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Biological hydrogen production using an anaerobic fluidised bed bioreactor

Thompson, Liam Jed

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Appendix 1 – Reagents, Equipment and Suppliers

Supplier	Chemical
Merck (South Africa)	Ethanol (Absolute), K_2HPO_4, $MgCl_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $CoCl_2 \cdot H_2O$, H_2SO_4, NaCl, Yeast Extract, Peptone, Lactose, Glucose, Agar, MacConkey Agar with Crystal Violet, Phenol red
BDH (Merck, South Africa)	$MnSO_4 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, KCl, Ferric citrate, Sodium Thiosulphate
Associated Chemical Enterprises	Hydrochloric Acid (32%), Granular Activated Carbon
Protea Industrial Chemicals (South Africa)	NH_4HCO_3, $NaHCO_3$, NaOH flakes,
Oxoid	Eosin Methylene Blue Agar, Potato Dextrose Agar, Lab Lemco' powder
Fluka	Resorcinol
Makro (South Africa)	Sucrose

Supplier	Equipment
Measurement and Control Solutions	*DataTaker* DataLogger
Perspex World	Feed Bins, Reservoir
Mazey's Plastics	Perspex for reactor construction
Leeways Garden Centre	Plumbing components
A-Z Technical Services	Electrode housing vessel
Wirsam Scientific	Autoclude Peristaltic Pumps
Swiss Lab	Barometer,
AP Electronics	Wiring, Resistors, Control System Housing
Aquapump	Boyser AMP-16 Peristaltic Pump
Laboratory Automation and Control	Grant Digital 60 Waterbath
Evna Industrial Products	Silicon Grease

Appendix 2 – Media and General Reagents

Unless otherwise specified, all media was made up to 1L with distilled H_2O and autoclaved at 121^oC for 15 minutes.

KLIGLER IRON AGAR

Formula	g/L
Lab Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
NaCl	5.0
Lactose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.05
Agar	12.0
pH 7.4 ± 0.2	

MODIFIED MACCONKEY BROTH

Formula	g/L
Peptone	20.0
Lactose	10.0
Bile salts	5.0
NaCl	5.0
Neutral red	0.075
Ferric Citrate	1.5
Sodium Thiosulphate	5.0
pH 7.4 ± 0.2	

EOSIN METHYLENE BLUE AGAR (MODIFIED) LEVINE

Formula	g/L
Peptone	10.0
Lactose	10.0
K_2HPO_4	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH	6.8 ± 0.2

INDOLE MEDIA

Formula	g/L
Tryptone	10.0
NaCl	5.0
Glucose	1.0
Agar	1.25

ENDO FORMULATION

NH_4HCO_3 and $NaHCO_3$ were both filter sterilised due to their low thermal decomposition temperatures. Sucrose and salts were autoclaved together at 121^{o}C for 15 minutes.

Chemical Component	(g/L)
Sucrose	17.80
NH_4HCO_3	5.240
$NaHCO_3$	6.720
K_2HPO_4	0.125
$MgCl_2 \cdot 6H_2O$	0.100
$MnSO_4 \cdot 4H_2O$	0.015
$FeSO_4 \cdot 7H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.005
$CoCl_2 \cdot H_2O$	1.24×10^{-4}

MODIFIED ENDO FORMULATION – REACTOR MEDIUM

The components for the reactor were not sterilized due to the volume of chemicals used in the concentrated feed. The final volume of the concentrated feed was 5x with a volume of 100L.

Chemical Component	(g/L)	Concentrated Feed (g/100L)
Sucrose	17.65	8820
NH_4HCO_3	3.490	1732
K_2HPO_4	0.699	3360
$NaHCO_3$	6.720	349.3
$MgCl_2 \cdot 6H_2O$	0.100	50
$MnSO_4 \cdot 4H_2O$	0.015	7.5
$FeSO_4 \cdot 7H_2O$	0.025	12.5
$CuSO_4 \cdot 5H_2O$	0.005	2.5
$CoCl_2 \cdot H_2O$	1.24×10^{-4}	0.062

