.70	137		138	262 Ca 300 sh
t-Cinnamic acid	17.52	149	149	148	322,286 sh, 233
Phenol	15.74		94	94	262, sh 300

Proposed pathway of coniferyl alcohol degradation by *Streptomyces coelicolor* strain COB



Proposed pathway of coniferyl alcohol degradation by *Bacillus megaterium* strain NOB.



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The degradation of coniferyl alcohol and the complementary production of chlorogenic acids in the growth culture of *Streptomyces albogriseolus* KF977548 isolated from decaying wood residues

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ABSTRACT

Coniferyl alcohol is one of the major precursors of lignin; the most abundant aromatic compound and a natural resource currently receiving attention because of the value-added metabolites resulting from its degradation. Growth study of *Streptomyces albogrtseolus* KF977548 (strain AOB) isolated from decaying wood residues in a tropical estuarine ecosystem was carried out using coniferyl alcohol as a sole carbon source. Cell growth and metabolite production were monitored at 24 h interval by dry weight measurements and HPLC, LC–MS-DAD analyses. Biochemical and PCR assays were carried out to detect the major catabolic enzymes of interest. Strain AOB utilized coniferyl alcohol completely within 72 h ($\mu = 0.204\ h^{-1}$, $T_d = 3.4$ h). Laccase and peroxidase were released into the growth medium up to 0.099 and 98 μmol/mL respectively. Protocatechuate 3, 4-dioxygenase and demethylase were detected in the genome whilst *ortho*-adipate pathway was clearly indicated. Growth on coniferyl alcohol or caffeic acid as mono substrates resulted in the production of secondary metabolites identified by HPLC–MS as 1-caffeoylquinic and 3,4,5-tricaffeoylquinic acids, known as chlorogenic acids, in the culture medium. The microbial production of chlorogenic acids from a lignin-related substrate base by strain AOB could arouse a plausible biotechnological process.

MICROBIAL DIVERSITY OF DECAYING WOOD COMMUNITY

1. Terminal restriction fragment length polymorphism (T-RFLP) (Tiedje, 2002)

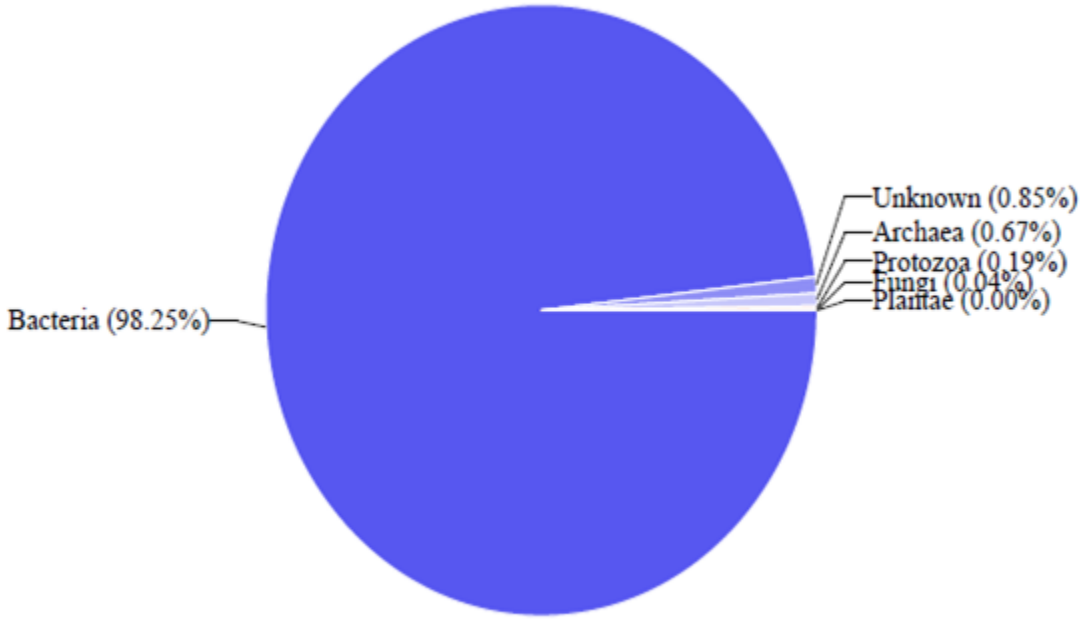
(OARDC, Ohio State University, Wooster, Ohio)

