Supporting Information

Understanding the mobilisation of a nitrification inhibitor from novel slow release pellets, fabricated through extrusion processing with PHBV biopolymer

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Table S1: Summary of the soil report provided by SWEP Analytical Laboratories, Keysborough, VIC, Australia for a soil supplied from a sugarcane field in Wangan, QLD, Australia

Cation Balance

ITEM			RESULT	DESIRABLE
pH(1:5 Water)			5.5	6.5-8.0
pH(1:5 0.01M $CaCl_2$)			4.91	
Electrical Conductivity	EC	µS/cm	298	< 780
TOTAL SOLUBLE SALT	TSS	ppm	983.4	< 2574
TOTAL ORGANIC MATTER %			4.44	4 - 6
TOTAL ORGANIC CARBON %			2.22	2 - 3

EXCHANGEABLE CATIONS			RESULTS	DESIRABLE LEVEL
CALCIUM	Ca	meq/100 of soil	3.08	8.05
MAGNESIUM	Mg	meq/100 of soil	1.05	1.86
SODIUM	Na	meq/100 of soil	0.05	< 0.62
POTASSIUM	K	meq/100 of soil	0.32	0.62
HYDROGEN	H	meq/100 of soil	10.1	
ADJ. EXCH. HYDROGEN	H	meq/100 of soil	7.88	< 1.86
CATION EXCHANGE CAPACITY	CEC	meq/100 of soil	14.6	
ADJUSTED CEC	Adj.CEC	meq/100 of soil	12.38	
SATURATION BASE PERCENTAGE	BSP		36	

EXCHANGEABLE CATION BALANCE		% OF ADJUSTED CEC	DESIRABLE
CALCIUM PERCENTAGE		24.9	65-70%
MAGNESIUM PERCENTAGE		8.5	12-15%
SODIUM PERCENTAGE	ESP	0.4	0.5-5%
POTASSIUM PERCENTAGE		2.6	3-5%
ADJ. HYDROGEN PERCENTAGE		63.7	<20%
CALCIUM / MAGNESIUM RATIO	Ca/Mg	2.95	2 - 4

Nutrient Balance

ITEMS			RESULTS	DESIRABLE LEVEL
AVAILABLE CALCIUM	Ca	ppm	778	1843
AVAILABLE MAGNESIUM	Mg	ppm	158.4	244
AVAILABLE SODIUM	Na	ppm	14.076	< 156
AVAILABLE NITROGEN	N	ppm	80.9	102
AVAILABLE PHOSPHORUS	P	ppm	15.8	75
AVAILABLE POTASSIUM	K	ppm	157.17	263
AVAILABLE SULPHUR	S	ppm	123	11 - 15
AVAILABLE COPPER	Cu	ppm	14.9	3
AVAILABLE ZINC	Zn	ppm	27.6	4 - 6
AVAILABLE IRON	Fe	ppm	10	> 30
AVAILABLE MANGANESE	Mn	ppm	6	> 20
AVAILABLE COBALT	Co	ppm	2.11	0.7-0.8
AVAILABLE MOLYBDENUM	Mo	ppm	0.13	0.3-0.4
AVAILABLE BORON	B	ppm	0.29	0.6-1.0
TOTAL PHOSPHORUS	TP	ppm	1080	
TOTAL NITROGEN	TN	%	0.18	

Biology Balance

ITEM			Result	% of TAP	Desirable	% Desirable
ACTIVE LACTIC ACID BACTERIA	cfu/g soil		120,000	38.7%	113,066	17.0%
Active Fungi	cfu/g soil	50,000				
Cellulose utilisers	cfu/g soil	70,000				
TOTAL ACTIVE FUNGI	cfu/g soil		120,000	38.7%	219,480	33.0%
ACTIVE YEASTS	cfu/g soil		100	0.0%	106,415	16.0%
ACTIVE ACTINOMYCETES	cfu/g soil		70,000	22.6%	139,669	21.0%
ACTIVE PHOTOSYNTHETIC BACTERIA	cfu/g soil		100	0.0%	86,462	13.0%
Total Active Population (TAP):	cfu/g soil		310,200		665,092	
CARBON/NITROGEN RATIO			12.3		10-15	

Table S2: Nutrient media used for leaching columns containing biologically active soil. The bulk nutrient media was diluted 1:100 with deionised water and pre-warmed to 30 °C before it was added to the column.