NUTRIENT BROTH

Component	g/L
Meat Extract	1.0
Yeast Extract	2.0
Peptone	5.0
Sodium Chloride	8.0

Appendix 3

Design of Fluidised Bed Reactor

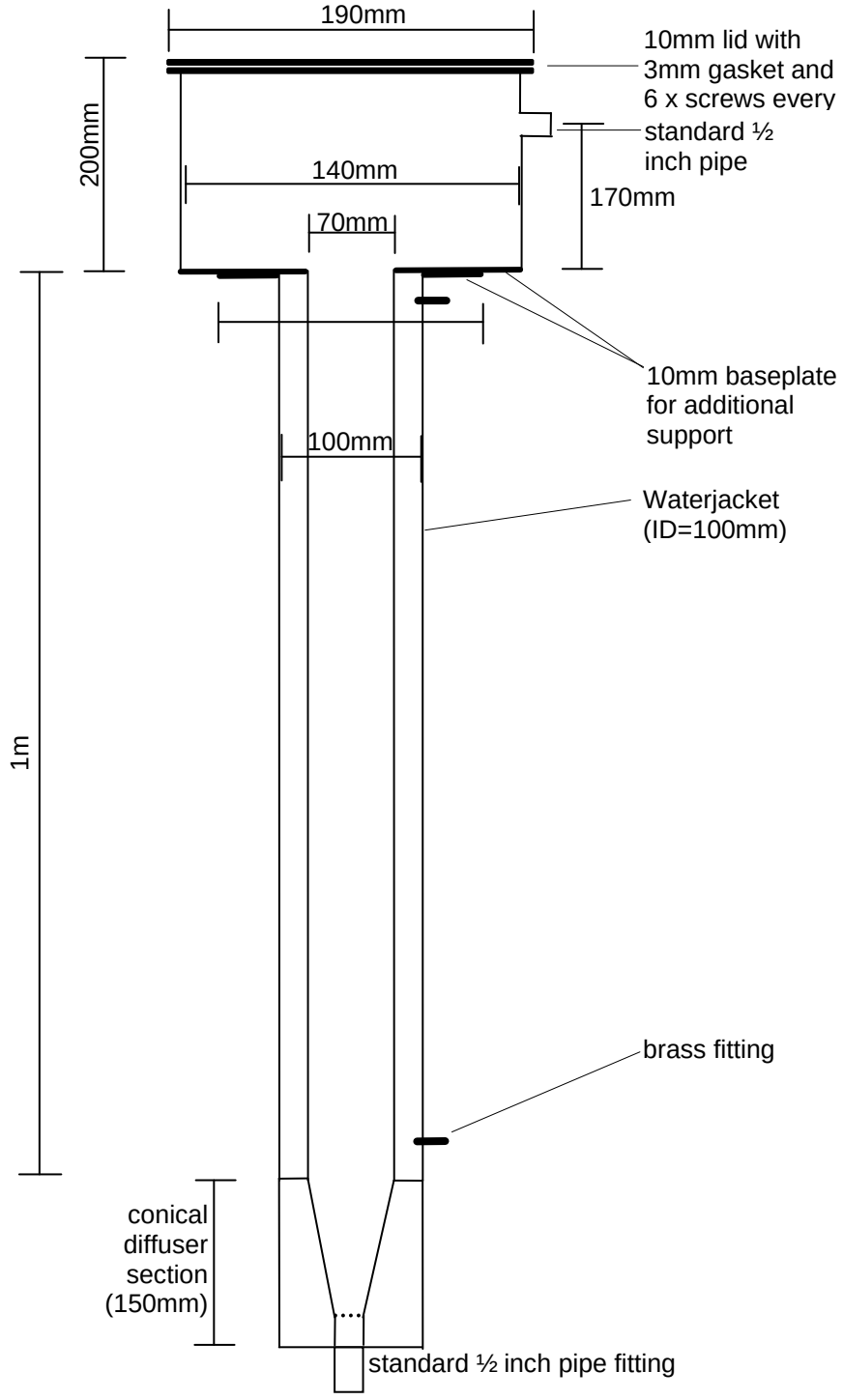


Figure c1: Technical schematic of entire fluidised bed reactor. Reactor was constructed from 5mm perspex unless otherwise stated.