There were about 103 named microbial genera and over 3,000 uncultured microorganisms associated with decaying wood community

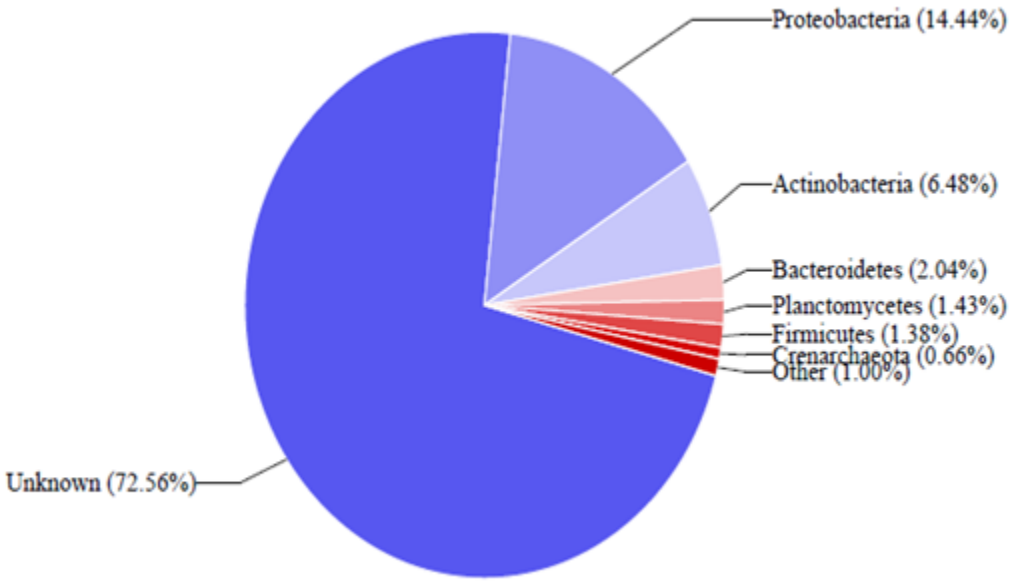
2. Illumina sequencing of metagenomic DNA of decomposing wood wastes in the Lagos lagoon, Nigeria.

(Inqaba Biotechnical Ltd., Hatfield, Pretoria, South Africa).

