Supporting Information for:

Evaluating the simultaneous retention of organic matter, organic contaminants, and *Escherichia coli* (*E. coli*) in biochar-amended biofilters

Johanna Jernberg,[1,2] Tadele Haile,[1] and Bridget Ulrich[1,*]

[1]Natural Resources Research Institute, University of Minnesota Duluth, Duluth, MN, 55811

[2]Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN, 55812

[*]Corresponding author; email: ulrichb@d.umn.edu

TABLE OF CONTENTS:

AMERICAN BIOCHAR COMPANY

Technical Data Sheet

NAKED Char® *Activated Biochar*

Purpose:

- Improves physical and biological soil characteristics
- Increases soil cation exchange capacity
- Improves water holding capacity
- Reduces leaching of fertilizer and nutrients
- Decreases soil compaction & has liming effects
- Promotes healthy soil microbiology
- Remediates contaminants and excess salts
- Sequesters carbon & reduces carbon footprint

General Information	
Composition	100% Wood BioChar
Feedstock	Southern Yellow Pine Species
Production Method	Pyrolysis, temp. range of 550-900° C
Pore Surface Area	557 acres/cf (225 hectares/cf)
Carbon Content	77.6% (USDA 95%)
Particle Size	.5mm – 2.0mm
Bulk Density	15.1 lbs/cu ft
Moisture Content	25 – 46%

TYPICAL ANALYSIS	
pH	7.5-9.0
Hydrogen:Carbon Ratio (H:C)	1:3 (.37)
Nitrogen (N)	.40% tdm
Phosphorous (P)	837 mg/kg
Potassium (K)	1215 mg/kg
Iron (Fe)	1014 mg/kg
Manganese (Mn)	457 mg/kg
Sodium (Na)	nd
Magnesium (Mg)	.36% dwt
Calcium (Ca)	2.22% dwt
Zinc (Zn)	14.1 mg/kg

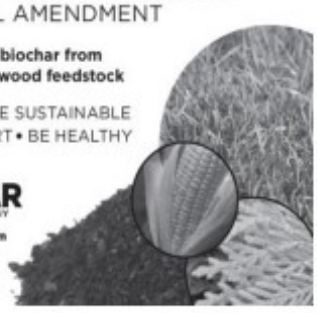
OMRI LISTED For Organic Use · USDA CERTIFIED BIOBASED PRODUCT · IBI Certified biochar · Made in USA

NAKED Char
SOIL AMENDMENT

Pure, activated biochar from single-sourced wood feedstock

• BE GREEN • BE SUSTAINABLE
• BE ECO-SMART • BE HEALTHY

AMERICAN BIOCHAR COMPANY
visit us at: www.ambiochar.com



Available sizes: 2 CY Tote
1.5 CF Bag (pending)

Application

Non-Ag Rates for 50% blended Biochar
Turf and Landscape bed rates: 1.5 - 3 gallons/ 1000 sf
Tree rates: half gallon/ inch dbh

Conventional Agriculture rates:
.25 -1.0 cf/ 1000 sf (10 - 44 cf/ acre)

Aggressive (Remediation) Agriculture rates:
1 -3 cf/ 1000 sf (45 – 130 cf/ acre)

Work into the soil, top-dress, broadcast or banding (may vary on per row acre), 1-2 times per year.

Note: Generally, biochar should not be used unless it has been pre-charged for non-agriculture applications.

Please contact the office for more specific application details.

American BioChar Company • P.O. Box 962 • Niles, MI 49120
Tel: 269-663-2224 • www.ambiochar.com

Figure S1. Technical specifications sheet for NAKED Char biochar (ABC-biochar), obtained from the American Biochar Company.

Table S1. Particle size distribution for concrete sand (provided by Plaisted Companies).

Sieve Size	Passing (%)
9.5 mm (3/8 inch)	100
4.75 mm (No. 4)	100
2.36 mm (No. 8)	96
1.18 mm (No. 16)	83
600 μm (No. 30)	60
300 μm (No. 50)	23
150 μm (No. 100)	4
75 μm (No. 200)	0.7

Table S2. Mineral composition of concrete sand determined by X-ray diffraction (includes illite, mica, kaolinite, and chlorite). Data was provided by Plaisted Companies.

