OUTLINES
Thursday 25th June

VANDENBOSSCHE Virginie (LCA) ~60min
Biomass pretreatment by extrusion and reactiv-extrusion

DE LEON Rizalinda (FETSL) ~60min
Bioethanol production from alkaline-pretreated sugarcane bagasse by consolidated bioprocessing using Phlebia sp.

Lunch time (pack lunch in Bio5)

Presentation of EAD8 (FAME) and
visit of experimental capacity ~60min

DE LEON Rizalinda ~30min
FETSL presentation to FAME Team

CAMELEYRE Xavier (LISBP) ~60min
Microbial valorization of hydrolysed or pretreated lignocellulosic biomass

LISBP LABORATOIRE D'INGÉNIERIE DES SYSTÈMES BIOLOGIQUES ET DES PROCÉDÉS
INRA SCIENCE & IMPACT — CNRS — INSA TOULOUSE — Université de Toulouse

Department of Chemical Engineering
University of the Philippines-Diliman

Ethanol and Biologically-Active Compounds from Sugarcane Bagasse

Le Duy Khuong
Rizalinda L. de Leon
Ryuichiro Kondo
To Kim Anh

Department of Chemical Engineering
University of the Philippines-Diliman

Substrate and Fermenting Fungi

Sugarcane Bagasse

- Obtained from Lam Son Company, Than Hoa City, Vietnam.
- Fibrous residue of mechanical extraction of sugarcane juice

- Used as substrate for producing enzymes, amino acids, drugs, ethanol, animal feed
- Lower ash content vs. rice/wheat straw

Phlebia sp. MG-60

- Marine fungus screened from mangrove stands (*MKFC40001: National Institute of Technology and Evaluation, NITE, Chiba, Japan*)
- Proven effective for lignin degradation with high selectivity
- Has been shown to have hydrolytic and fermentative ability.
- Stock cultures maintained on a potato dextrose agar slant at 4^{o}C in the dark.

Department of Chemical Engineering
University of the Philippines-Diliman

THIS SPECIFIC STUDY:

BIOETHANOL PRODUCTION FROM SUGARCANE BAGASSE (SCB) BY CONSOLIDATED BIOPROCESSING (CBP) USING *Phlebia* sp. MG-60

Pretreatment Methods

Pretreatment Method	Process Conditions	Reported Results
Mechanical Method	Normal milling, nano-sized milling	• Increase available contact surface between enzyme and substrate. • Reduced DP of cellulose & lignin. • Reduced crystalline index of cellulose
Hydrothermal Method	200-230 C, 15-60 minutes	• Can dissolve 80-90% hemicellulose • Open cellulosic vessel (increased matrix pore size) • Considerably dissolves lignin • Reduced crystallinity index of cellulose • Good dissolution of pentose • Low levels of toxic chemicals produced • Minimal substrate losses
Steam Explosion	Saturated steam at high P with sudden drop in P (40 bar for pure water, for several seconds to several minutes)	• Effective hydrolysis: 90% • Fermentation-Inhibitory side products (e.g., furfural)

Pretreatment Methods

Pretreatment Method	Process Conditions	Reported Results
Microwave	2.45 MHz, 400 W magnetron, constant stirring @ 900 rpm, followed by washing with water	• Accelerated reaction rate, better yields, uniform and selective heating, greater reproducibility of reactions. • Reduced process energy requirements.
Dilute Acid	H_2SO_4 0.25-7% (w/v), 15-240 min, 121 C, 1.5 kg/cm2 HCl (has environmental impact) H_3PO_4 (removes separation process), 1%, 60 min, 145 C	• Dissolves hemicellulose, leaving matrix of cellulose and lignin • By-products of oligosaccharide, acetic acid, yeast-toxins (furfural, hydroxylmethylfurfural) • 198 mg reducing sugar/g dried bagasse (H_3PO_4)
Alkaline	Ca(OH)2 0.8 to 1.2 g/10 g dried SCB, 90-120 C, 60 minutes NaOH: 0.1 g/g dried bamboo	• Dissolves most of the lignin and part of hemicellulose • Swelling cellulose microfibrils • Reduced DP and crystallinity index of cellulose • 689 mg reduced sugar/g $Ca(OH)_2$-treated dried bagasse • 568 mg reduced sugar/g NaOH-treated bamboo

Pretreatment Methods

Pretreatment Method	Process Conditions	Reported Results
Wet oxidation	Water with air (or oxygen) @ 185 C, 12 bar, 5 mins, acidic pH on sugarcane Water w N-methyl-N-oxit @ 130 C, 1 hour on sugarcane	• Lignin ozonized or oxidized to lower DP • Reduced lignin content by 40-50% • Dissolved hemicellulose • Low reducing sugar in liquid which mainly contains oligosaccharides of hemicellulose • 95% of cellulose converted to glucose after hydrolysis (using N-methyl-N-oxit)
Ammonia fiber explosion (AFEXP)	Concentrated liquid ammonia-water, high pressure, high T followed by sudden P-drop	
Catalyzed steam explosion	Catalyst: SO_2 or H_2SO_4, 150-200 C, 2% H2SO4, 5 min	• Completely dissolved hemicellulose • Increased hydrolytic effectiveness • Reduced production of chemicals (with H2SO4, but not with SO2)

Pretreatment Methods

Pretreatment Method	Process Conditions	Reported Results
Organosolv	Organic solvent (formic acid 90% v/v), atmospheric P, 80 min Dimethyl formamide	
Oganosolv + supercritical CO2	Alcohol with Supercritical CO2 @ 7MPa, 190 C, 105 min, 60% 1-butanol	• Decreasing pulp yield as alcohol chain length increased • High lignin removal (up to 94.5%) • Low selectivity (high polysaccharide loss) as alcohol content increased
Biological	Fungi, actinomyces	• Lignin removal • Low energy consumption • Slow hydrolysis rate

Physical

- Increased surface area, pore size, reduced DP & crystallinity
- Energy Cost
- Incomplete removal of lignin, loss of hemicellulose

Chemical

- High lignin removal, high reaction rates
- Energy cost + chemical cost + environmental cost
- Sugar losses & inhibitory by-products, corrosion

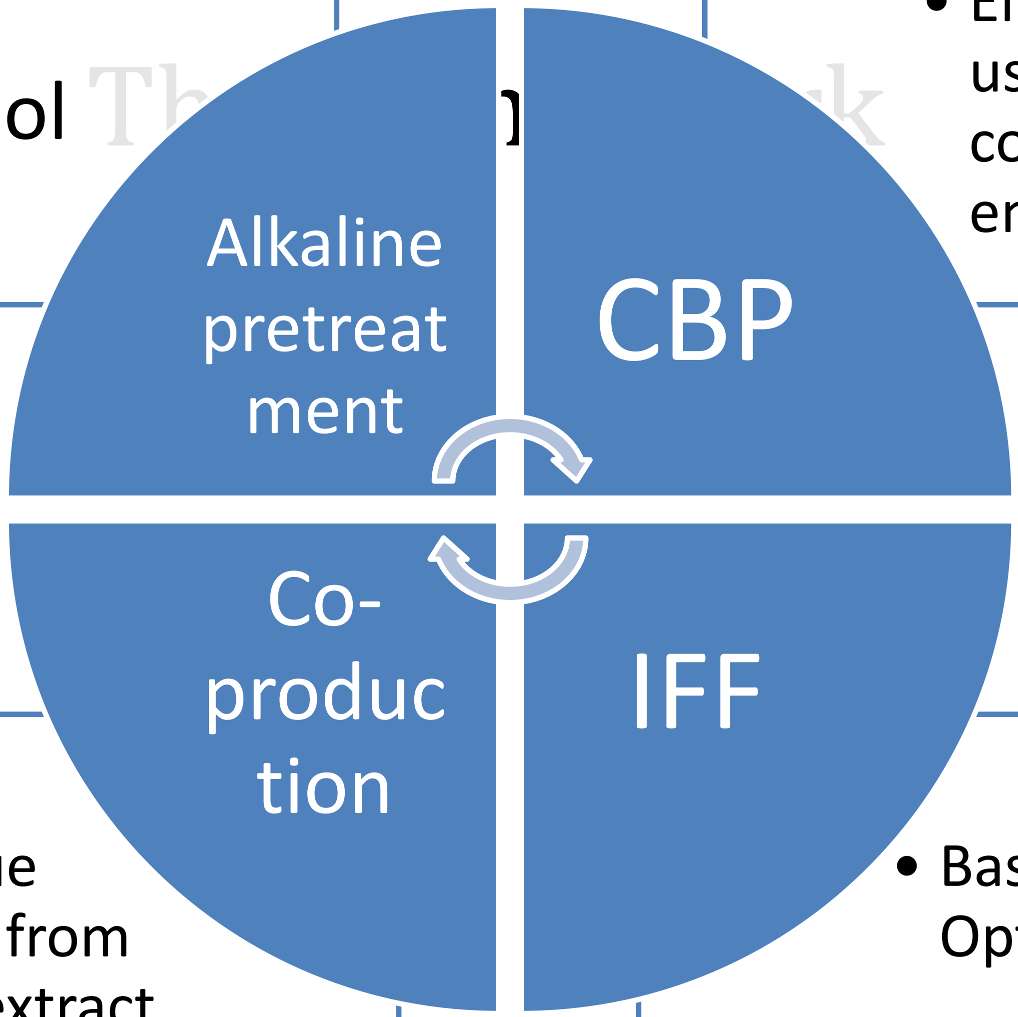
Biological

- Low energy consumption, low chemical requirement, no corrosion
- Long processing time
- High cost of commercial enzymes

Department of Chemical Engineering
University of the Philippines-Diliman

- High ethanol yield

- Eliminate use of commercial enzyme



- High-value products from alkaline extract

- Basal Optimization

Topic Outline

- **Study 1:** Bioethanol from Alkaline-pretreated Sugarcane Bagasse by Consolidated Bioprocessing with *Phlebia* sp. MG-60
- **Study 2:** Effect of Chemical Factors on Ethanol production by Integrated fungal fermentation sugarcane bagasse with *Phlebia* sp. MG-60
- **Study 3:** Bioactivity of NaOH extracts of sugarcane bagasse
- **Study 4:** Bioactive compounds in NaOH extracts of sugarcane bagasse

Study 1: Bioethanol from Alkaline-pretreated Sugarcane Bagasse by Consolidated Bioprocessing with *Phlebia* sp. MG-60

OBEJCTIVES:

1. To determine the effect of alkaline pretreatment at different concentrations on chemical composition of sugarcane bagasse.
2. To evaluate the performance of *Phlebia* sp. MG-60 for production of sacharification enzymes and ethanol concentration *via* consolidated bioprocessing.

Study 1: Results

Table 1.2.The decrease in chemical components of sugarcane bagasse

Samples	Residual Glucan (%) *	Residual Xylan (%)*	Residual Lignin (%) *	Residual Fructan (%) *
Initial bagasse	100	100	100	100
0.0 % NaOH	100.0 ± 0.2	99.6 ± 0.3	91.9 ± 0.1	83.3± 0.0
0.2 % NaOH	99.9± 0.5	82.1 ± 0.7	90.4 ± 0.3	107.1± 0.6
0.4 % NaOH	99.8± 0.2	78.8 ± 0.3	75.8 ± 0.2	103.3± 0.3
0.6 % NaOH	97.6± 0.1	55.4 ± 0.1	55.3 ± 0.2	88.8 ± 0.3
0.8 % NaOH	98.3 ± 0.3	50.4± 0.1	36.1± 0.3	98.9± 0.3
1.0 % NaOH	81.9 ± 0.2	34.9 ± 0.1	20.5± 0.1	56.6± 0.2
5.0 % NaOH	81.6 ± 0.1	3.8 ± 0.1	11.0± 0.0	41.9 ± 0.0

*Each value calculated from composition of sulfuric acid hydrolysate of each substrate.