Taxonomic distribution based on kingdom



Taxonomic distribution based on phylum

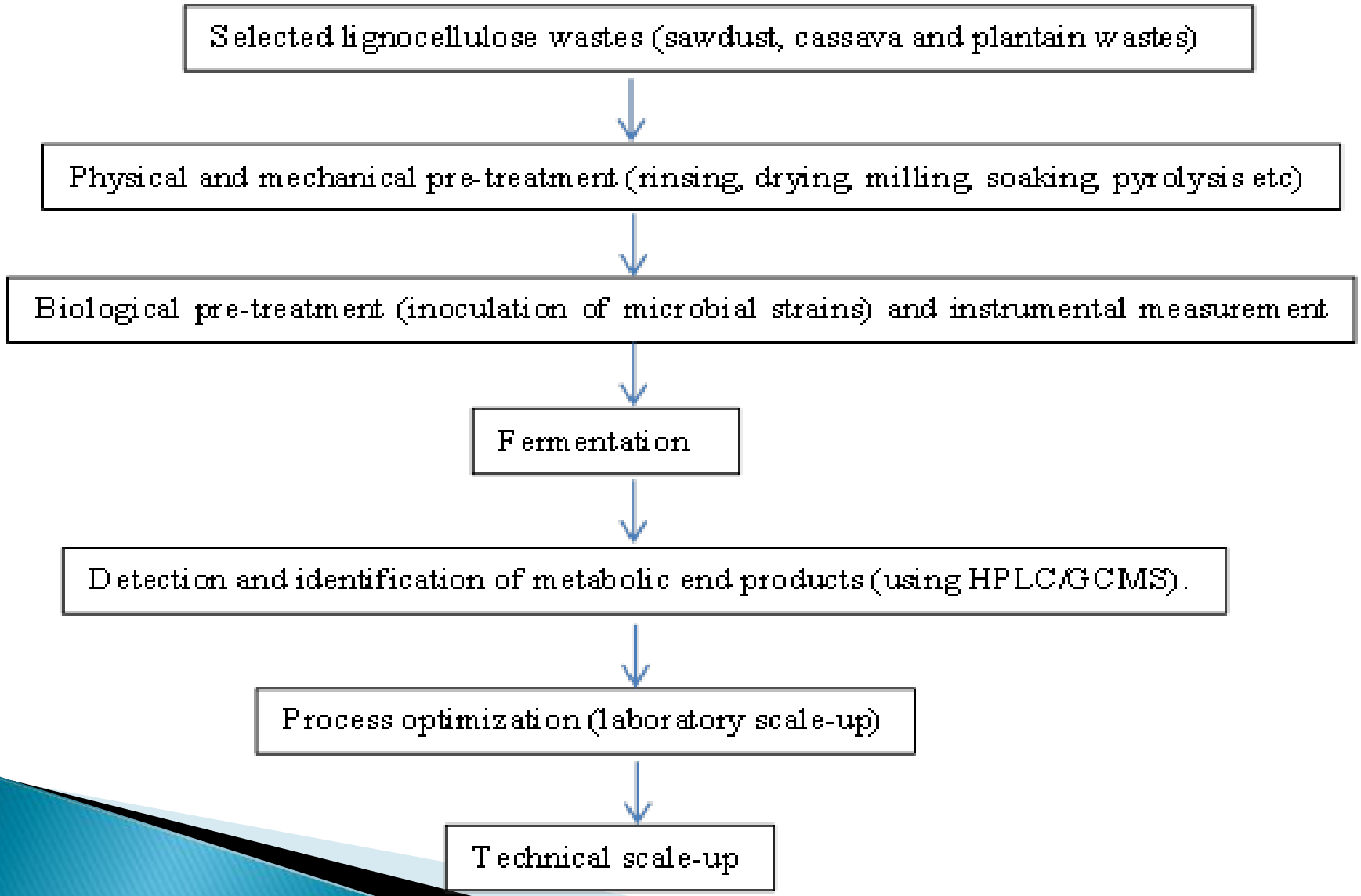


- Preliminary Production of biochemicals (bioethanol and organic acids of industrial importance) from renewable agricultural wastes for possible biotechnological applications

Renewable substrates ("Wastes") available in Nigeria

- Sawdust, wood shavings, wood residues
- Plantain peels
- Cassava peels
- Cassava waste water
- Sugarcane bagasse
- Grasses
- Corn cobs
- Rice husks/straws