NMS (for methanotrophs)		
Composition per liter		
Agar	12.5	g
$MgSO_4.7H_2O$	1.0	g
KNO_3	1.0	g
$Na_2HPO_4.H_2O$	0.7	g
KH_2PO_4	0.3	g
$CaCl_2.6H_2O$	0.2	g
Ferric ammonium EDTA	4.0	g
Trace elements solution	0.5	ml
pH 6.8 ± 0.2 at 25°C		
Trace elements solution		
Disodium EDTA	0.5	g
$FeSO_4.7H_2O$	0.2	g
H_3BO_3	0.03	g
$CoCl_2.6H_2O$	0.02	g
$ZnSO_4.7H_2O$	0.01	mg
$MnCl_2.4H_2O$	3.0	mg
$Na_2MoO_4.2H_2O$	3.0	mg
$NiCl_2.6H_2O$	2.0	mg
$CaCl_2.2H_2O$	1.0	mg
Preparation of trace elements solution:		
Add components to distilled/deionized water and bring volume to 1 L. Mix thoroughly.		
Preparation of medium:		
Add components to distilled/deionized water and bring volume to 1 L. Mix thoroughly. Gently heat and bring to boilling. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 121°C. Pour into sterile Petri dishes or leave in tubes.		

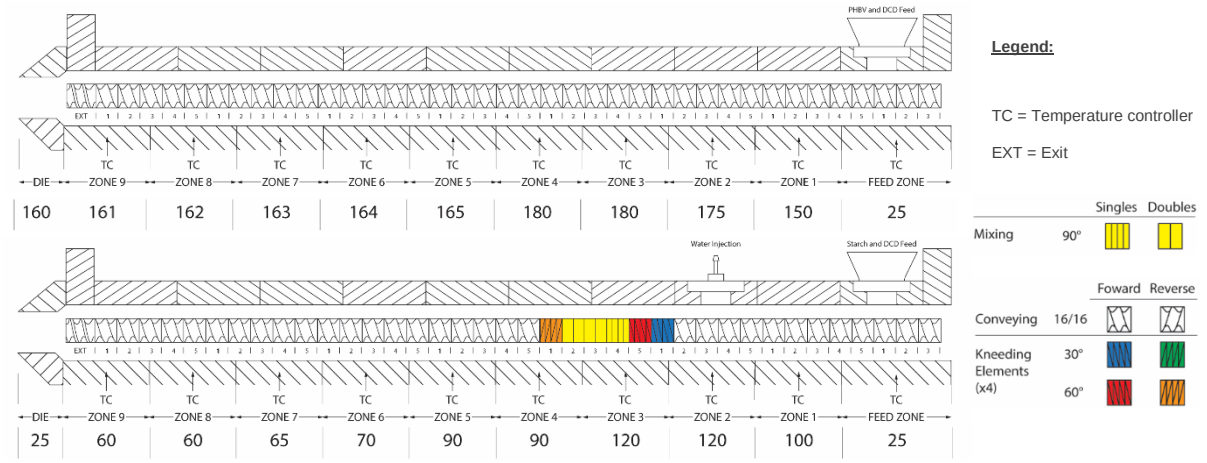

Figure S1: Extruder schematic illustrating the screw profile and temperature for each zone (shown along the bottom) used during the processing of DCD with A) PHBV and B) PS. Material flows from right to left. The starch is plasticized within the extruder with water injected in zone 2 with a peristaltic pump to make a 60: 40 water: starch weight ratio. Kneading and mixing zones in zones 3, 4 and 5 aid the hydration and plasticization of the of the starch molecules. The mixing section included a 30º and a 60º kneading section, followed by four single and four double mixing screw elements and finally a 60º reverse kneading element.