Study 1: Results

Ethanol production from alkaline-pretreated sugarcane bagasse by MG-60 during CBP

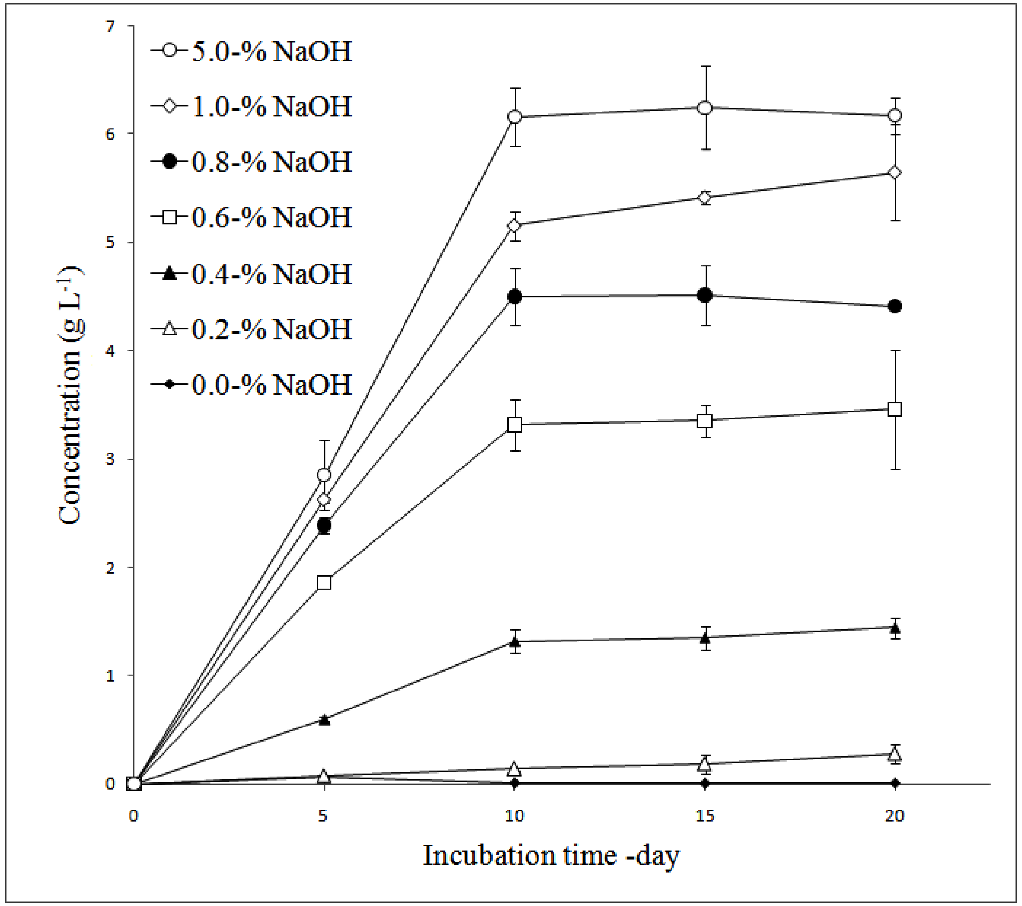


Fig 1.1. Time courses of ethanol production from alkaline-pretreated sugarcane bagasse by *Phlebia* sp. MG-60 under semi-aerobic conditions. Ethanol yield was calculated from the concentration in g L^{-1}.

Ethanol production plateaued after 10 days.
Alkaline pretreatment has significant effect on ethanol production.

Department of Chemical Engineering
University of the Philippines-Diliman

Study 1: Results

Enzyme activities during the CBP process

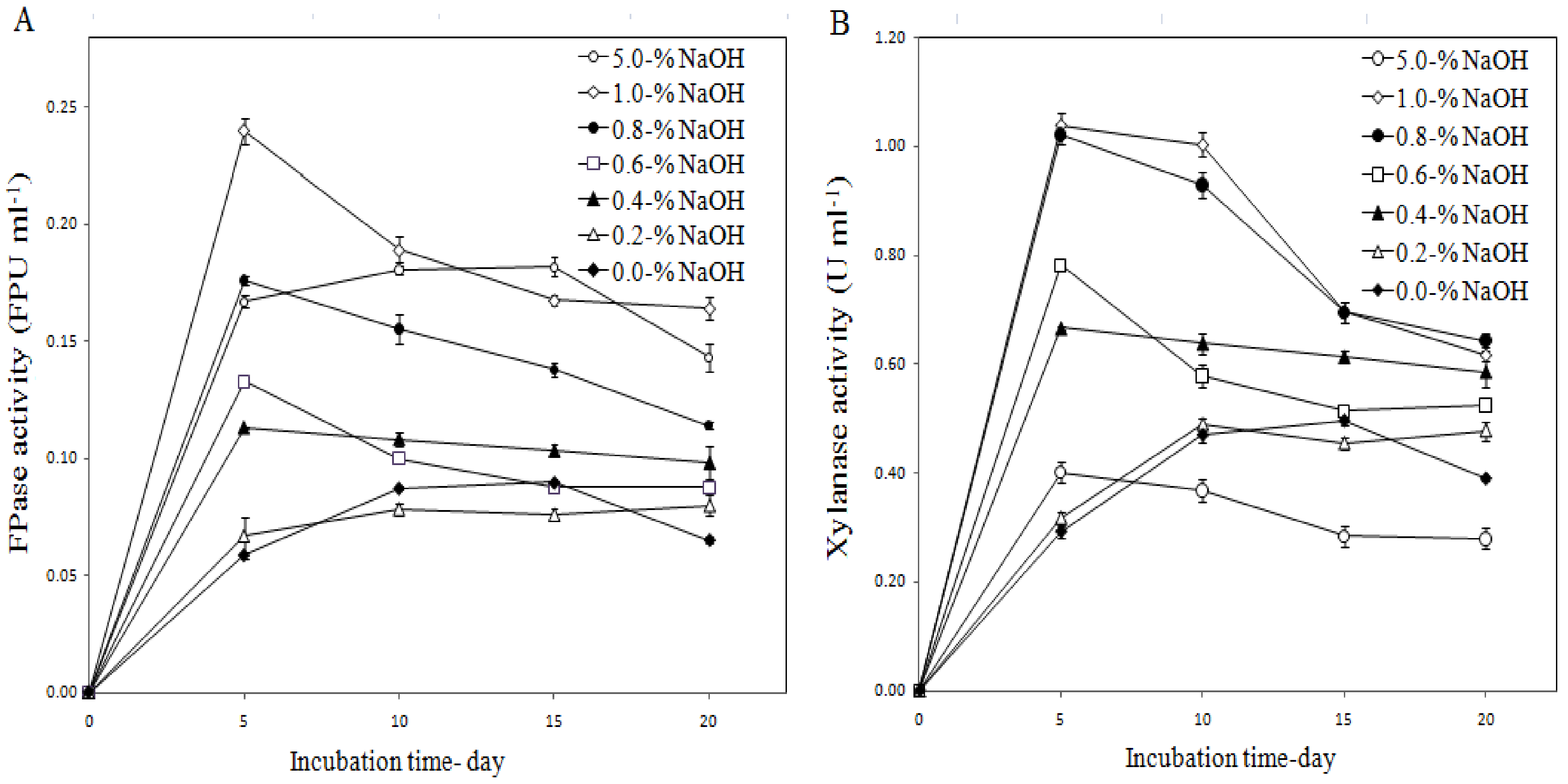
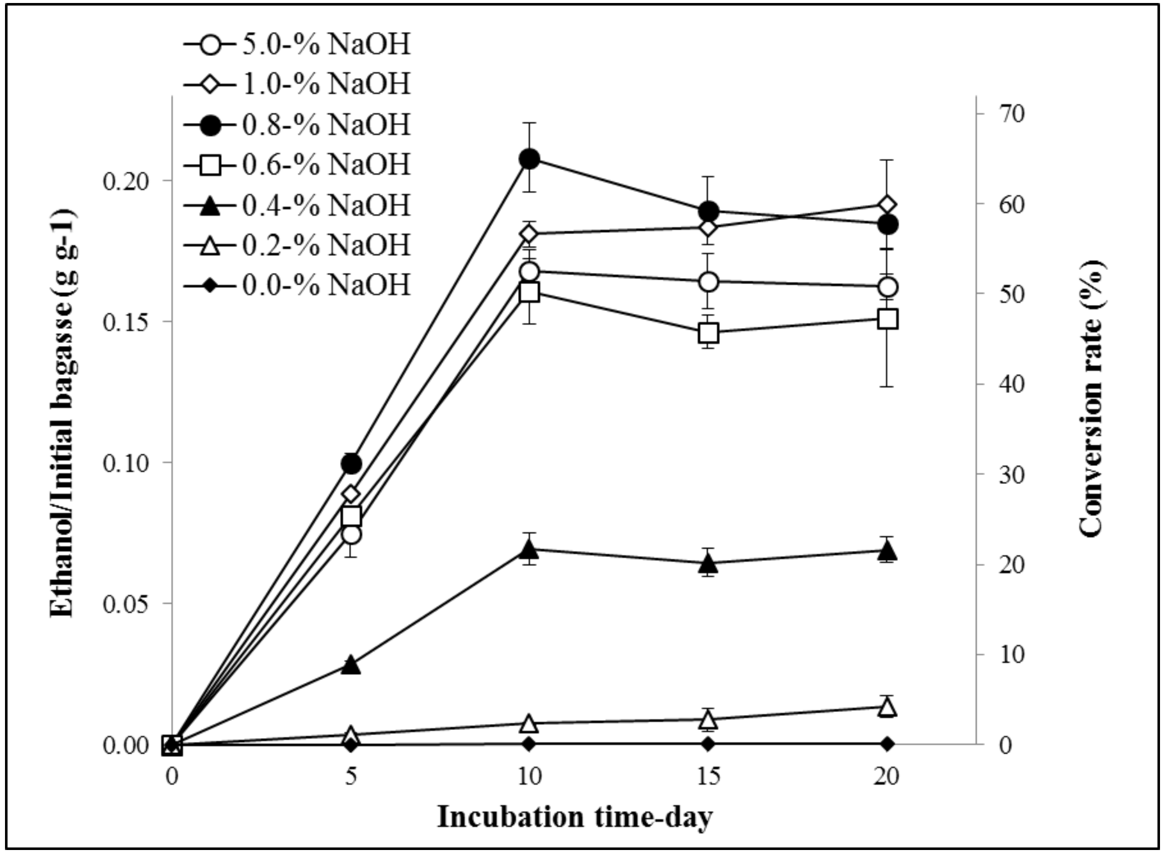


Figure 1.3. Time courses of FPase (A) and xylanase (B) activities under semi-aerobic conditions . The data represent the average of four independent experiments.

Alkaline pretreatment important for effective production of cellulose in CB fermentation using MG-60.

Study 1: Results

Enzyme activities during the CBP process



Alkaline pretreatment is effective for improving fermentation by MG-60 however weight loss at higher alkaline concentrations can significantly reduce ethanol yield per g of raw bagasse.

Figure 1.3. Time courses of ethanol production from alkaline-pretreated sugarcane bagasse by Phlebia sp. MG-60 under semi-aerobic conditions. Ethanol yield based on original untreated bagasse (primary axis), and theoretical maximum conversion rate based on original untreated bagasse (secondary axis)

Study 1: Conclusion

- Alkaline pretreatment and CBP fermentation using *Phlebia* sp. MG-60 is recommended for high ethanol yield from cellulosic materials.

- Alkaline pretreatment decreased the lignin content of sugarcane bagasse, leading to effective ethanol production.

- *Phlebia* sp. MG-60 is capable of direct ethanol production from pretreated sugarcane bagasse without addition of other enzymes.

- The ethanol production by *Phlebia* sp. MG-60 from pretreated sugarcane bagasse has the potential for ethanol production from cellulosic materials in the near future.

Study 2: Effect of chemical factors on integrated fungal fermentation of sugarcane bagasse for ethanol production by a white-rot fungus, *Phlebia* sp. Mg-60

OBJECTIVE:

To determine the effects of initial moisture content and chemical factors in shortening the time and/or improving the yield of ethanol production from sugarcane bagasse using only the white-rot fungus *Phlebia* sp. MG-60.

Part I (INITIAL MOISTURE CONTENT of SCB)

- To 1-g samples, add water to make 60, 65, 70, 75, 80% w water.

Part II (CHEMICAL FACTORS)

- Inorganic basal = basal medium (Kirk et al, 1978) – (glucose, tween-80, ammonium tartrate)
- Inorganic basal low nitrogen (LN) = inorganic + 0.22 g/L ammonium tartrate
- Other basal media = basal medium + (Malt extract, glucose, Fe2+, Mn2+, Cu2+ OR Ca2+)

Effect of initial moisture content of bagasse

Integrated Fungal Fermentation (IFF)

=

Aerobic Delignification

+

Semi-aerobic CBP

Effect of chemical factors

Using MG-60

Study 2: Part I. Experiment design

1g sample bagasse
42-100 mesh size

↓

Add amounts of distilled water to produce different moisture content

↓

Autoclave at 121 ^{o}C, 15 min

↓

Add 1 disc of *Phlebia* sp. MG-60, kept in aerobic conditions

↓

Step I

Delignification at 28 ^{o}C, dark condition for 2 and 4 weeks

↓

Step II

Add 20 mL fermentation medium and changed to anaerobic conditions

↓

CBP performance at 28 ^{o}C, dark condition for 20 days

↓

Centrifuged and used the broth to analyze ethanol content

Biological pretreatment using *Phlebia* sp. MG-60

Measured chemical composition of delignified bagasse

CBP

↓

IFFP

Study 2: Part I Results

Table 2.1. Chemical composition of initial and 2–week incubated MG-60 pretreated bagasse

Samples	Composition (% by wt)					
	Glucan*	Xylan*	Fructan*	Lignin*	L/G	Recovery
Initial bagasse	40.7 ±0.2	16.9 ±0.2	2.9 ±0.3	23.4 ±0.4	0.57	83.8
60%	41.2 ±0.3	13.6 ±0.1	2.6 ±0.1	21.9 ±0.2	0.53	79.3
65%	40.4 ±0.4	13.4 ±10	2.5 ±0.2	21.2 ±0.2	0.53	77.6
70%	39.3 ±0.8	13.0 ±0.7	2.3 ±0.2	20.7 ±0.3	0.53	75.3
75%	37.4 ±0.4	12.6 ±0.1	2.5 ±0.1	20.4 ±0.1	0.55	72.9
80%	35.4 ±0.4	11.3 ±0.4	2.4 ±0.3	20.9 ±0.2	0.59	69.9

*Each value calculated from composition of sulfuric acid hydrolysate of each substrate.
L/G: lignin–to–glucan ratio.