Proposed fermentation process



Methodology

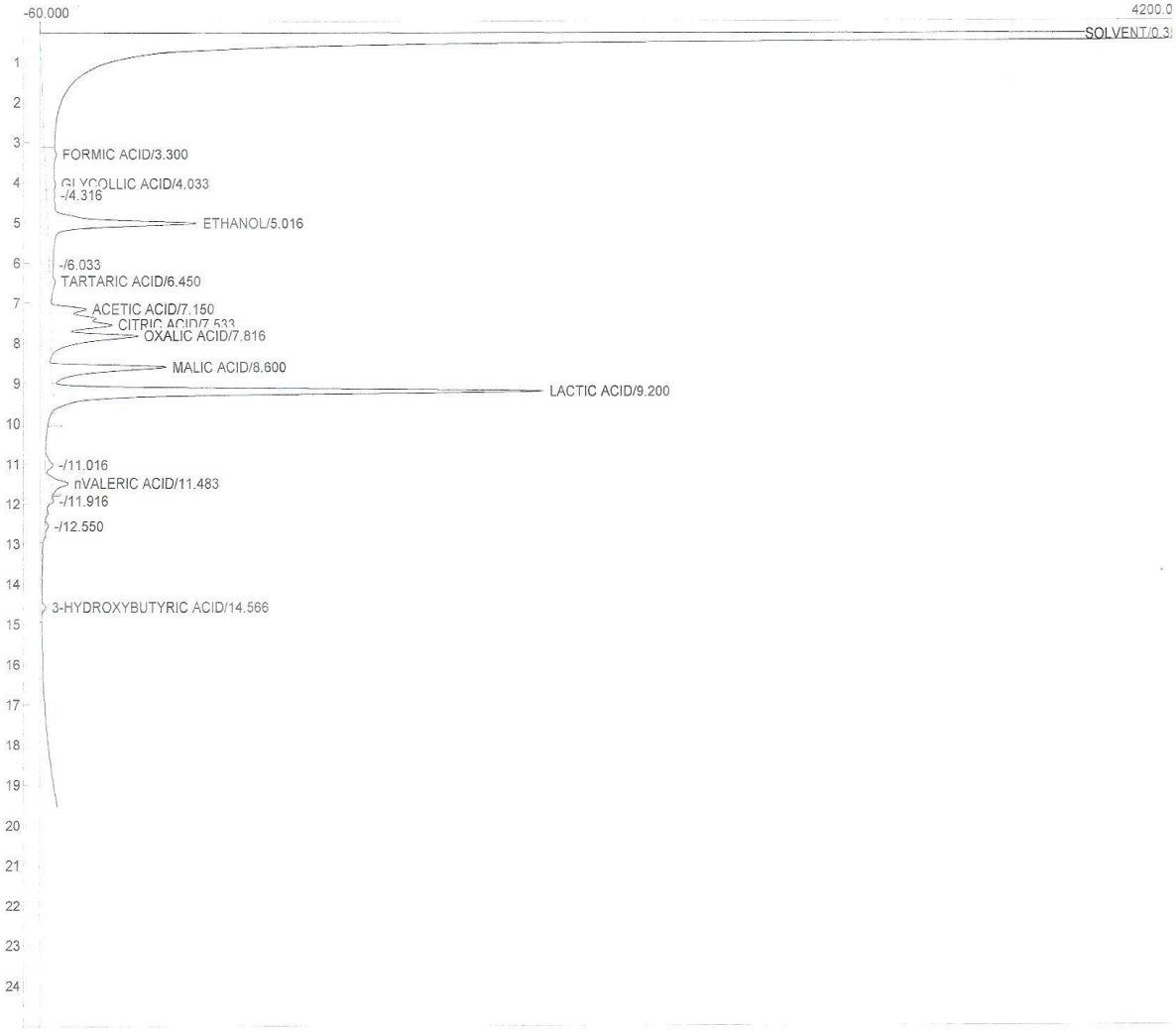
- Preparation of wastes- (rinsing, drying, grinding etc.)
- Growth of the test strains were performed under aerobic batch (submerged) fermentation.
- 2 ml of each organism was grown in separate Erlenmeyer flasks (250 ml) containing mineral salts medium (100 ml, pH 7.2) which was supplemented with 1.0 g (w/v) substrate (sugarcane bagasse or plantain peel) as sole carbon source.
- A flask containing substrate and medium but without organism serves as the control.
- The flasks were 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
- The metabolic products were determined using GC-FID

Gas chromatographic profiles for the 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