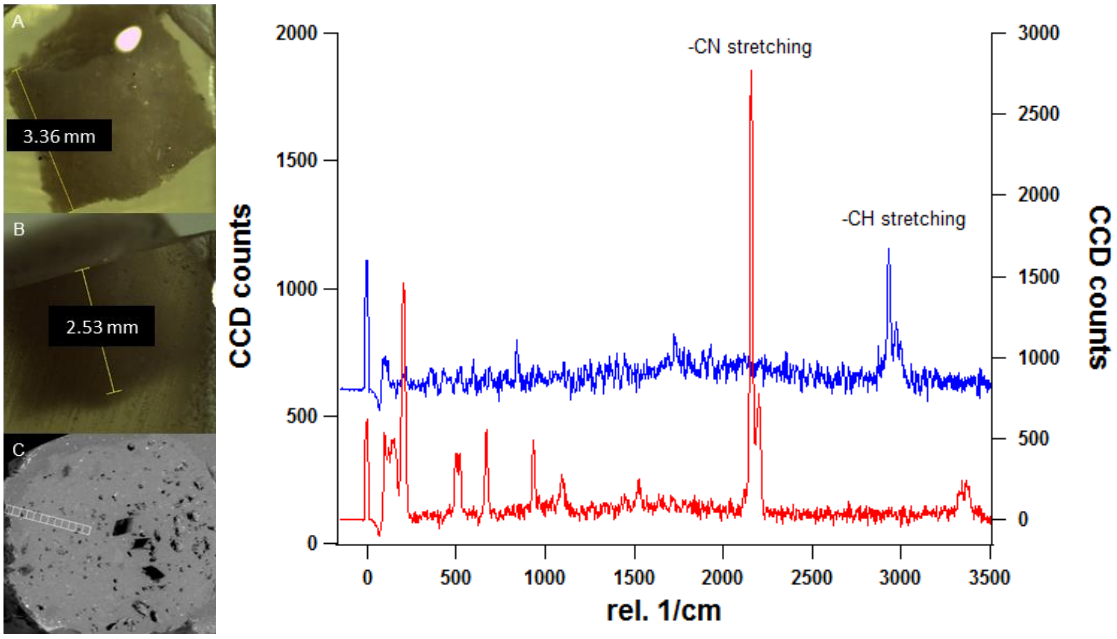

Figure S2: Left - an example of a DCD-PHBV pellet embedded in resin A) before and B) after microtoming. C) is a top view backscattered SEM micrograph of the embedded, microtomed pellet, showing the region of interest (ROI) where the 12 optical images and Raman maps were acquired from. The ROI started at the outer edge and progressively moved toward the centre of the pellet. Right - Raman signature of DCD (red/lower spectrum, right y-axis) and PHBV (blue/upper spectrum, left y-axis) calculated from the microtomed pellet. Raman maps were acquired on an Alpha 300 Raman/AFM (WITec GmbH, Ulm, Germany) equipped with a frequency-doubled continuous-wave Nd:YAG laser to obtain a 532 nm excitation line through a collar corrected objective (Nikon 40X, N.A. 0.6, CFI S Plan Fluor ELWD objective). The back-scattered Raman signal was collected with a 100 µm optical fiber, employing an Andor Raman spectrometer (600 grooves per mm grating) with an electron-multiplier charge-coupled device (EMCCD) spectroscopic detector. Raman maps were generated by binning the CN vibration mode at 2154 rel. cm^{-1}..