Study 2: Part I Results

Table 4.1.2. Effect of Initial moisture content on Chemical composition of initial and 4–week incubated MG-60 pretreated bagasse

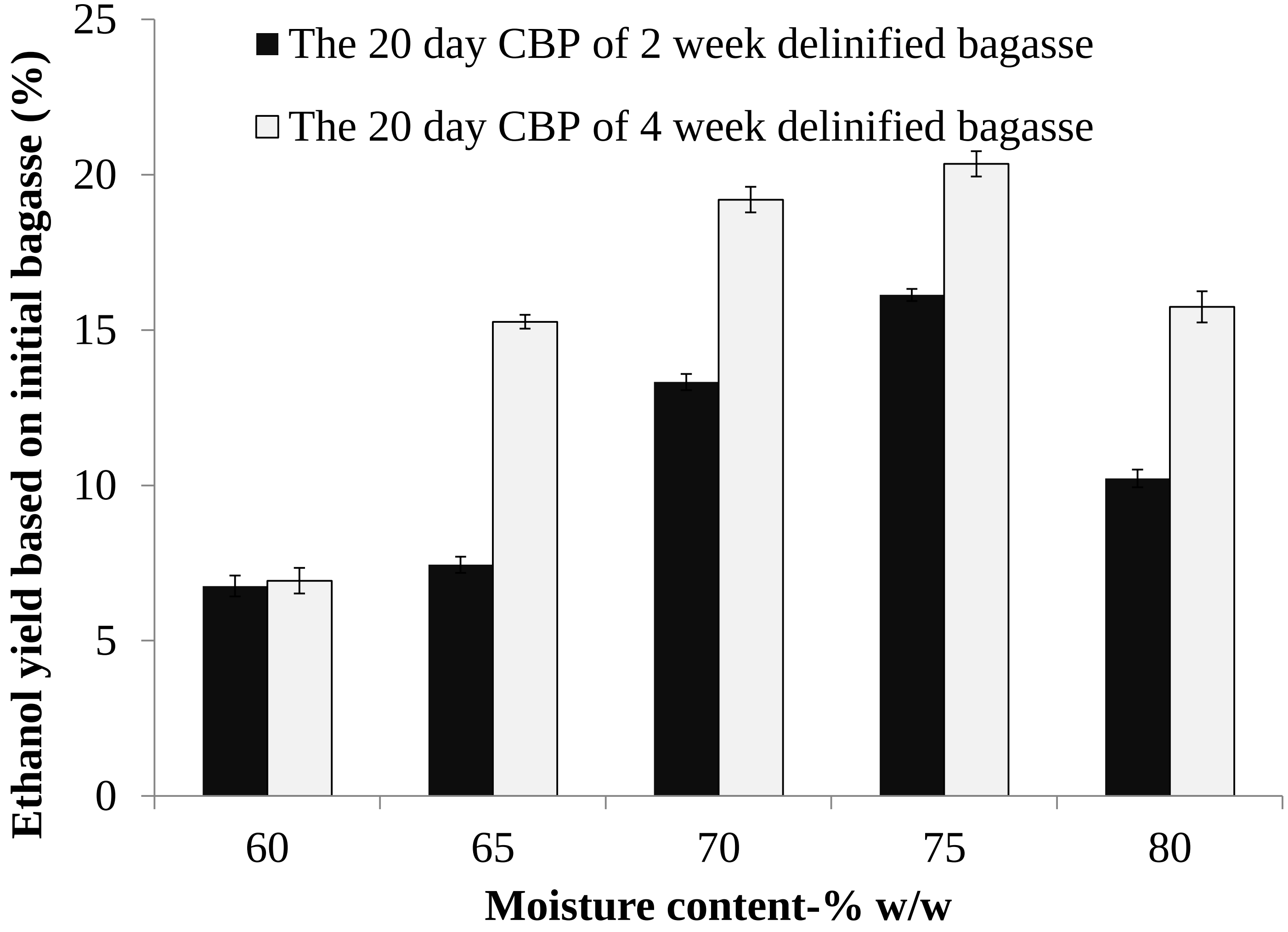
Samples	Composition (% by wt)					
	Glucan*	Xylan*	Fructan*	Lignin*	L/G	Recovery
Initial bagasse	40.7 ±0.2	16.9 ±0.2	2.9 ±0.3	23.4 ±0.4	0.57	83.8
60%	42.4 ±0.6	15.4 ±0.1	2.7 ±0.3	21.7 ±0.1	0.51	82.2
65%	41.3 ±0.1	15.1 ±0.2	2.6 ±0.1	20.9 ±0.3	0.50	79.8
70%	39.9 ±0.9	12.8 ±0.2	2.0 ±0.5	20.6 ±0.5	0.52	75.2
75%	39.0 ±0.7	13.5 ±0.5	0.9 ±0.1	17.1 ±0.3	0.43	70.5
80%	37.7 ±0.6	10.7 ±0.1	2.6 ±0.1	19.7 ±0.1	0.52	70.7

*Each value calculated from composition of sulfuric acid hydrolysate of each substrate.
L/G: lignin–to–glucan ratio.

L./G values significantly changed after 4-week incubation.
Initial moisture content affects MG-60 selectivity to degrade lignin compared to glucan.
75% initial moisture content of bagasse provided the most available substrate for subsequent fermentation.

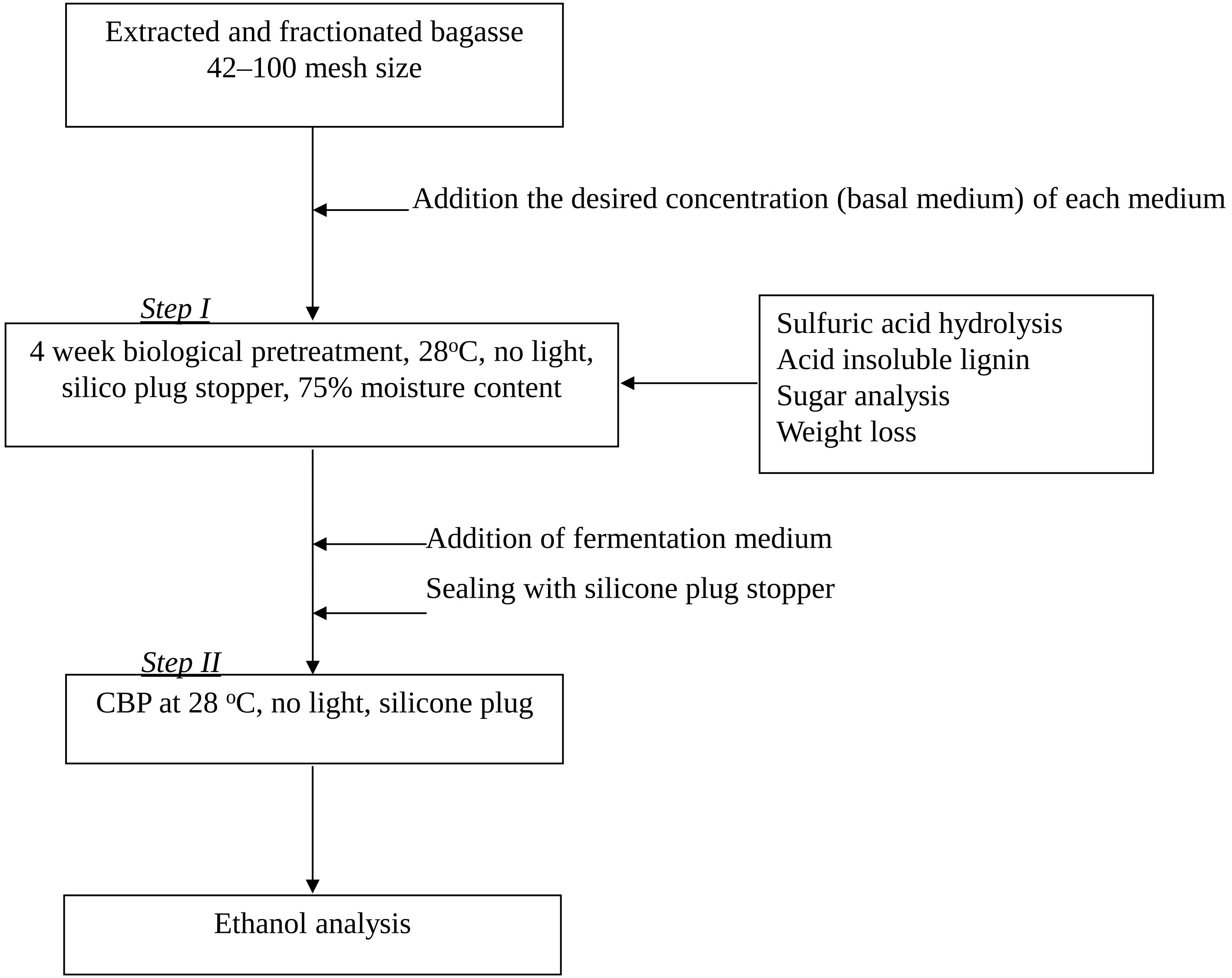
Study 2: Part I Results

Effect of moisture content on ethanol conversion rate by using MG-60



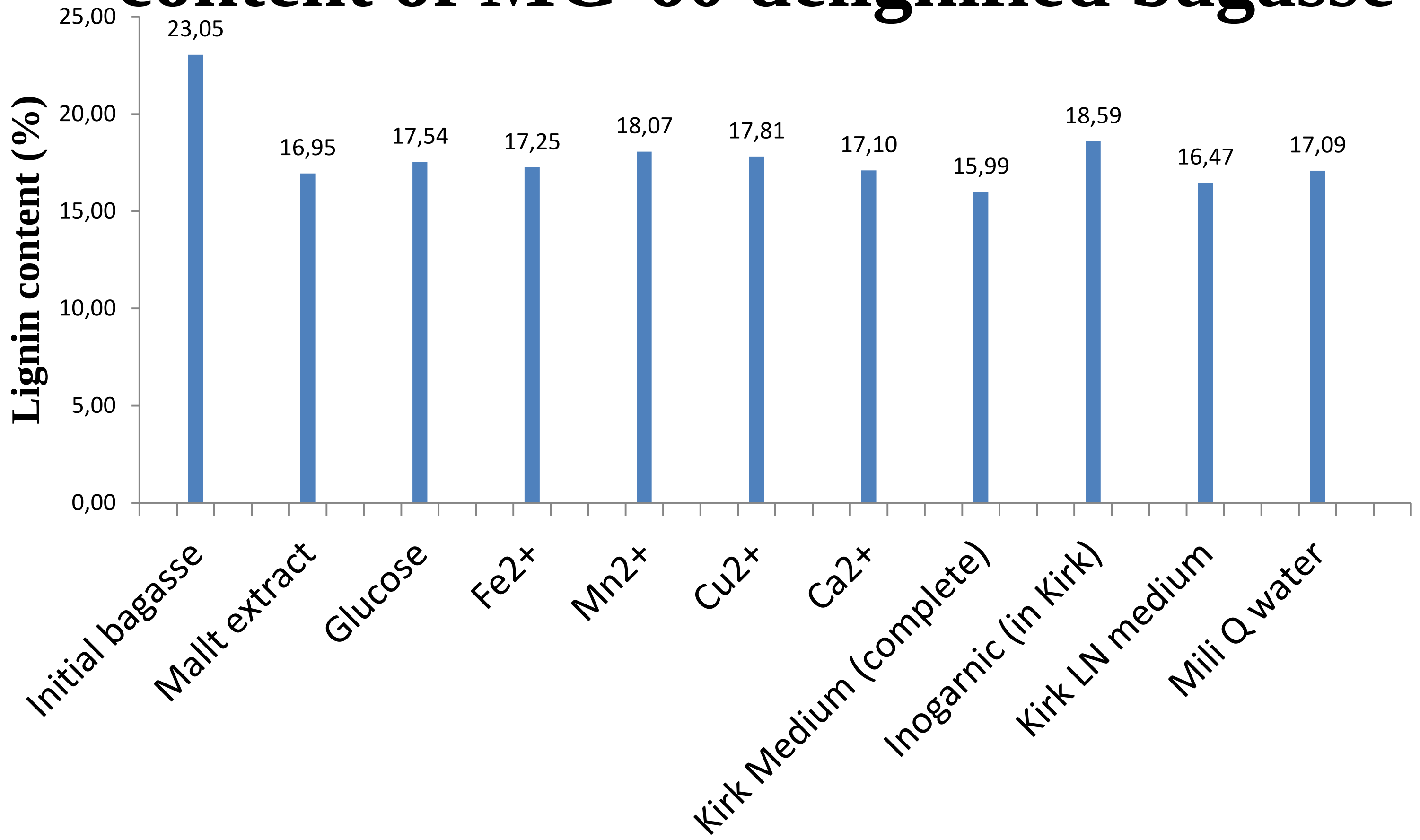
Initial moisture content significantly affects ethanol yield by MG-60 from MG-60 pretreated bagasse. However ethanol yield is low.