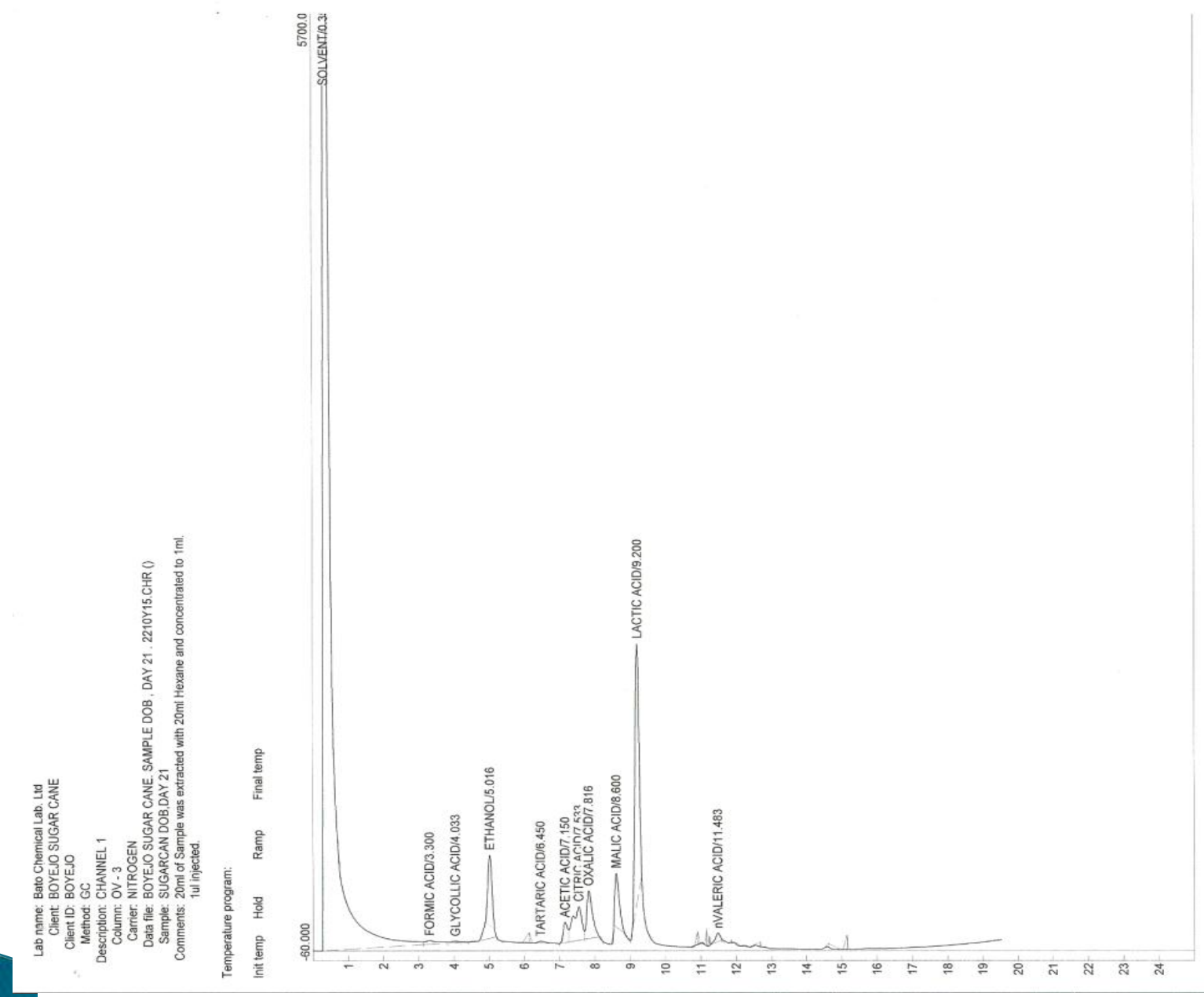
Lab name: Bato Chemical Lab. Ltd
Client: BOYEJO SUGAR CANE
Client ID: BOYEJO
Method: GC
Description: CHANNEL 1
Column: OV - 3
Carrier: NITROGEN
Data file: BOYEJO SUGAR CANE. SAMPLE COB , DAY 21 . 2210Y15.CHR ()
Sample: SUGARCAN COB,DAY 21
Comments: 20ml of Sample was extracted with 20ml Hexane and concentrated to 1ml.
1ul injected.

Temperature program:

Init temp	Hold	Ramp	Final temp



Component	Retention	Area	Height	External	Units
SOLVENT	0.350	96619.9080	5682.236	0.0000	%
FORMIC ACID	3.300	1517.8650	43.018	0.0000	
GLYCOLLIC ACID	4.033	747.6520	34.742	0.0000	
ETHANOL	5.016	8045.8830	546.478	0.0000	
TARTARIC ACID	6.450	510.7750	19.515	0.0000	
ACETIC ACID	7.150	1139.2510	129.776	0.0000	
CITRIC ACID	7.533	4041.0130	221.694	0.0000	
OXALIC ACID	7.816	4293.5395	315.423	0.0000	
MALIC ACID	8.600	4854.7350	423.663	0.0000	
LACTIC ACID	9.200	18308.8960	1793.565	0.0000	
nVALERIC ACID	11.483	747.2620	62.680	0.0000	
3-HYDROXYBUTYRIC	14.566	166.3655	15.596	0.0000	
		140993.1450		0.0000	



Gas chromatographic profiles for generation of diverse biochemicals from medium containing sugarcane bagasse during aerated batch culture of *Streptomyces albus* strain DOB by day 21.