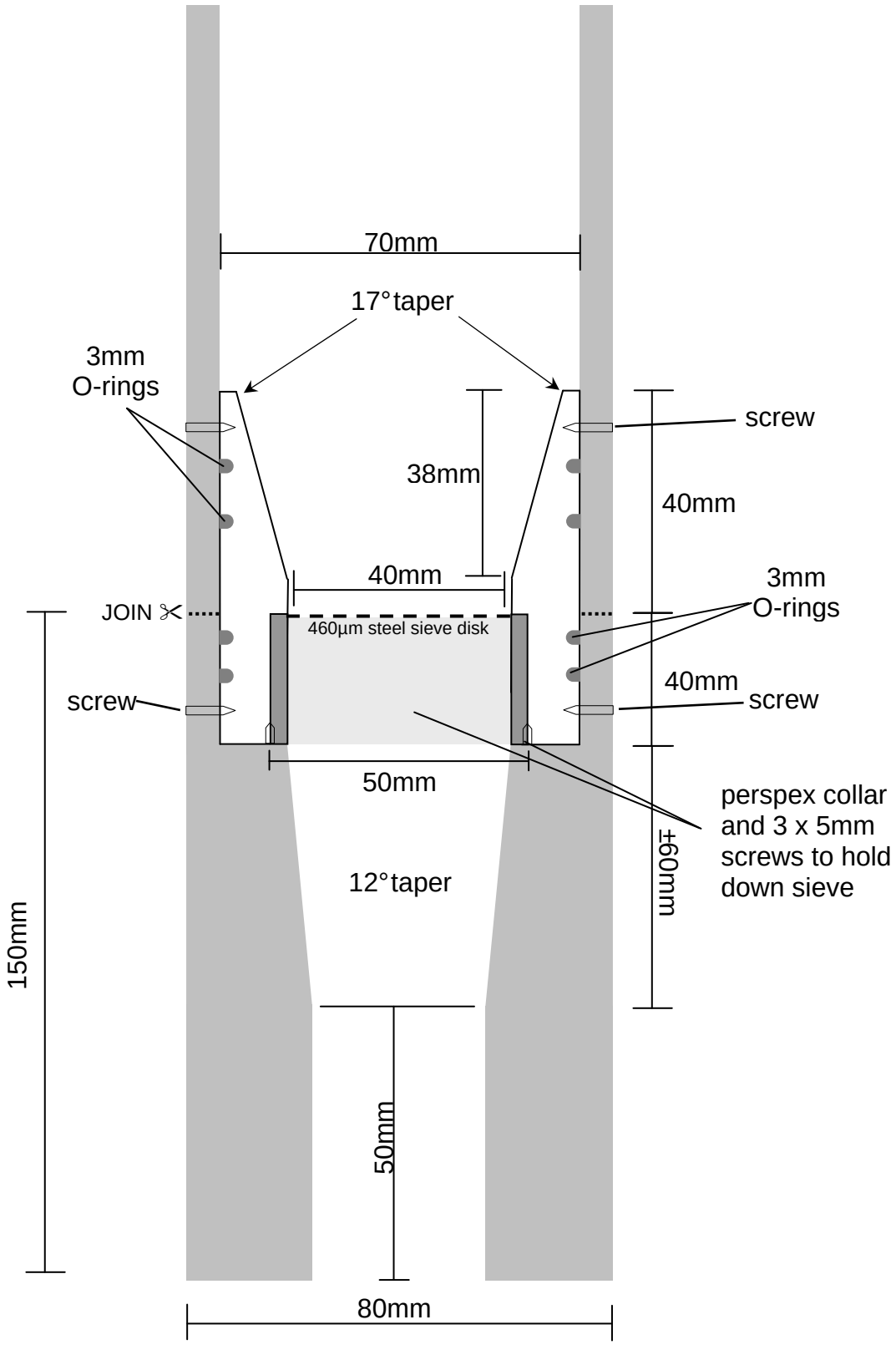


Figure c2: Dimensions of the conical diffuser portion of the fluidised bed reactor. The bottom section of the diffuser was machined out of a solid perspex rod. Double O-rings were included to prevent any escape of liquid from within the reactor.