Study 2. Part II Experiment design



Study 2: Part II Results

Effect of Chemical Factors on Lignin content of MG-60 delignified bagasse



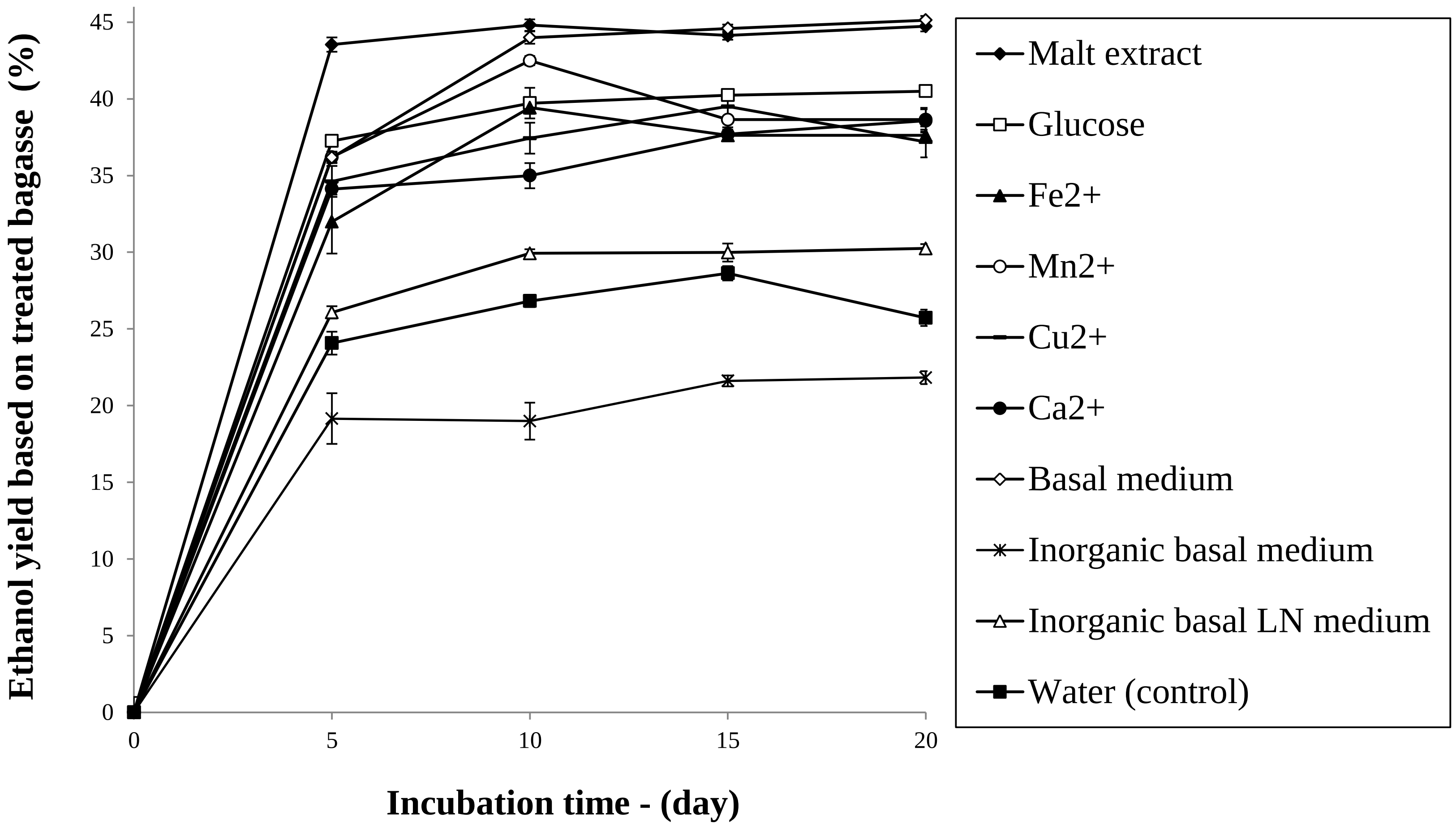
Study 2: Part II Results

Chemical composition of initial and pretreated bagasse with 10 different media

Media	Dry weight of pretreated bagasse (mg)	Composition (% by wt)					
		Glucan*	Xylan*	Fructan*	Lignin*	L/G	Recovery
Malt extract	819 ±15	40.5 ±0.3	14.5 ±0.1	0.9 ±0.2	16.9 ±0.4	0.42	72.8
Glucose	841 ±11	41.9 ±0.5	15.4 ±0.2	1.0 ±0.2	17.5 ±0.3	0.42	75.8
Fe^{2+}	815 ±11	44.7 ±1.0	16.1 ±0.6	1.0 ±0.2	17.3 ±0.1	0.39	79.1
Mn^{2+}	821 ±11	43.1 ±0.6	14.3 ±3.2	1.0 ±0.2	18.1 ±0.4	0.42	76.4
Cu^{2+}	816 ±26	46.8 ±1.1	17.3 ±0.5	1.1 ±0.2	17.8 ±0.5	0.38	82.9
Ca^{2+}	810 ±9	41.2 ±0.7	14.7 ±0.6	0.9 ±0.1	17.1 ±0.1	0.41	73.8
Basal medium	794 ±5	43.7 ±0.5	15.2 ±0.2	0.9 ±0.1	15.9 ±0.2	0.37	75.8
Inorganic basal	841 ±21	39.6 ±0.4	15.5 ±0.2	0.9 ±0.1	18.6 ±0.1	0.47	74.6
Inorganic basal LN	805 ±3	43.5 ±1.3	16.1 ±0.5	1.0 ±0.1	16.5 ±0.2	0.38	77.1
Water	813 ±8	39.0 ±0.7	13.5 ±0.5	0.9 ±0.1	17.1 ±0.3	0.44	70.5
Initial bagasse (*)	939 ±8	40.7 ±0.2	16.9 ±0.2	2.9 ±0.3	23.4 ±0.4	0.57	83.8

Chemical additives have significant effects on delignification.
Low weight losses durin MG-60 delignification.

Study 2: Part II Results



Chemical Factors have significant effect on the ethanol yield.

Chemical factors effective in shortening overall culture time.

IFF with basal medium exhibited the highest ethanol yield.

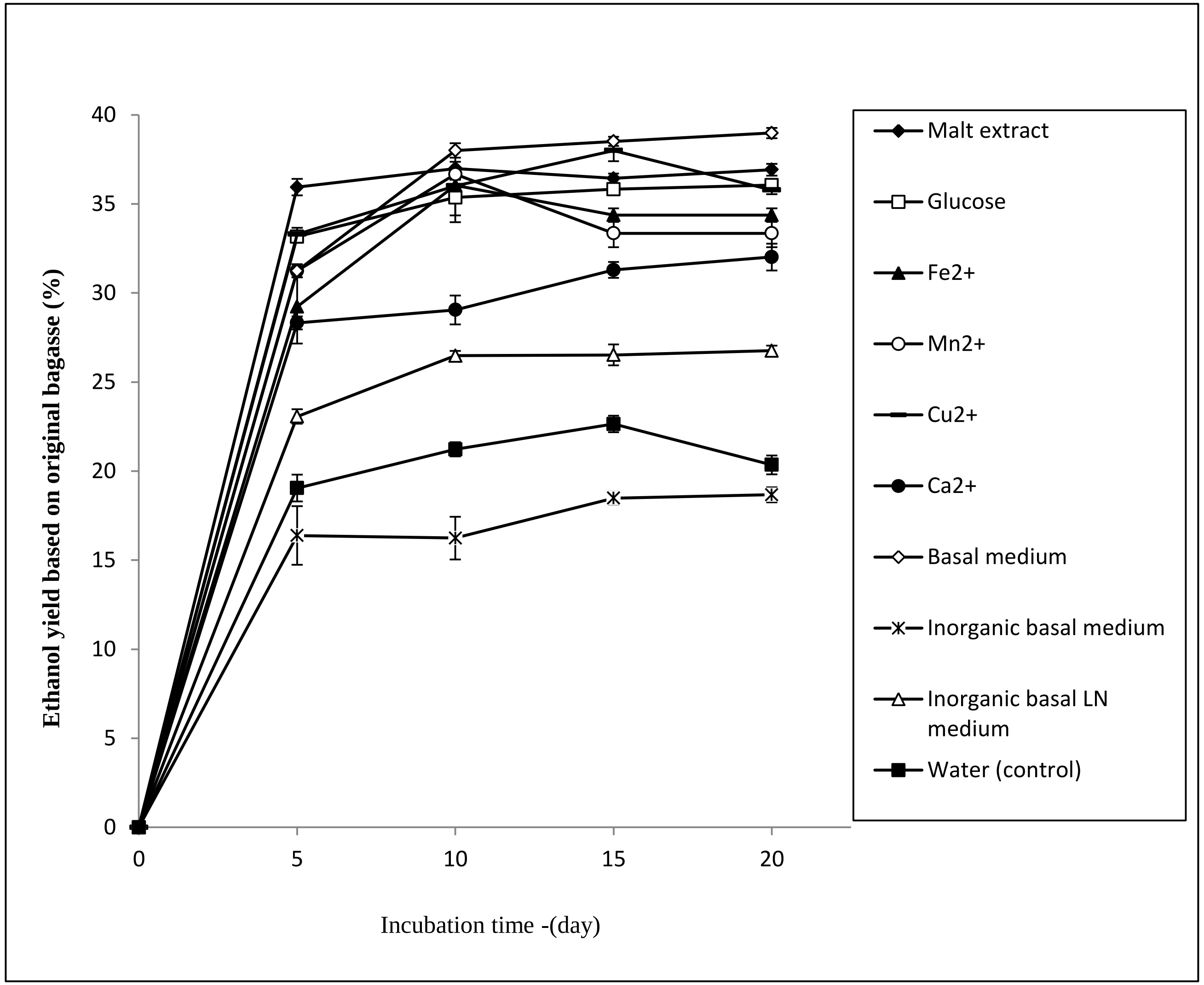
Time course of ethanol yield based on treated bagasse from 4-week incubations of pretreated sugarcane bagasse with *Phlebia* sp. MG-60 under semi-aerobic conditions.

Study 2: Part II Results

Table 2.1. Residual bagasse chemical components with different media

Media	Coefficient	Residual components (% by wt)			
		Residual glucan (%)	Residual xylan (%)	Residual fructan (%)	Residual lignin (%)
Malt extract	0.86	85.8 ±0.2	73.9 ±0.1	27.0 ±0.1	72.6 ±0.4
Glucose	0.89	91.2 ±0.4	80.6 ±0.2	31.2 ±0.1	66.5 ±0.2
Fe^{2+}	0.86	94.4 ±0.8	81.7 ±0.5	30.9 ±0.2	63.4 ±0.1
Mn^{2+}	0.86	91.6 ±0.1	72.9 ±2.8	29.4 ±0.2	66.9 ±0.3
Cu^{2+}	0.86	98.7 ±0.9	87.8 ±0.4	32.3 ±0.2	65.5 ±0.4
Ca^{2+}	0.85	86.4 ±0.6	73.9 ±0.5	25.9 ±0.1	62.4 ±0.1
Basal medium	0.84	89.8 ±0.4	75.0 ±0.2	26.9 ±0.1	57.3 ±0.2
Inorganic basal	0.89	86.2 ±0.4	81.0 ±0.2	27.0 ±0.1	70.5 ±0.1
Inorganic basal LN	0.85	90.7 ±1.1	80.7 ±0.4	28.7 ±0.1	59.8 ±0.2
Water	0.86	82.1 ±0.6	68.5 ±0.4	26.3 ±0.1	62.9 ±0.2
Sugarcane bagasse	1.00	100.00	100.00	100.00	100.00

Study 2: Part II Results



- Time course of ethanol yield based on original bagasse from 4-week incubations of pretreated sugarcane bagasse by *Phlebia* sp. MG-60 under semi-aerobic conditions. Ethanol yield is based on initial untreated bagasse, and data represent average of four independent experiments.

Poor ethanol yield of IFF compared to CBP with alkaline pretreatment

Study 2: Part II Conclusion

- The initial moisture content and a range of additives significantly affected IFF efficiency by shortening the required duration of the biological processing.
- After 4 weeks of aerobic incubation, the L/G value decreased from 0.57 to 0.43, and then, after 10 d of semi-aerobic conditions, 44.0% of the most effective ethanol yield was achieved.
- We suggest here that varying the additives for IFF could effectively improve bagasse fermentation by a single biological process using *Phlebia* sp. MG-60.
- Further studies will focus more deeply on the causes of changes observed in MG-60 fermentation by these additives, which allow higher ethanol yields.