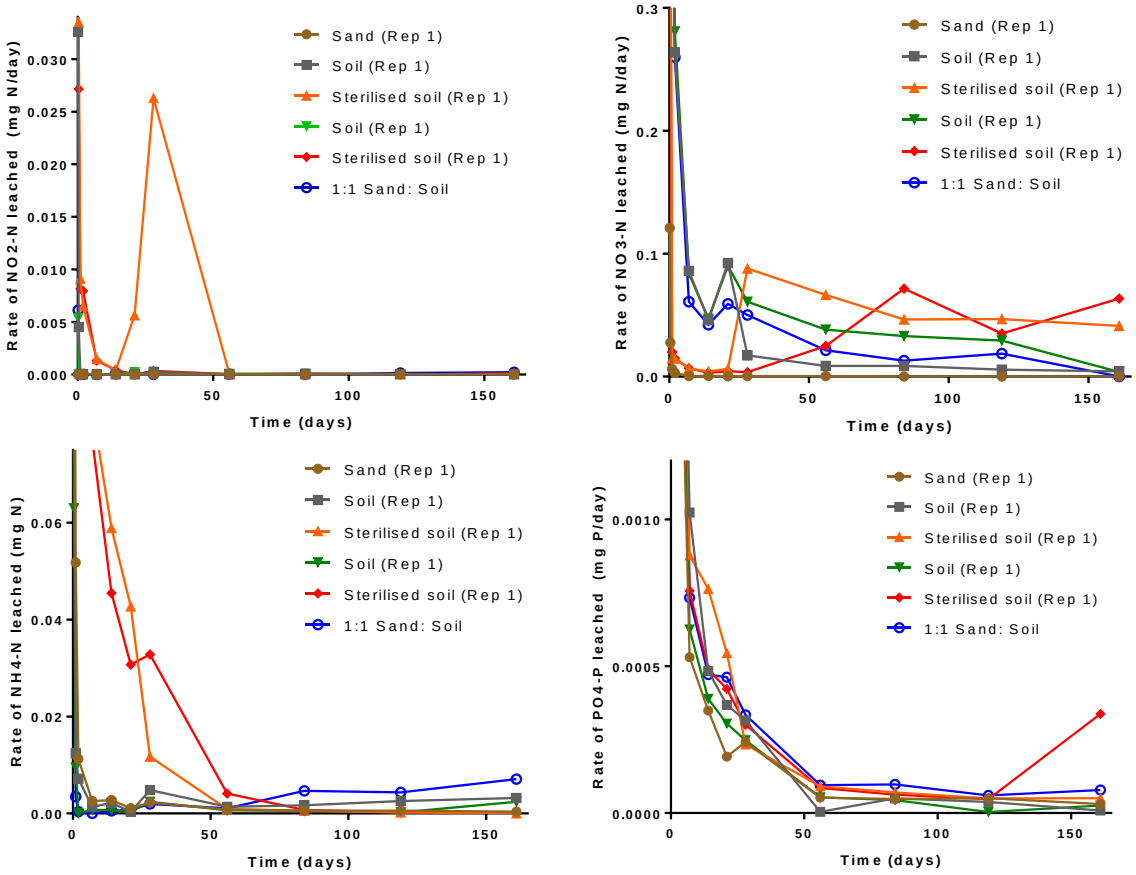

Figure S3:The rate of leaching of nitrite (NO_2^-), top left, nitrate (NO_3^-), top right, ammonia (NH_4^+), bottom left and phosphate (PO_4^-), bottom right, from the incubation columns calculated from FIA results.