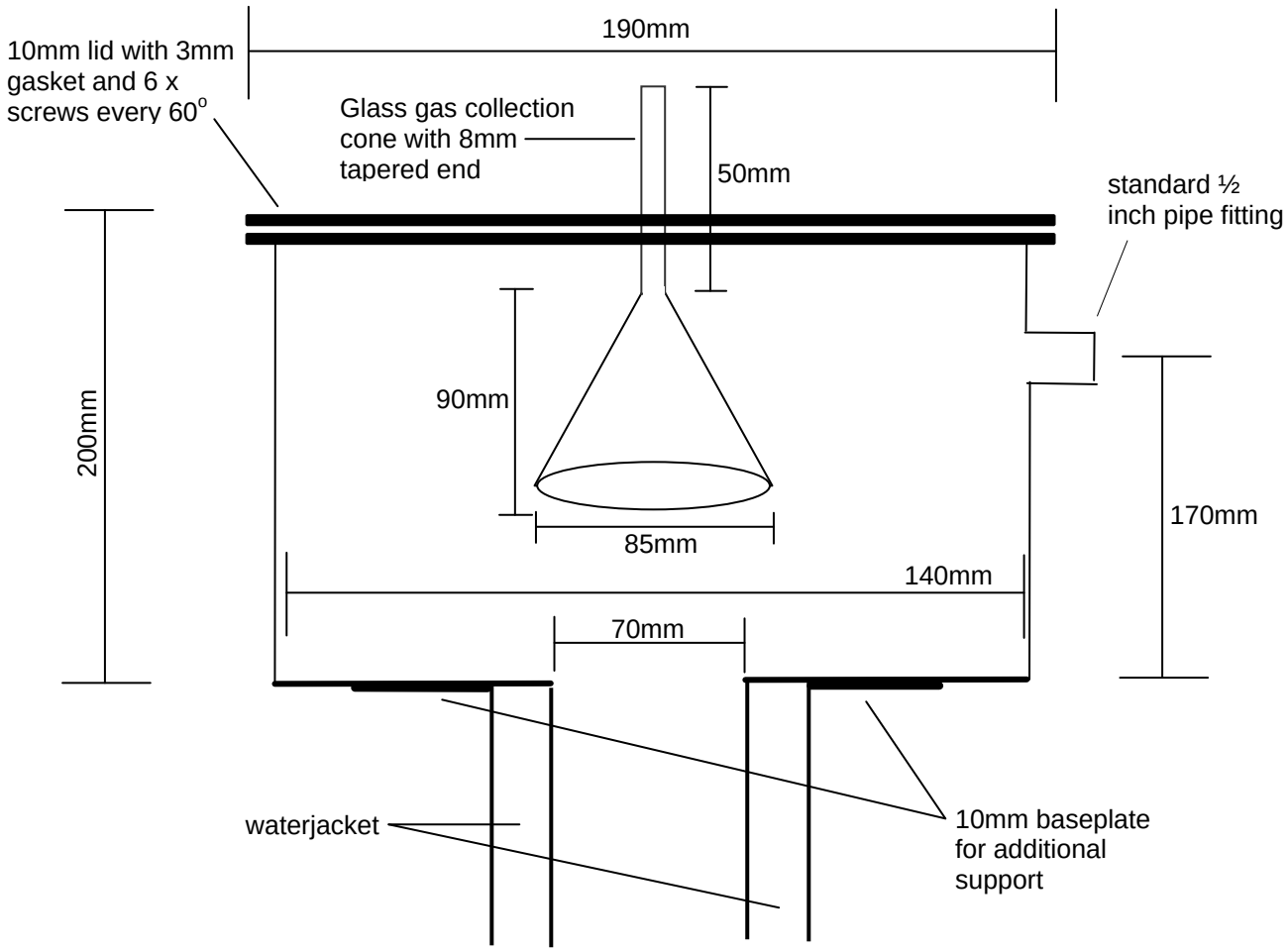


Figure c3: Technical schematic of the gas-solid separator portion of the fluidised bed. The glass cone is inserted and glued through the lid of the reactor. The glass cone is positioned so that it is able to catch all gas coming out of the central core of the reactor from the GAC bed.