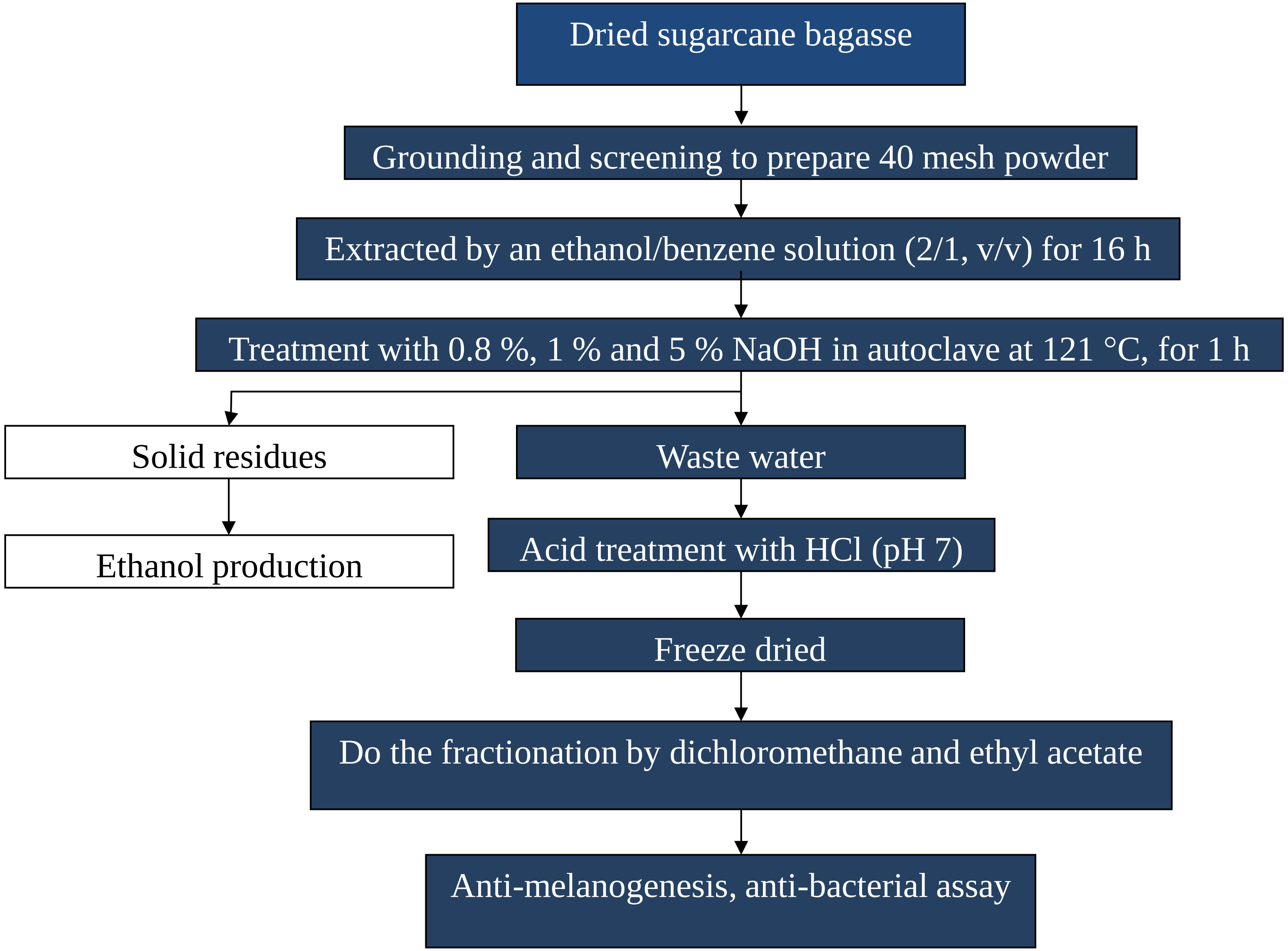
Study 3: Bioactivity of NaOH extracts of sugarcane bagasse

OBJECTIVE:

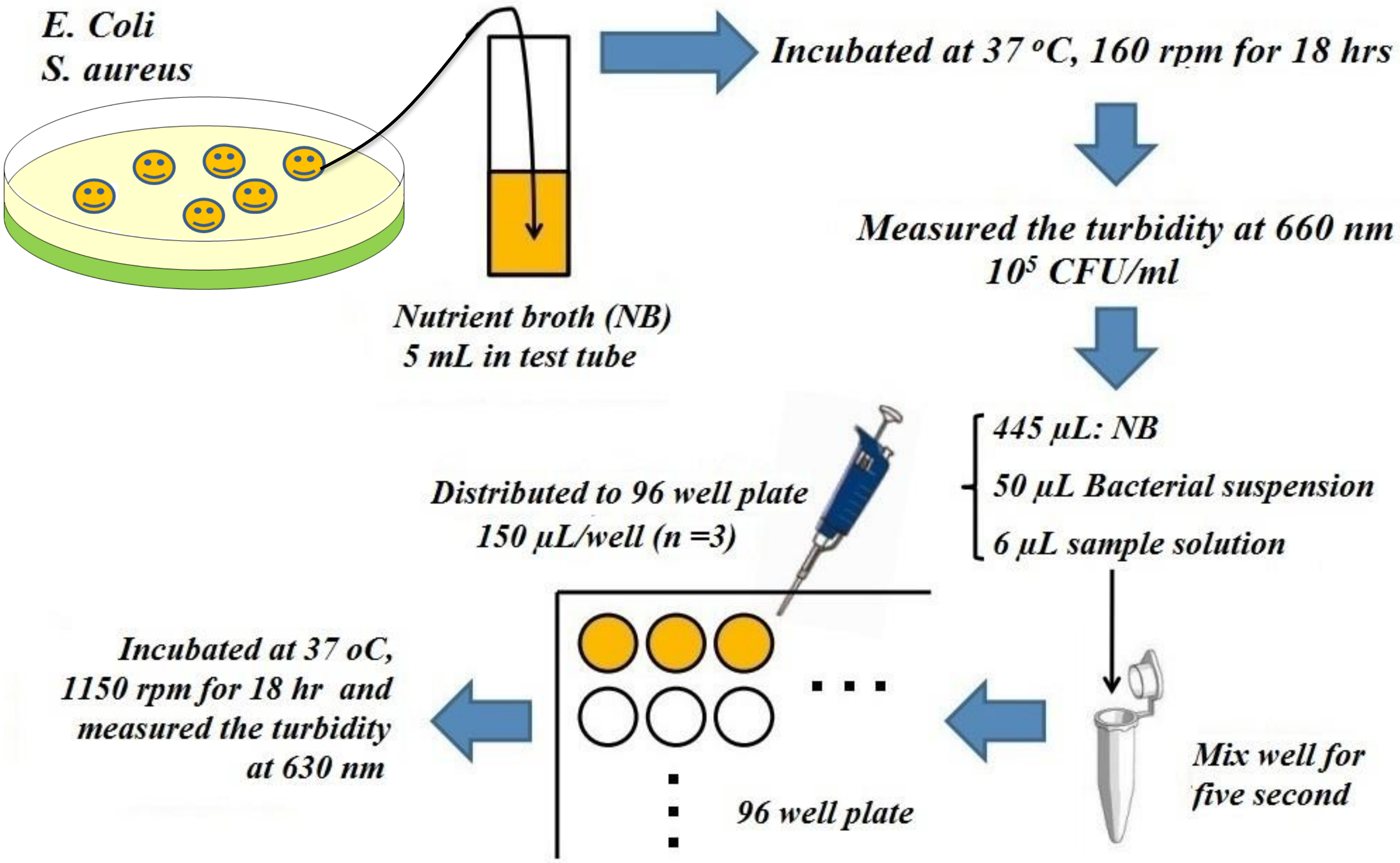
To analyze potential usage of alkaline pretreatment waste water by checking two biological activities; anti-melanogenesis assay and anti-bacterial assay