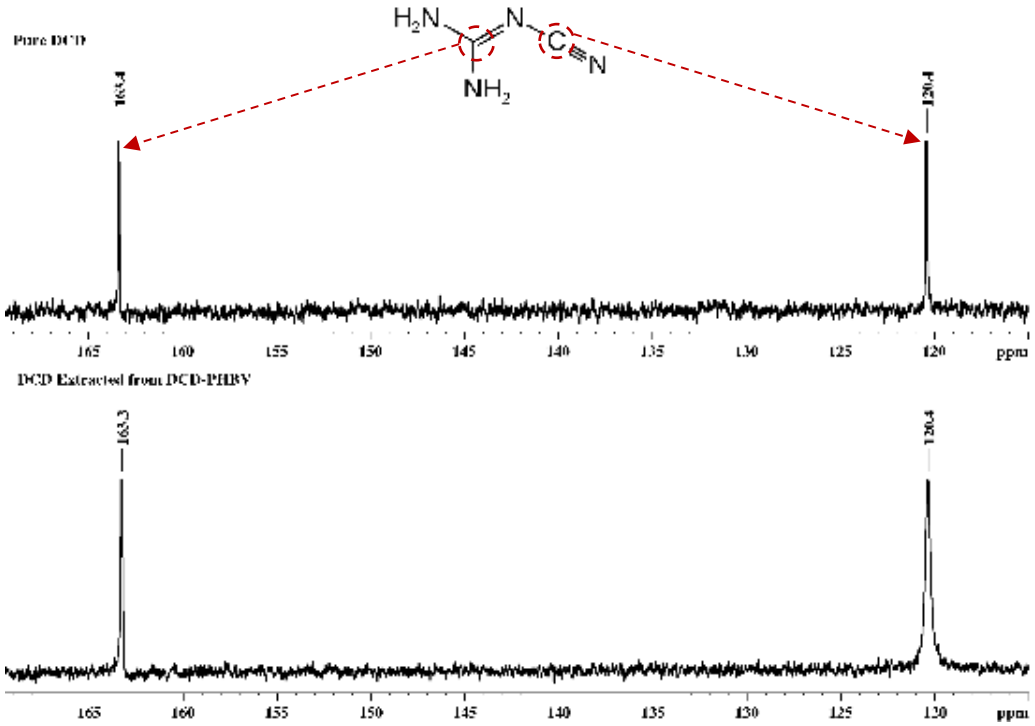

Figure S4: ^{13}C-NMR of pure DCD (top) and DCD extracted from an extruded DCD-PHBV pellet (bottom).

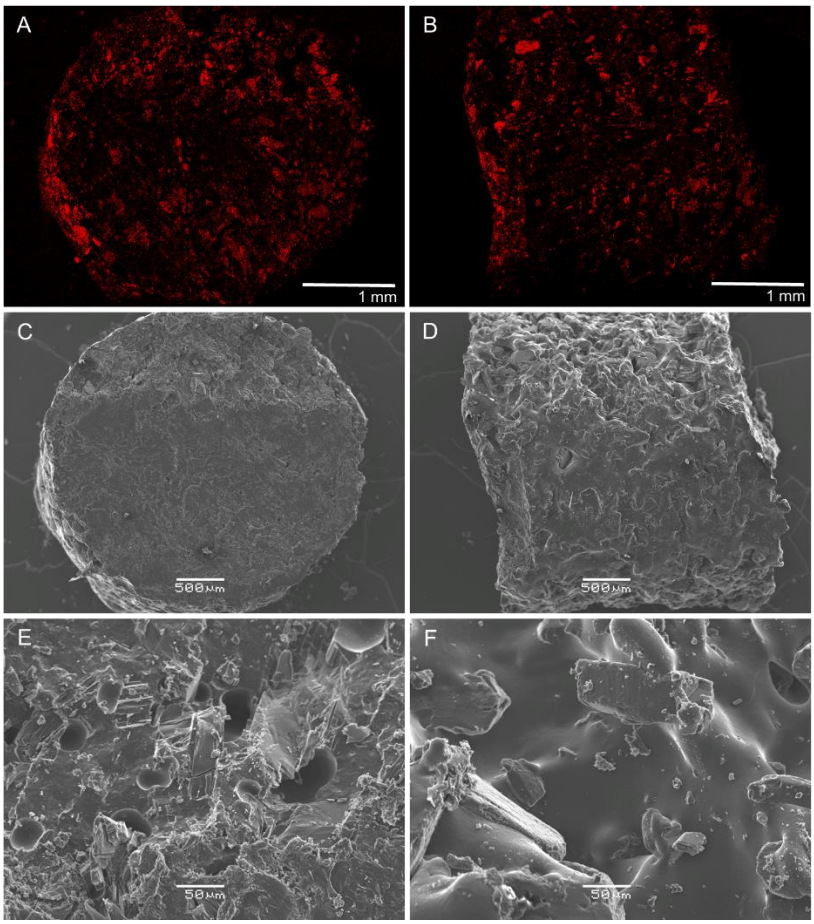

Figure S5: EDX nitrogen map overlays (A and B) and SEM micrographs at 100X (C and D) and 300X (E and F) magnification of the transverse (A, C and E) and lateral (B, D and E) face of a DCD-PHBV pellet. Red regions on the EDX indicates domains rich in nitrogen associated with dicyandiamide crystals.