Design of Acidified Water Gas Collector

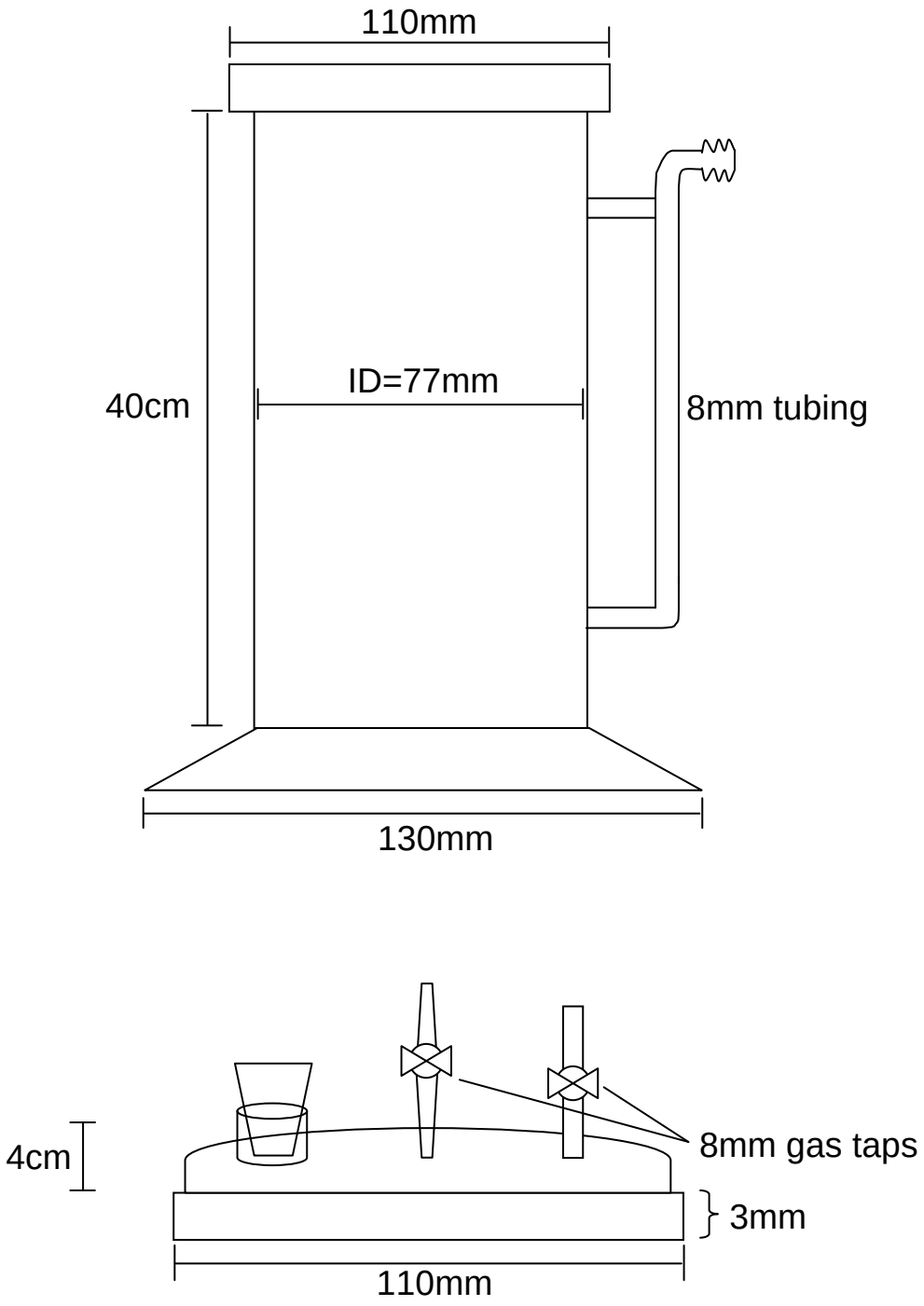


Figure c4: Technical schematic of portion A of the gas collector in which the acidified water from Standard Methods is placed (Franson, Clesceri *et al.* 1998). The internal diameter of the vessel is 77mm making every 2.18cm equal to 100ml based on the calculation of $(100cm^3)/\pi(3.8)^2$).