Department of Chemical Engineering
University of the Philippines-Diliman

Study 3. Experiment design

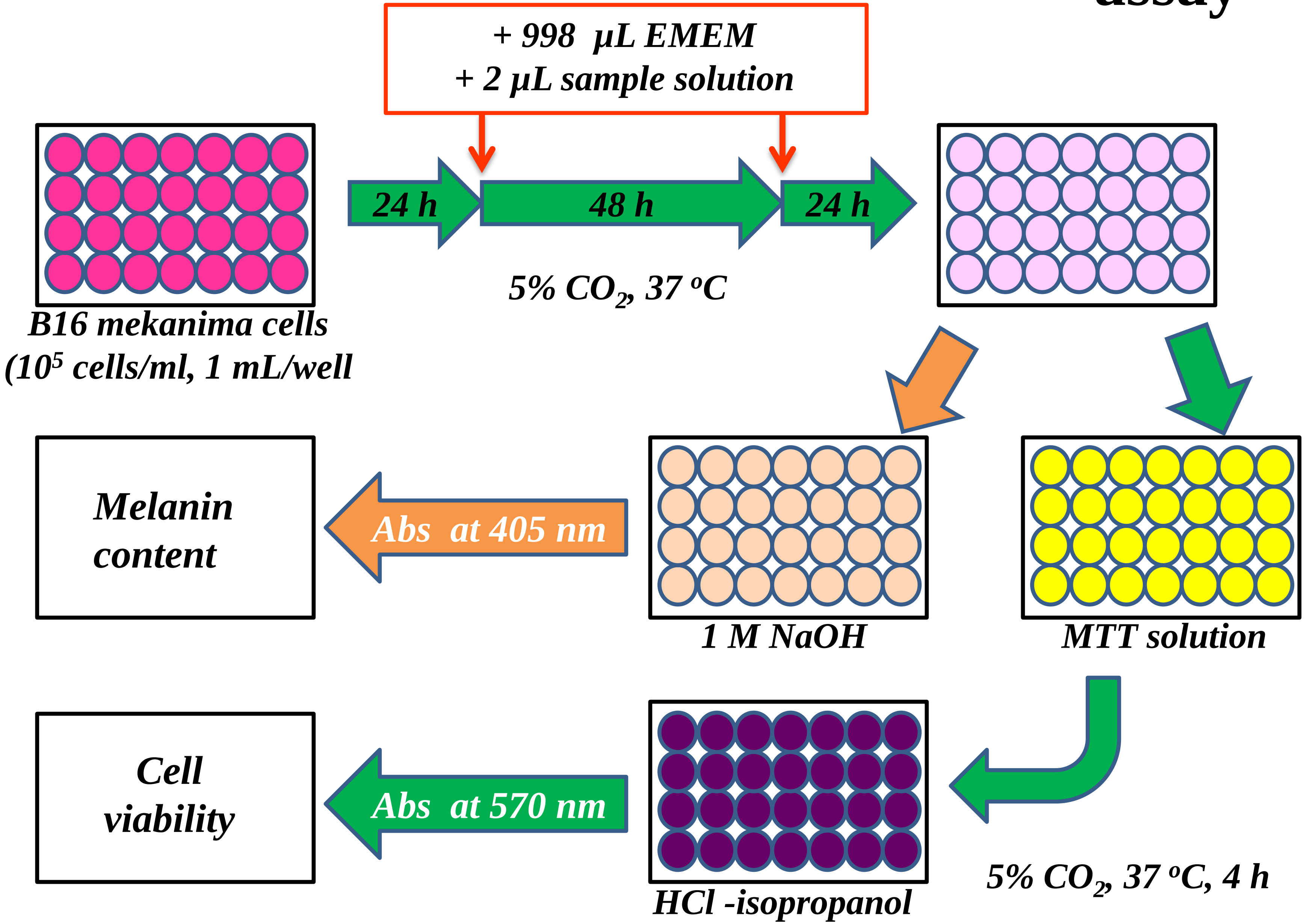


Study 3: Antibacterial assay



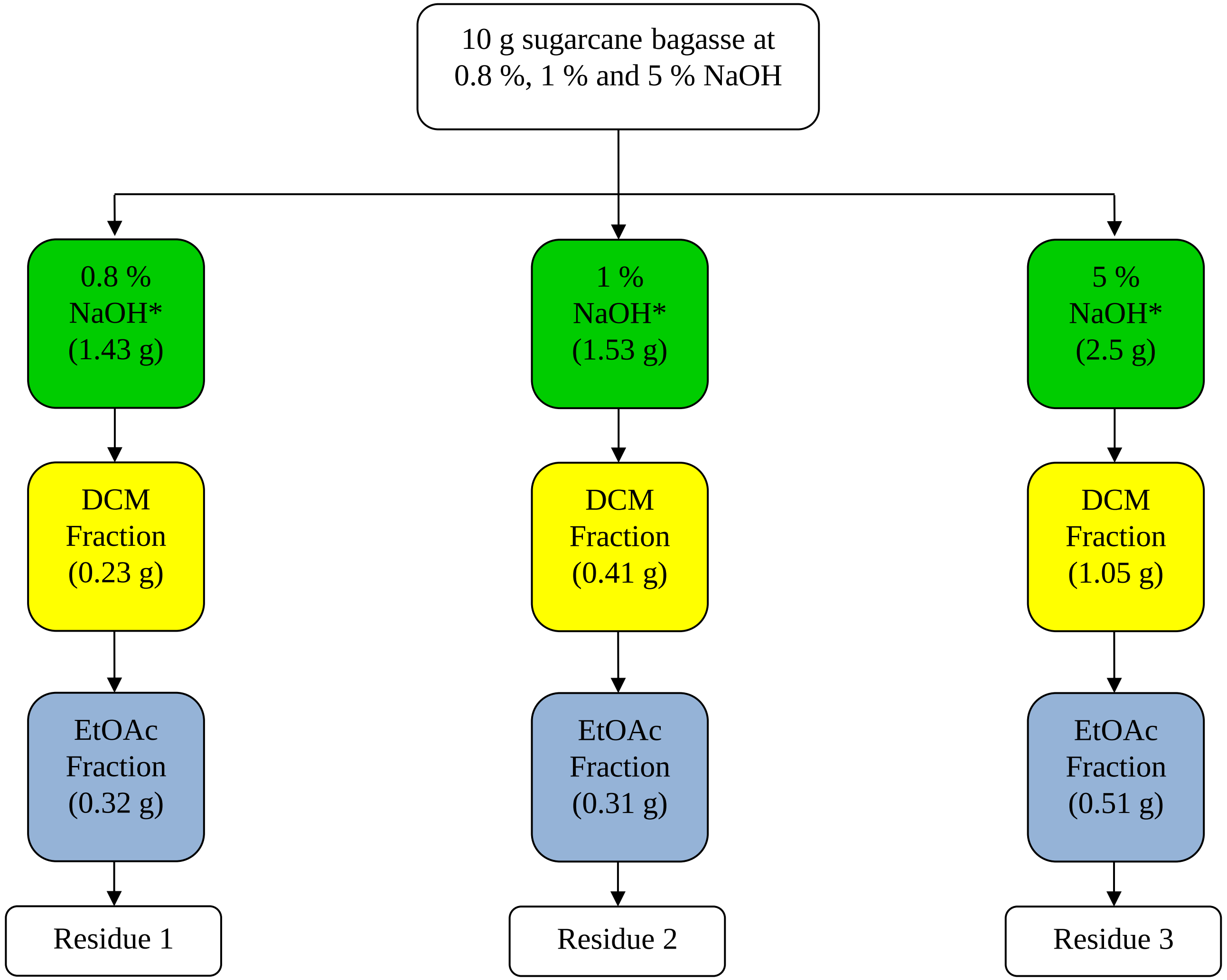
Department of Chemical Engineering
University of the Philippines-Diliman

Study 3: Melanin synthesis assay



Study 3: Results

The weight of fractionations from NaOH hydrolysis extracts



Without formed NaCl by calculation

Study 3: Results

B16- Dichloromethane fractions

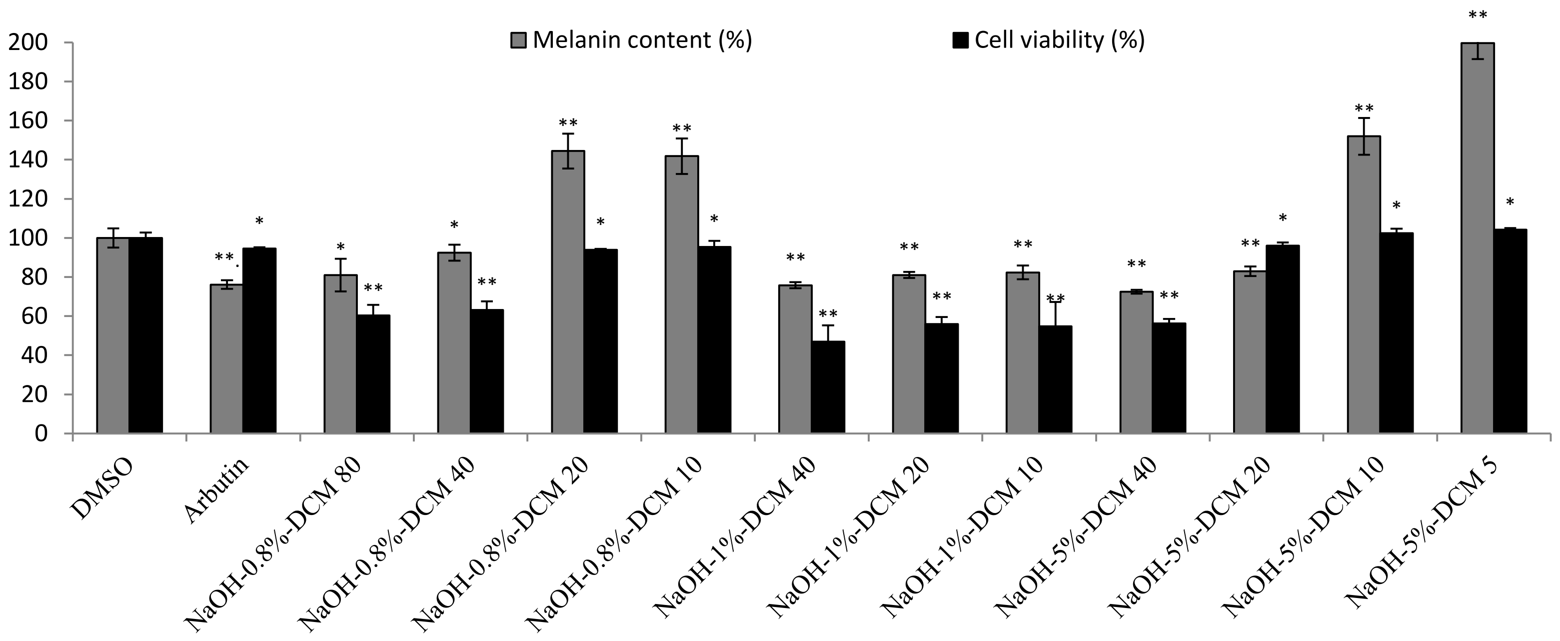


Fig. 5.1. Inhibitive effect of DCM fractionations of NaOH pretreatment waste waters at three different NaOH concentrations on melanin formation in B16 melanoma cells

Department of Chemical Engineering
University of the Philippines-Diliman

Study 3: Results

B16-Ethyl acetate fractions

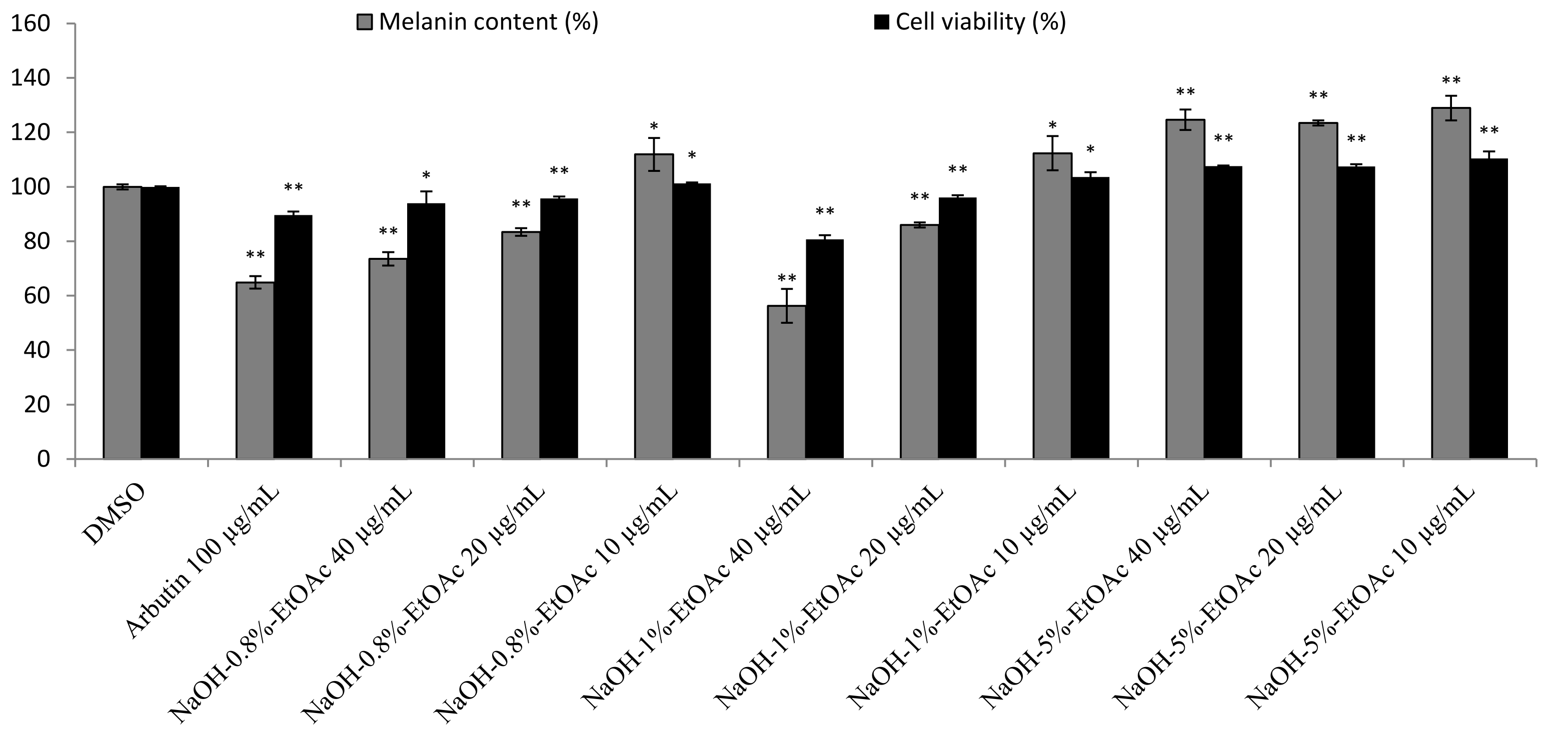


Fig. 5.2. Inhibitive effect of EtOAc fractionations of NaOH pretreatment waste waters at three different NaOH concentrations on melanin formation in B16 melanoma cells

Anti-bacterial activity

Study 3: Results

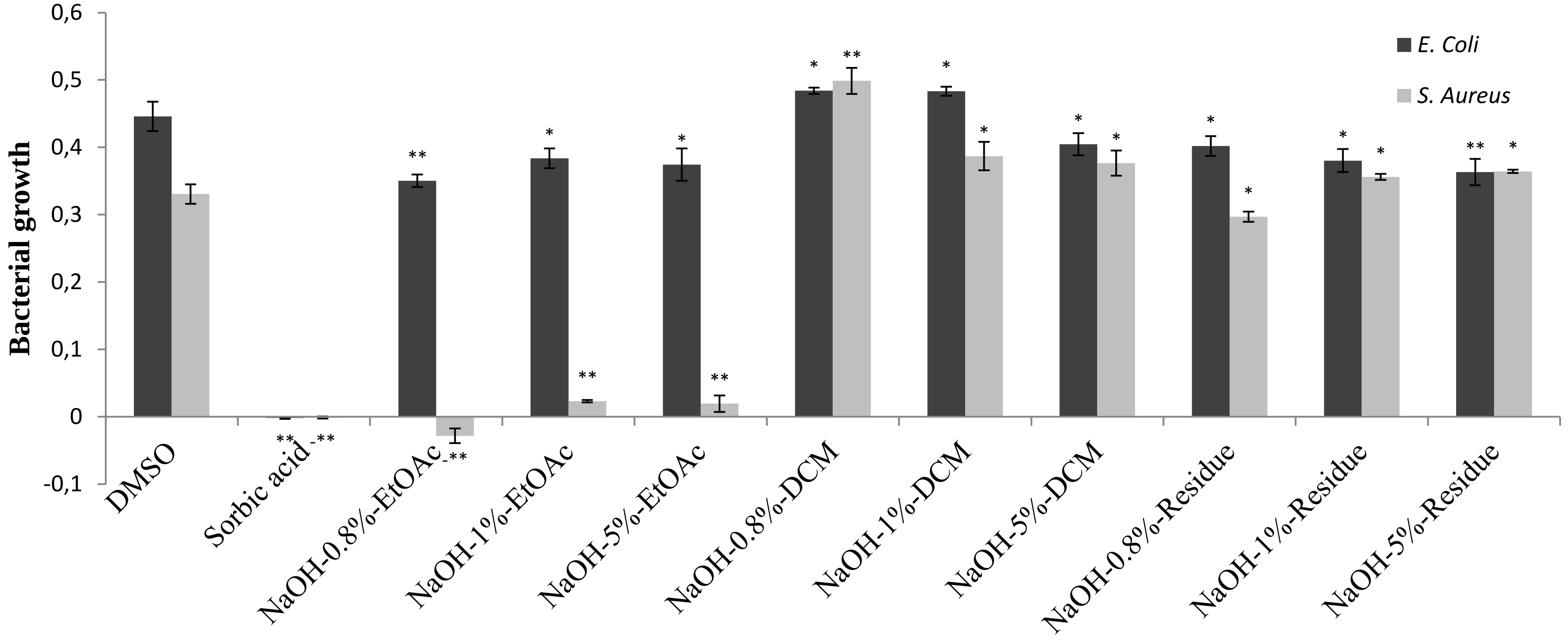


Fig. 5.3. Anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus*.The values are represented as the means ± standard deviation (SD), n = 3. Final concentration is 160 μg/mL equivalents to maximum solubility for extracts. Significant difference between 1% DMSO and each extract was determined by Student's t-test: *p<0.05, **p<0.01