Table 6: Ethanol generation (mg/L) with time by strains COB and DOB in medium containing sugarcane bagasse as sole carbon and energy source

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

Table 7: Optical density and pH levels of the growth medium of *Streptomyces albus* strain DOB KF977551 on plantain peel

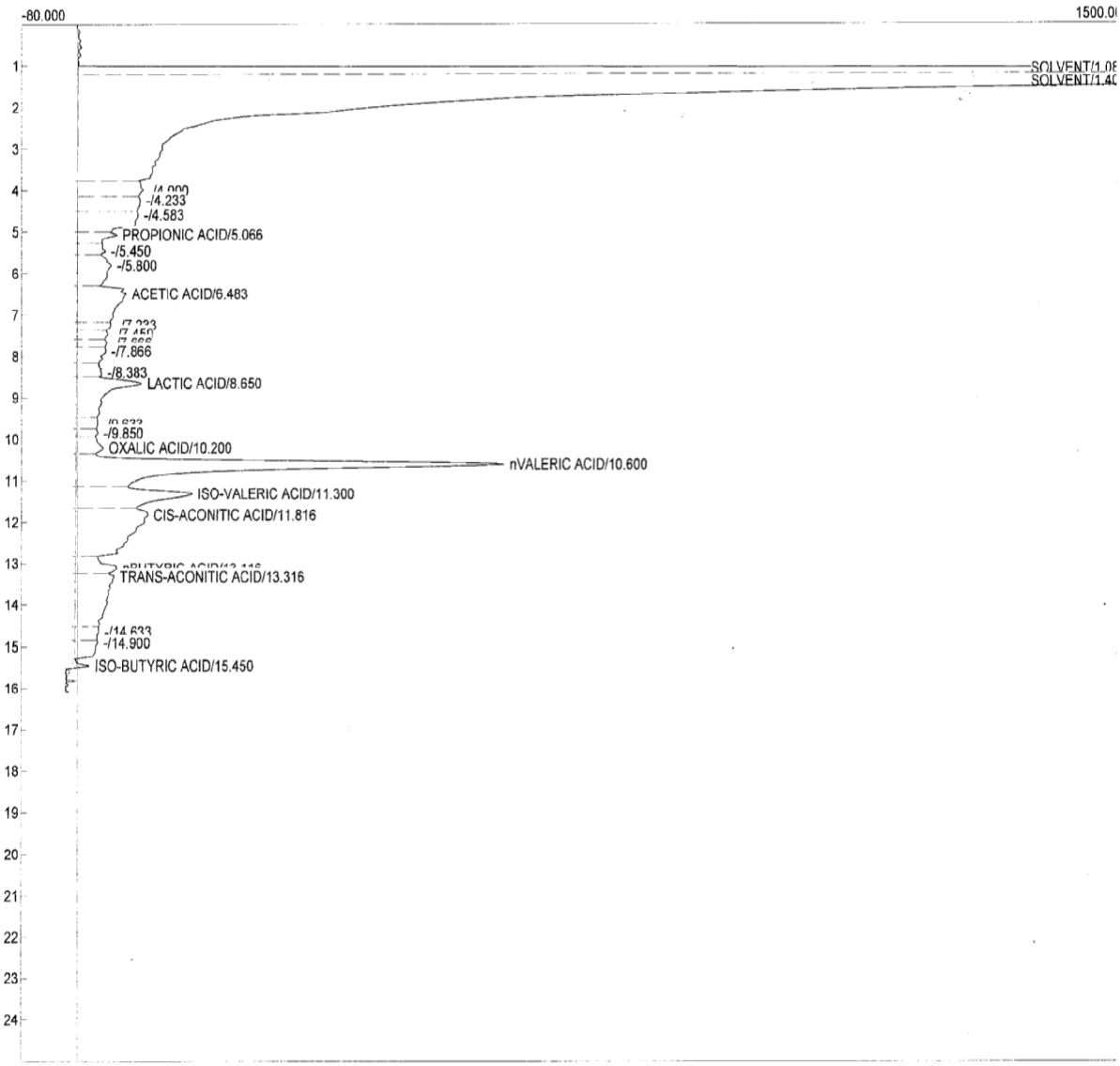
DAYS	O.D (nm)	pH
O	o.o8	7.2
3	1.03	6.08
6	1.11	6.54
9	1.07	6.41
12	2.20	6.48
15	1.13	6.53
18	1.07	6.64
21	1.11	6.87