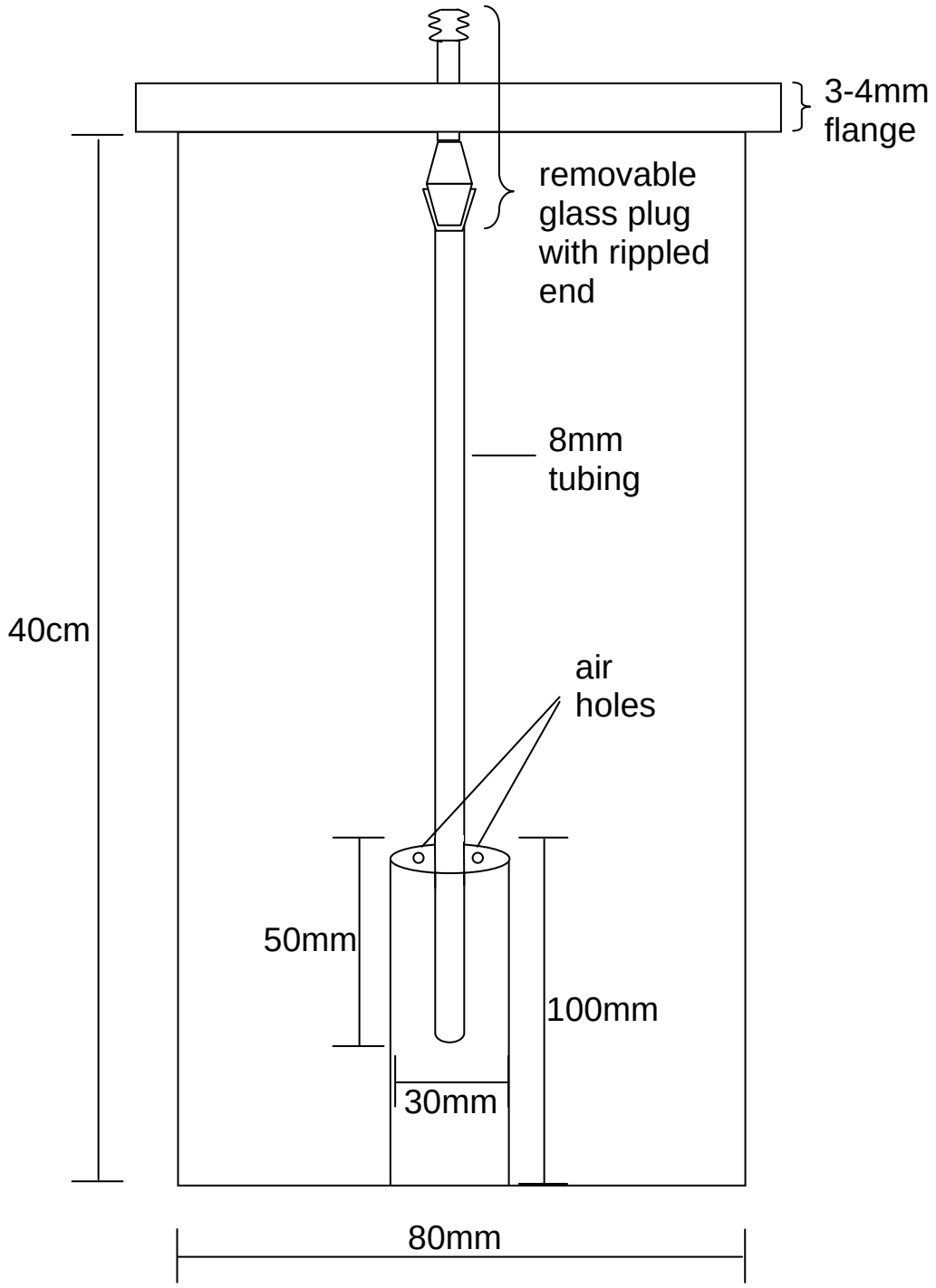


Figure c5: Technical schematic of portion B of the gas collector in which the acidified would flow into from portion A.