Study 3: Conclusion

- The results of the study indicated that fractions of extracts that originated from the NaOH pretreated waste water of sugarcane bagasse, some of them have which have a strong inhibition of melanin synthesis and strong *S. Aureus* activity, could be safely used as a source for skin preparations.
- ***Our results need future study for bioactive compounds responsible for each activity.***

Study 4: Bioactive compounds in NaOH extracts of sugarcane bagasse

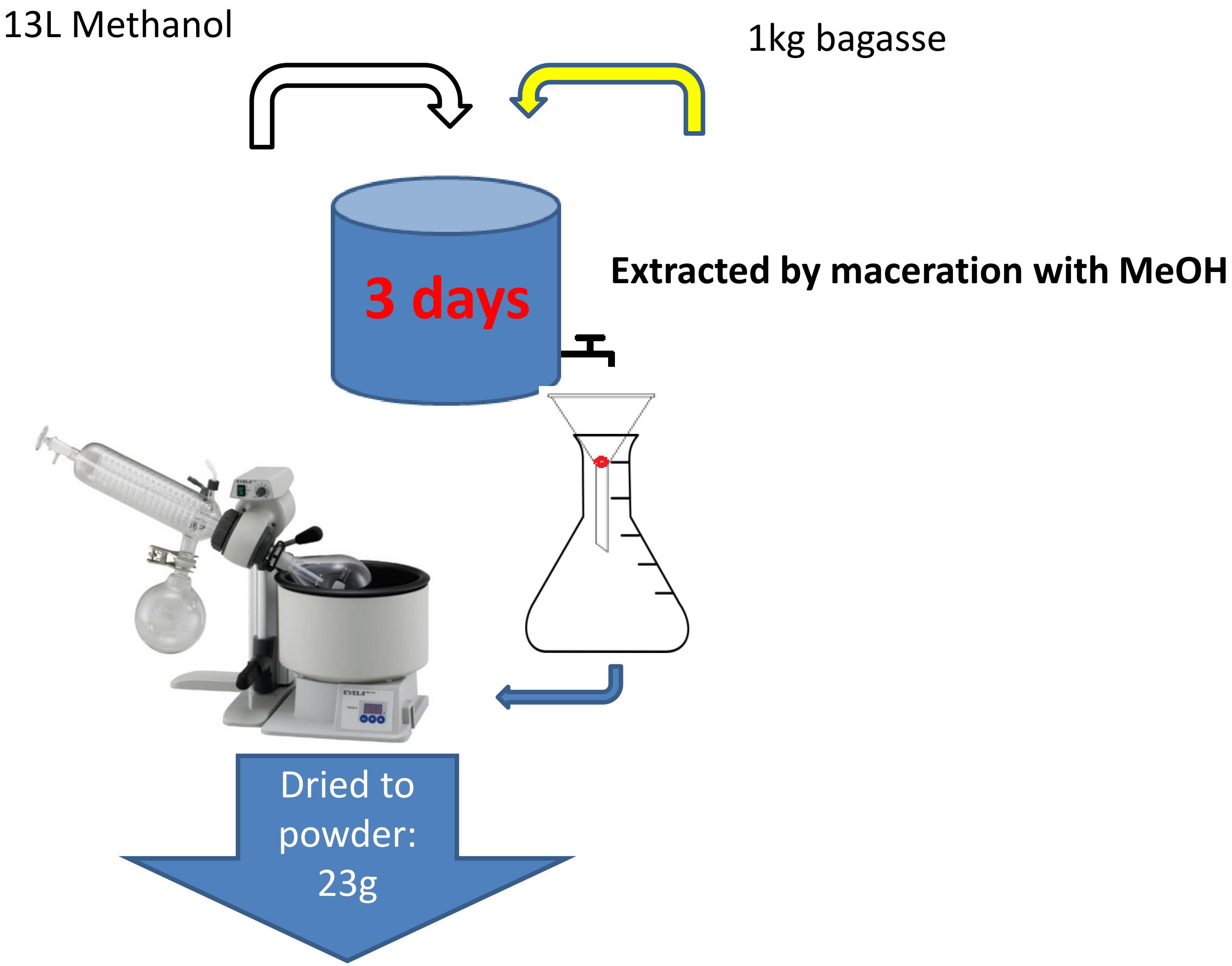
OBJECTIVE:

To isolate and identify aromatic compounds responsible for anti-melanin inhibitory from solvent extractives

Study 4: Methods

- Methanol extraction
- Fractionation by using liquid-liquid partition
- Silica gel chromatography
- Thin layer chromatography
- Preparative-HPLC
- Melanin synthesis inhibitory assay
- Antibacterial assay

Extracted by maceration with MeOH



Fractionation of sugarcane bagasse

Dried to powder: 23g

Fractionation using liquid-liquid partition

n- hexane | Dicloro-methane | EtOAc | MeOH

Melanin biosynthesis inhibitory effect of fractionations

n-hexane fractionation showed good inhibition

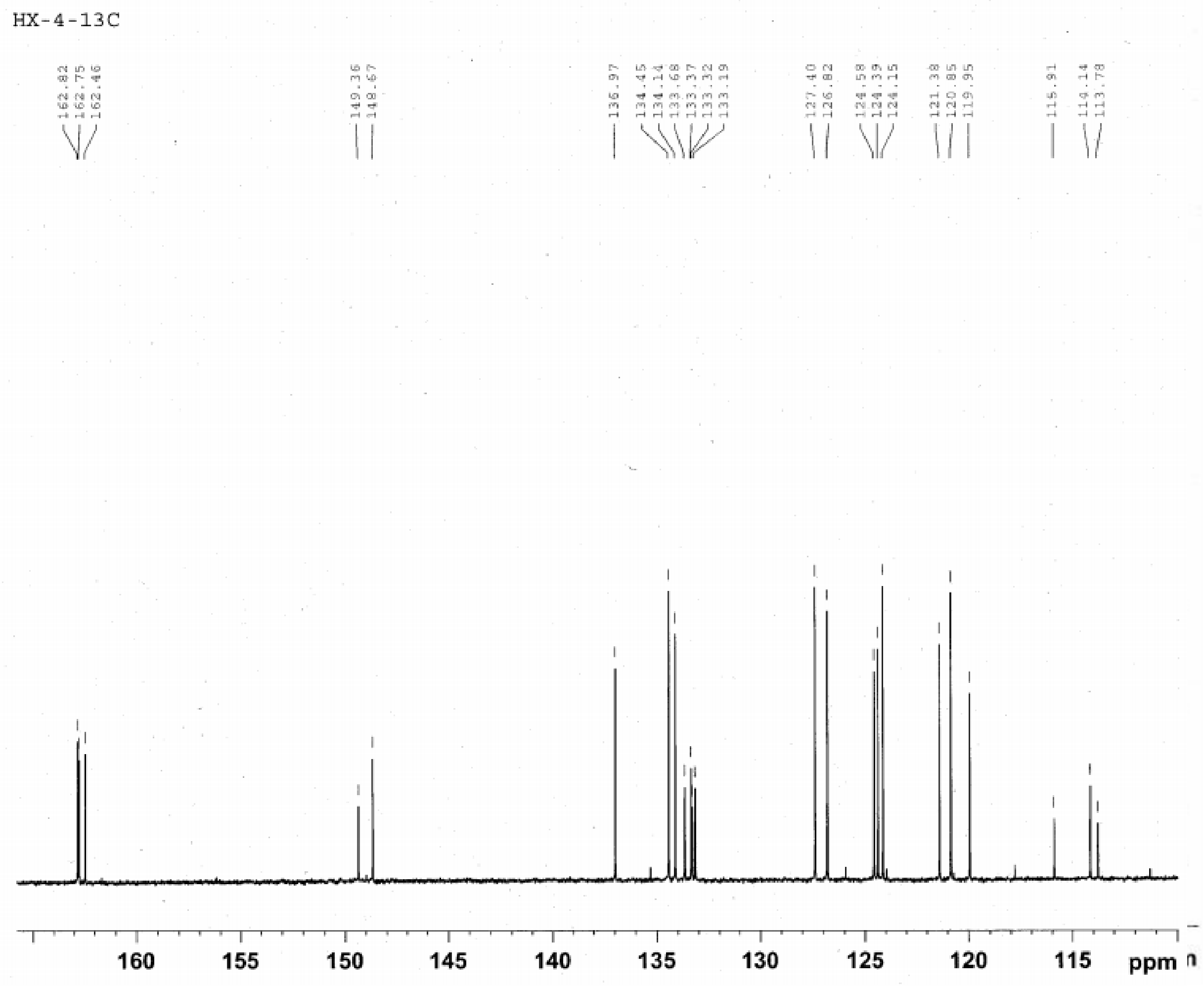
SGCC of n-hexane fractionation

Sub-fractionation	Fraction	*n*-hexane/EtOAc	Powder (mg)
1	1	100-0	17.62
	2	95-5	50.76
2	3	90-10	58.24
3	**4**	**85-15**	**142.63**
4	5	80-20	262.41
5	6	75-25	175.75
6	7	70-30	110.36
7	8	65-35	59.67
8	9	60-40	64.81
	10	55-45	68.22

Melanin biosynthesis inhibitory effect of 8 sub-fractionations

TLC of compounds

^{13}C-NMR of sample



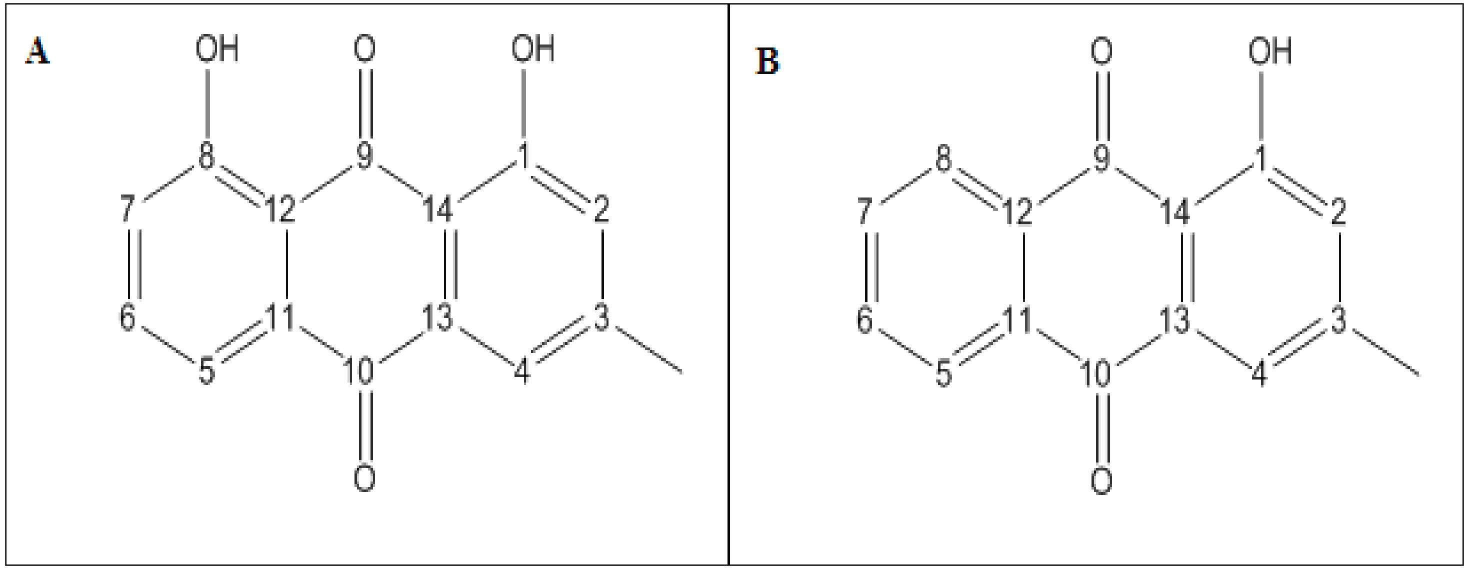
^{13}C-NMR spectroscopic data for crysophanol (A) and pachybasin (B).