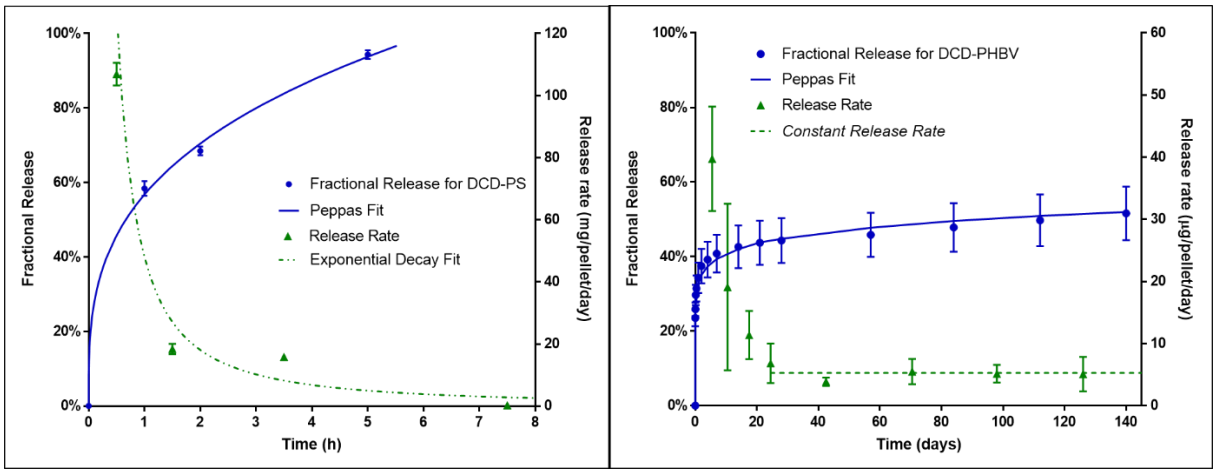

Figure S6: Fractional release and release rate of DCD from DCD-PS pellets (left) and DCD-PHBV (right) into DI water. Data points are the mean of three triplicates with error bars representing one standard deviation from the mean.

Table S3: Results from fitting the Korsmeyer-Peppas equation to the aqueous release data.

	k (d^{-n}) (± 95% confidence limits)	**n (± 95% confidence limits)**	**R^2**
DCD-PHBV	32.9 (± 1.7)	0.09 (± 0.02)	0.874
DCD-PS	153 (± 10)	0.31 (± 0.03)	0.997

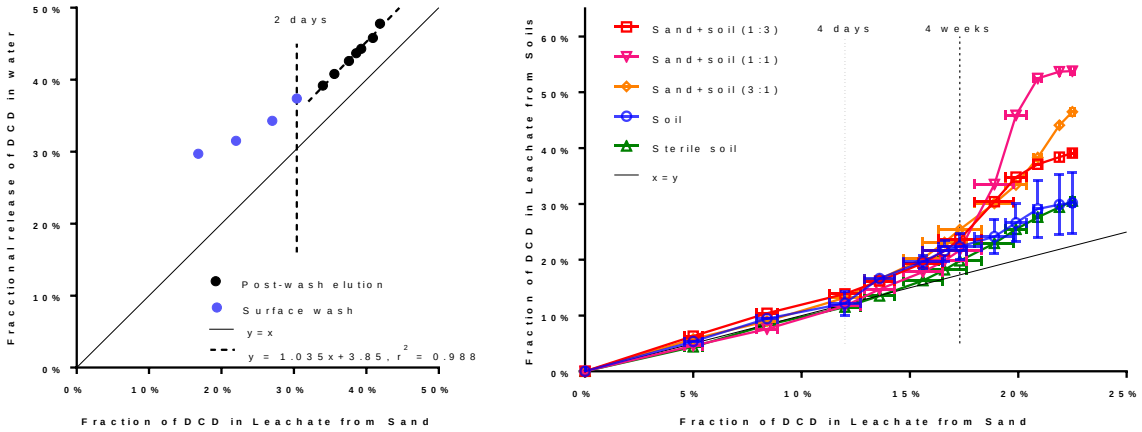

Figure S7: Left - fractional release data for water plotted against sand, with a linear regression fitted to the post-surface wash data. Data points in blue represent time points of 5 h, 10 h, 1 d and 2 d, while data points in black correspond to time points from 4 d out to 12 weeks. Right - the post-surface wash (after 10 h incubation) fraction of DCD accounted for in the leachate for sand plotted against soil and sand: soil mixtures of 1:3, 1:1 and 3:1. Error bars show one standard deviation from the mean.