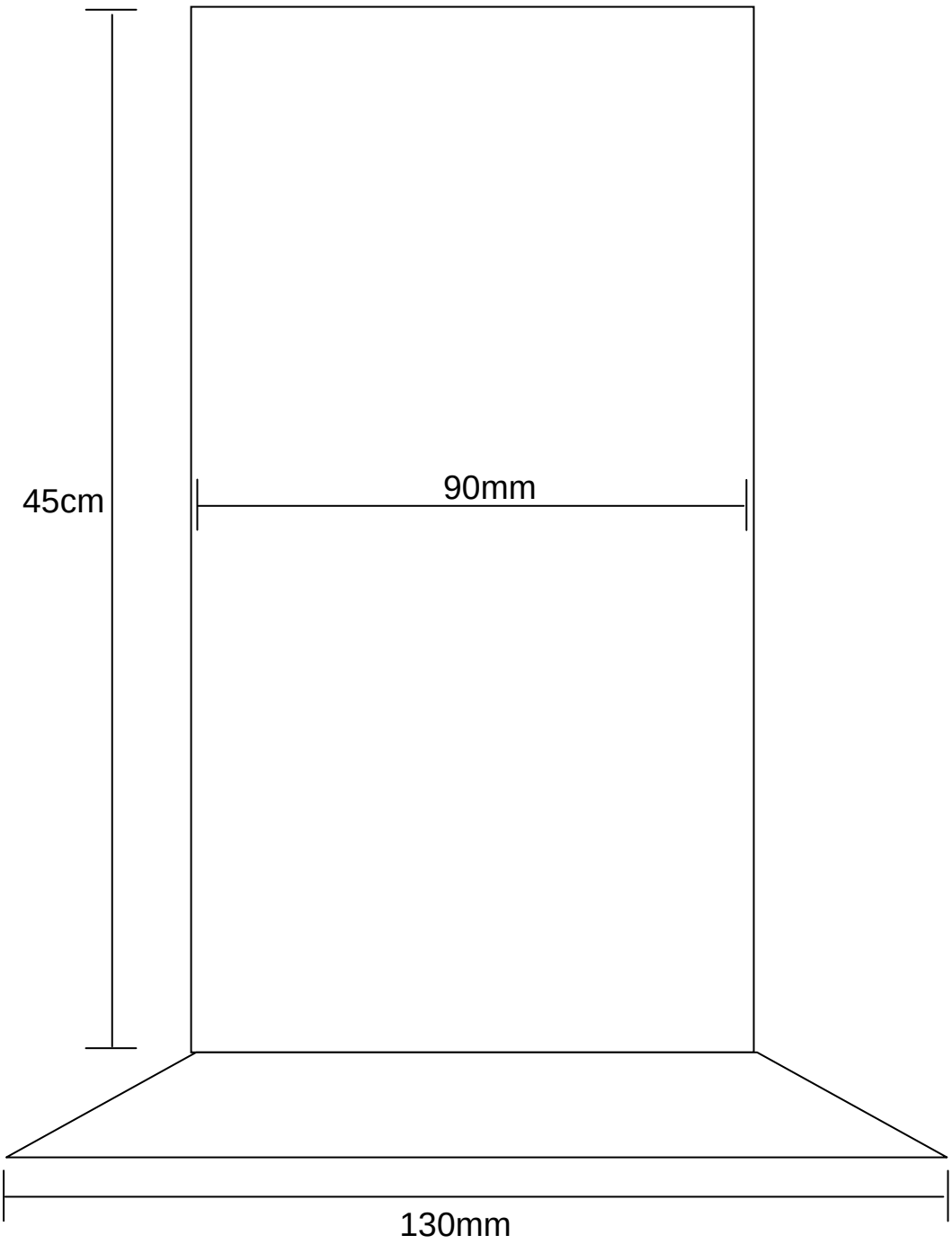


Figure c6: Technical schematic of portion C of the gas collector in which portion B would be placed while suspended on water. This portion is placed inside a bucket to collect the water that flows out of the top of the vessel.

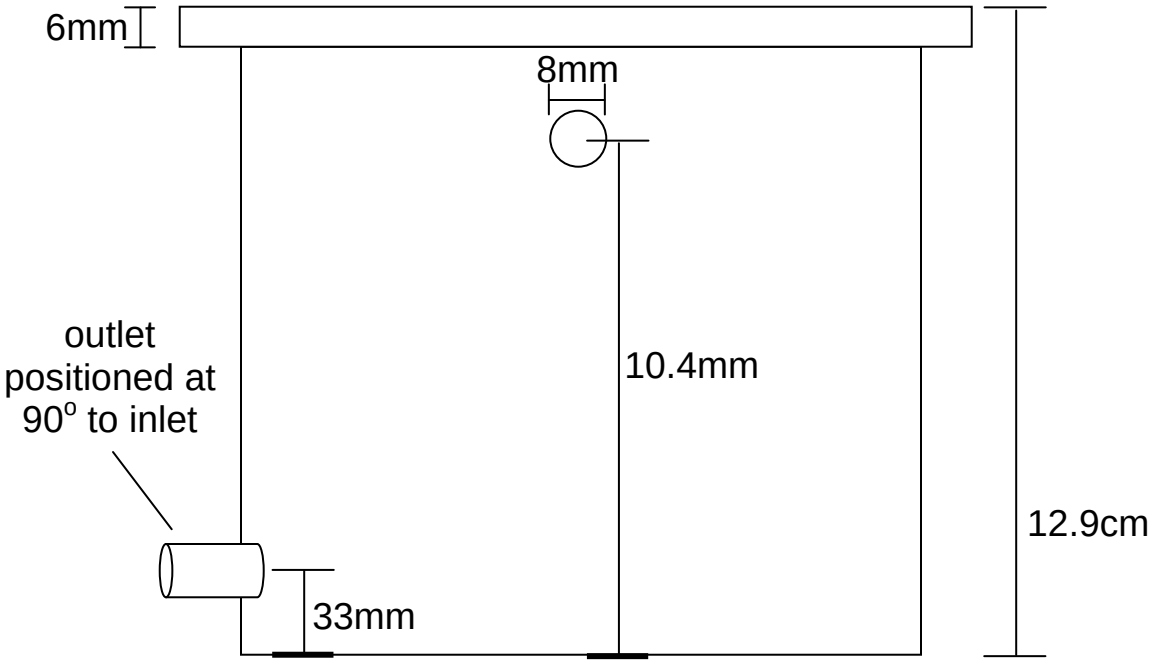


Figure c7: Dimensions of the electrode vessel used to house the monitoring electrodes.