A

δ_C-Value	Position
162.4	1
124.6	2
149.4	3
121.4	4
124.4	5
137.0	6
119.9	7
162.7	8
192.6	9
182.0	10
133.2	11
115.9	12
113.8	13
133.7	14
22.3	CH_3

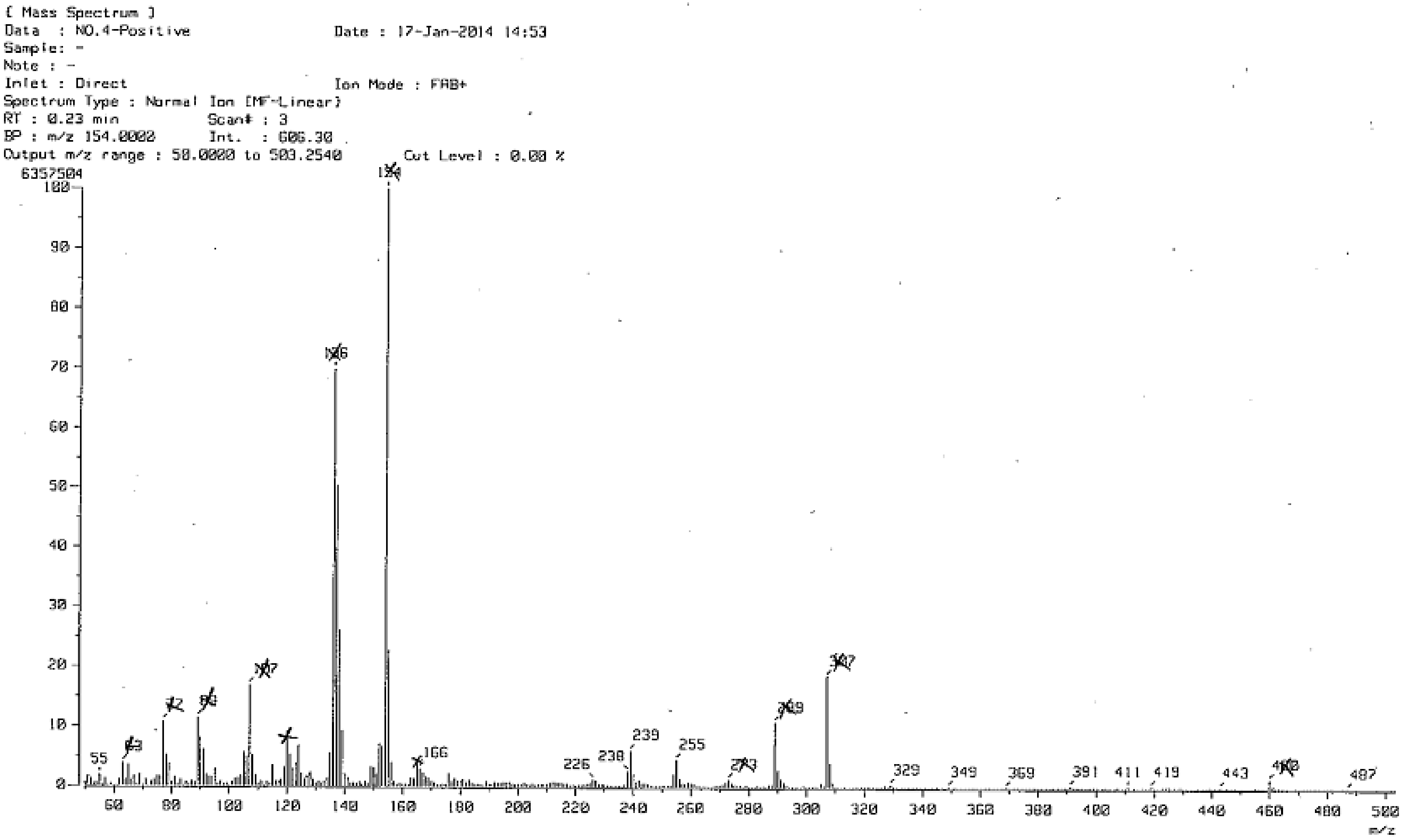
B

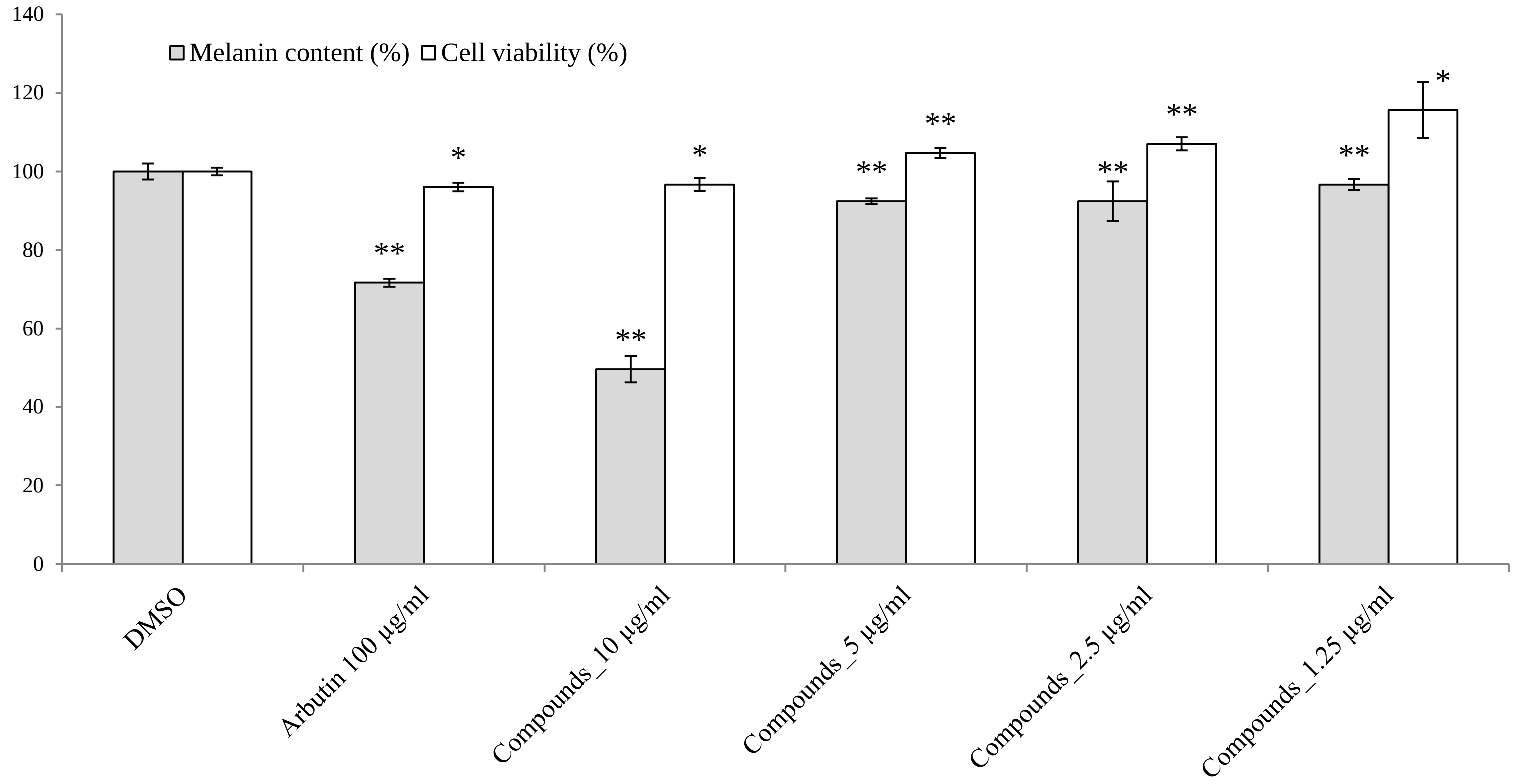
δ_C-Value	Position
162.4	1
124.6	2
149.4	3
121.4	4
124.4	5
137.0	6
119.9	7
162.7	8
192.6	9
182.0	10
133.2	11
115.9	12
113.8	13
133.7	14
22.3	CH_3



Crysophanol (1,8-dihydroxy-3-methyl- anthracene-9,10-dione) (A)
Pachybasin (1-hydroxy-3-methyl- anthracene-9,10-dione) (B)

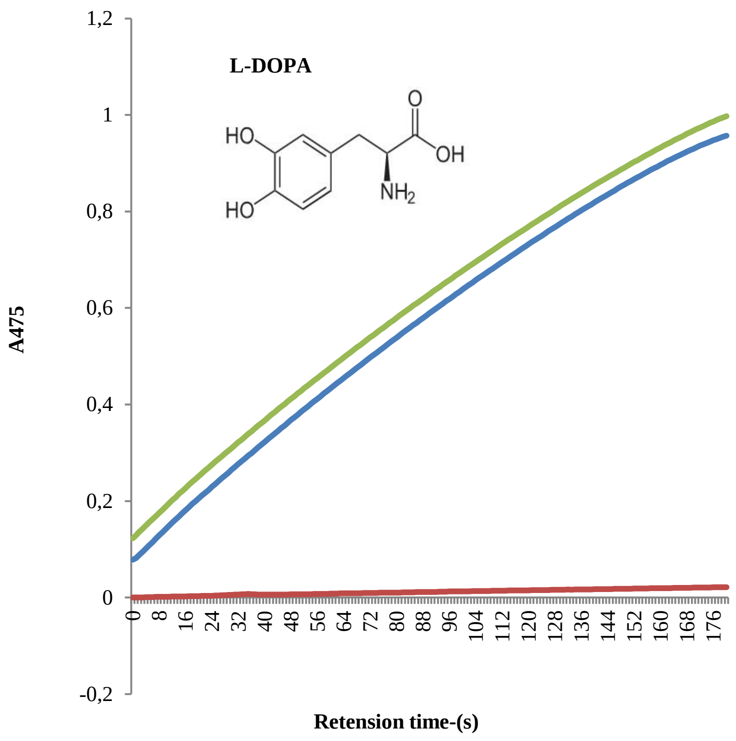
FAB-MS of sample

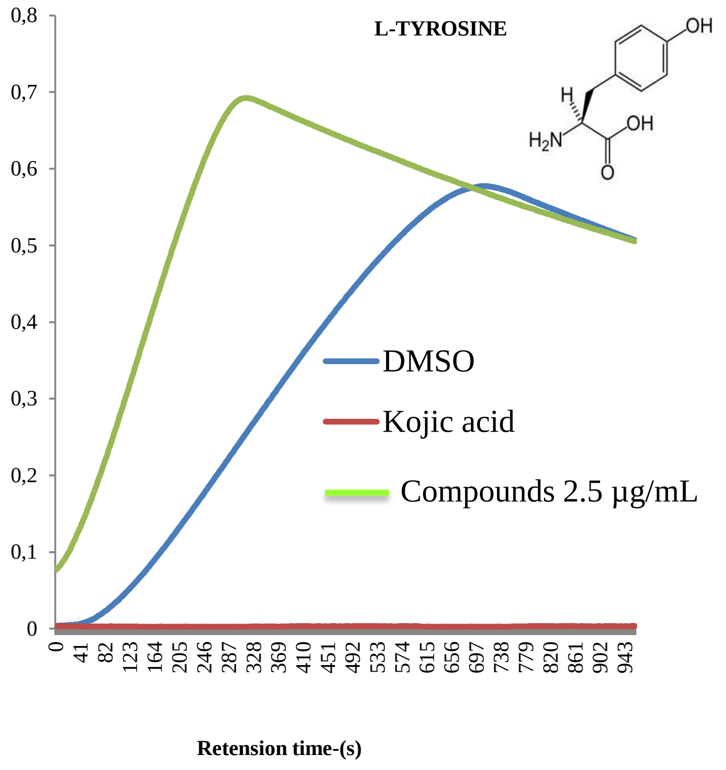




Melanin formation inhibition in B16 melanoma cells. The values were expressed as the means ±SDs, n=3. By the Dunnett test, treatments were significantly different from the control group (* p <0.05 and ** p <0.01).

Anti-tyrosinase activity with L-tyrosine and L-DOPA as substrates, using the mixture of crysophanol and pachybasin as inhibitor to compare with Kojic acid.





Study 4: Conclusion

Methanol extracts of the sugarcane bagasse exhibit a strong inhibition of melanin synthesis.

Two anthaquinone derivatives were identified and their combination showed strong melanin biosynthesis inhibitory activity.

These compounds do not have ability to inhibit the growth of *Escherichia coli and Staphylococcus aureus*, the results are not showed.

Future study to clarify which compound is responsible for this activity, and to test other biological activities, which could become an alternative to source for cosmetics as well medicals.

General conclusion and recommendations

- First, successfully applied a fungal MG-60 and NaOH for pretreatment, and fermentation processes to convert sugarcane bagasse into ethanol using single fermenter and *Phlebia*. sp MG-60.
- Second, ethanol production through integrated fungal fermentation (IFF), involving a unified process for biological pretreatment with CBP by *Phlebia* sp. MG-60, was employed to sugarcane bagasse.
- The evaluation of the waste liquid derived from alkaline pretreatment was performed by checking anti-melaanogenesis assay and anti-bacterial assay.
- Two valuable chemicals in sugarcane bagasse responsible for antimelanogenesis of B16 melanoma cells was identified are Chrysophanol and Pachybasin.

Further study

1. **Screening of other lignolytic helper enzymes.**

   In the natural degradation of biomass have many microorganism to work together. Moreover, with different materials due to their different chemical compositions, the required enzyme systems are specific for each material. Therefore, we suggest the screening of several other lignocellulosic biomasses, such as rice straw, switchgrass and corn stover to determine the best enzymes for each, and then we can use one appropriate cocktail of these enzymes for degradation.

2. **Searching for high value added chemicals from lignocellulosic materials**

   Another possible process improvisation is to isolate valuable chemicals from sugarcane bagasse and other materials. Each material has unique chemical composition, and because of in their structure contains specific compounds, which compound is major is needed to clarify to have exact evaluation of these compounds for other goals, such as cosmetics or medical industry.

Thank you

&

Mabuhay!