Gas chromatographic profiles for the generation of diverse biochemicals from the medium containing plantain peel as the sole carbon and energy source during aerated batch culture of *Streptomyces albus* strain DOB KF977551 by day 21

Lab name: Bato Chemical Lab. Ltd
Client: BOYEJO PLANTAIN
Client ID: BOYEJO
Method: GC
Description: CHANNEL 1
Column: OV - 3
Carrier: NITROGEN
Data file: BOYEJO PLANTAIN. SAMPLE DOB , DAY 21 . 2410Y15.CHR ()
Sample: PLANTAIN DOB , DAY 21
Comments: 20ml of Sample was extracted with 20ml Hexane and concentrated to 1ml.
1ul injected.

Temperature program:

Init temp	Hold	Ramp	Final temp



R

Component	Retention	Area	Height	External	Units
SOLVENT	1.083	17207.9875	1912.104	0.0000	%
SOLVENT	1.400	73647.3130	1966.479	0.0000	%
PROPIONIC ACID	5.066	699.1790	56.334	0.0000	
ACETIC ACID	6.483	3054.6510	70.287	0.0000	
LACTIC ACID	8.650	2750.1345	92.758	0.0000	
OXALIC ACID	10.200	807.3250	38.528	0.0000	
nVALERIC ACID	10.600	9655.9525	610.851	0.0000	
ISO-VALERIC ACID	11.300	3760.0370	165.835	0.0000	
CIS-ACONITIC ACID	11.816	5691.0310	103.200	0.0000	
nBUTYRIC ACID	13.116	1131.6325	59.367	0.0000	
TRANS-ACONITIC AC	13.316	3462.9550	55.385	0.0000	
ISO-BUTYRIC ACID	15.450	144.6870	26.942	0.0000	
		122012.8850		0.0000	

TABLE 8: Diversity and quantities (mg/L) of organic acids detected in the growth medium of *Streptomyces albus* strain DOB KF977551 using plantain peel as the sole carbon source

METABOLITES	DAY 0	DAY 9	DAY 21
Propionic acid	0.03	0.06	0.65
Acetic acid	0	0.94	2.86
Lactic acid	0	0.70	2.57
Oxalic acid	0	0.16	0.76
nVALERIC	0	6.24	9.04
Iso valeric	0	0.94	3.52
Cis aconitic acid	0	0.42	5.33
nButyric acid	0	0.31	4.93
Trans aconitic	0	0.86	3.24
Iso-butyric acid	0	0.13	0.13

Summary of findings

- *Streptomyces coelicolor* strain COB and *S. albus* strain DOB efficiently utilized sugarcane bagasse and plantain wastes for growth.

- The highest O.D of 2.20 nm was achieved by day 12 at pH 6.48 by strain DOB when grown on plantain peel

- 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 when grown on sugarcane bagasse .

- Strains COB and DOB yielded 43.08 and 28.80 mgL^{-1} of ethanol respectively from of sugarcane bagasse

- Various acids of industrial importance were generated from plantain and sugarcane bagasse wastes

Further works

- Optimization of processes for the production of biobased products,
- determination of the kinetics of degradation of the biomass components as well as the product formation,
- laboratory scale - up of the bio processes,
- modelling of the fermentative processes and technical scale-up
- Isolation and characterization of enzymes from metagenomic DNA

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