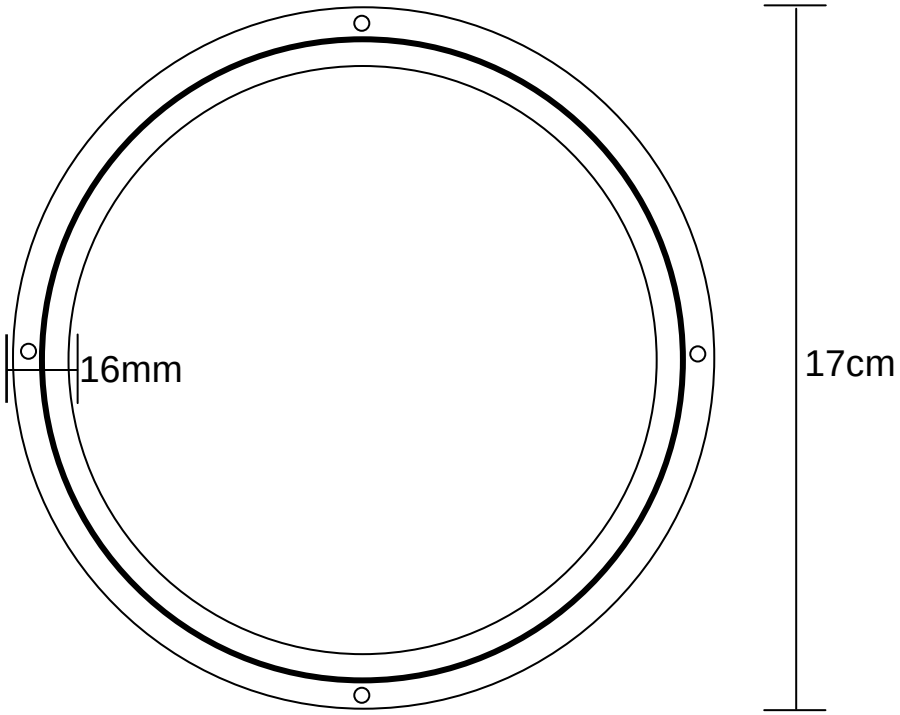


Figure c8: Top view of the electrode vessel used to house the electrodes.

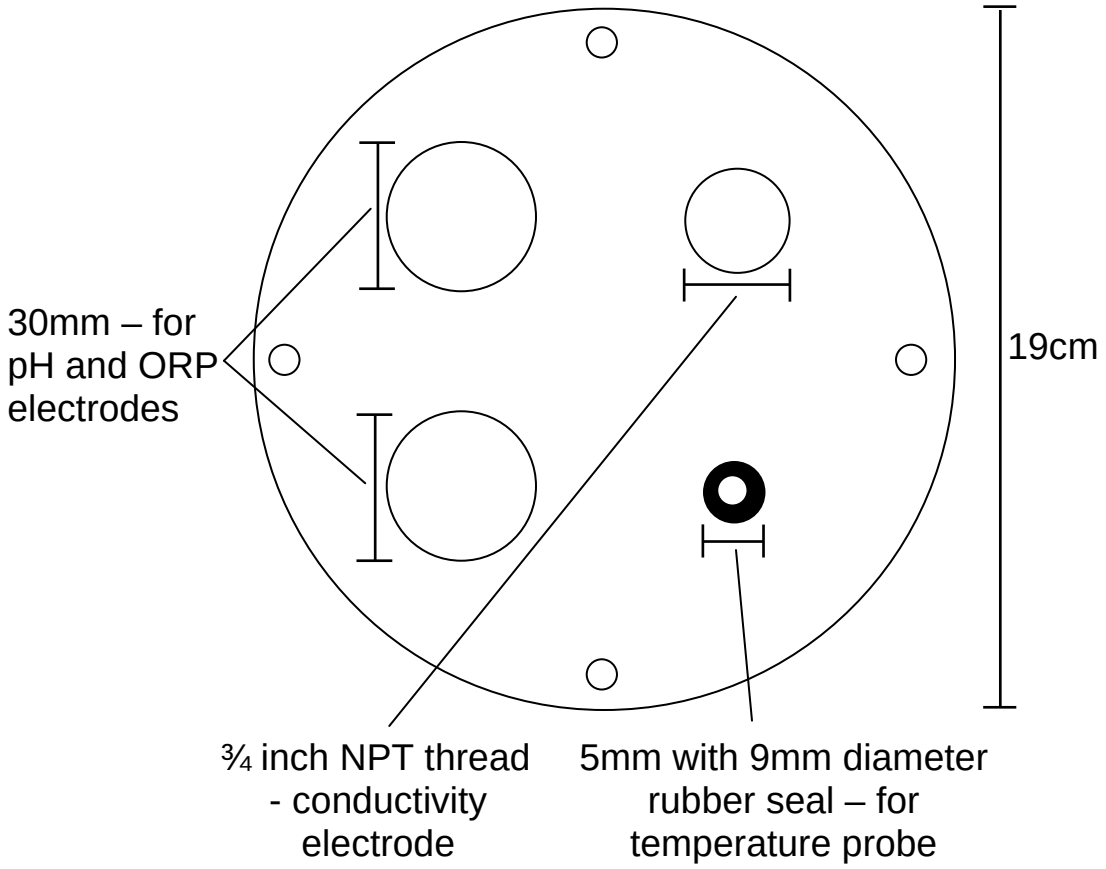


Figure c9: Lid of the electrode vessel into which the electrodes were fitted during the monitoring process.

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