Mineral	**Weight percent**
Quartz	65.7
K-feldspar	9.8
Plagioclase	17.6
Calcite	1.3
Dolomite	1.1
Pyrite	0.2
Total Clay Minerals	4.3

Table S3. Results for ultimate and proximate analysis, cumulative pore volume, and pH for commercial biochars. Biochars were analyzed as received, and composition results for dry weight for proximate analysis results were calculated based on results that included moisture content for comparison purposes.

Parameter	Method	ABC-biochar, wet	ABC-biochar, dry	WF-biochar, wet	WF-biochar, dry
Moisture total (wt%)	ISO 18134-1	13.52	-	2.12	-
Ash (wt%)	ISO 18122	9.42	10.89	74.90	76.52
Volatile matter (wt%)	ISO 18123	5.86	6.77	8.29	8.47
Fixed Carbon (wt%)	By difference	71.19	82.3	14.63	14.95
Sulfur (wt%)	ISO 16994	0.01	0.01	0.06	0.06
Carbon (C) (wt%)	ISO 16948	73.54		21.27	
Hydrogen (H) (wt%)	ISO 16948	0.26		0.21	
Nitrogen (N) (wt%)	ISO 16948	0.22		0.11	
O (O) (wt%)	ISO 16948	3.01		1.33	
H/C	NA	0.0421		0.118	
O/C	NA	0.0307		0.0472	
(O+N)/C	NA	0.0333		0.0516	
pH in DI water	NA	9.67		7.17	
Cumulative pore vol.	NA	0.443		0.0721	
Micropore vol. (cm^3/g)	NA	0.214		0.0405	
Mesopore vol. (cm^3/g)	NA	0.222		0.0316	

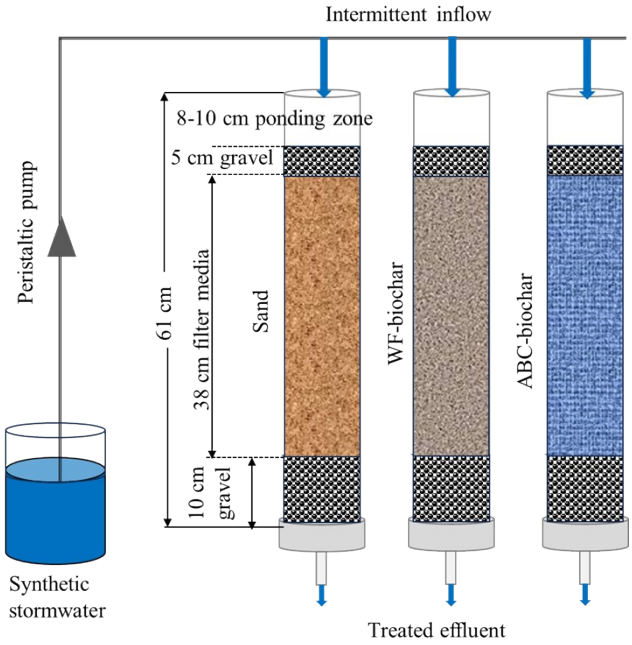


Figure S2. Generalized schematic of column apparatus used for dosing tests (conditions tested in triplicate).

Calculation S1. Dosing volume calculations. Bioretention column experiments were designed as recommended in the MN Stormwater Manual[1] based on historical rainfall characteristics for the Duluth, MN area (which receives an annual average precipitation depth of 76 cm).[2] Briefly, column tests simulated a bioretention system sized at 5% of the contributing impervious catchment area. Each experimental column had a 5 cm diameter, yielding a surface area of 19.625 cm². The impervious catchment area was calculated as the column surface area divided by the bioretention sizing factor (0.05), resulting in 392 cm².

The average annual stormwater volume entering a bioretention column was estimated using the Rational Method:[3]

$V=P\times A\times C$

where:

- V = Annual stormwater runoff volume (m³/year)
- P = Annual precipitation (cm/year)
- A = Catchment area (m²)
- C = Runoff coefficient (assumed 0.95)

Substituting values: $V=76\ \text{cm/year}\times 392\ \text{cm}^2\times 0.95$, which is 28.3 L/year.

Over a six-month experimental period, each column received 71.4 L of synthetic stormwater (2.1 L per event over 34 storm events). The equivalent treatment duration was calculated as:

$$Equivalent\ years = \frac{Vtreated}{Vrunoff} = \frac{71.4\ L}{28.3\frac{L}{year}} = 2.5\ years$$

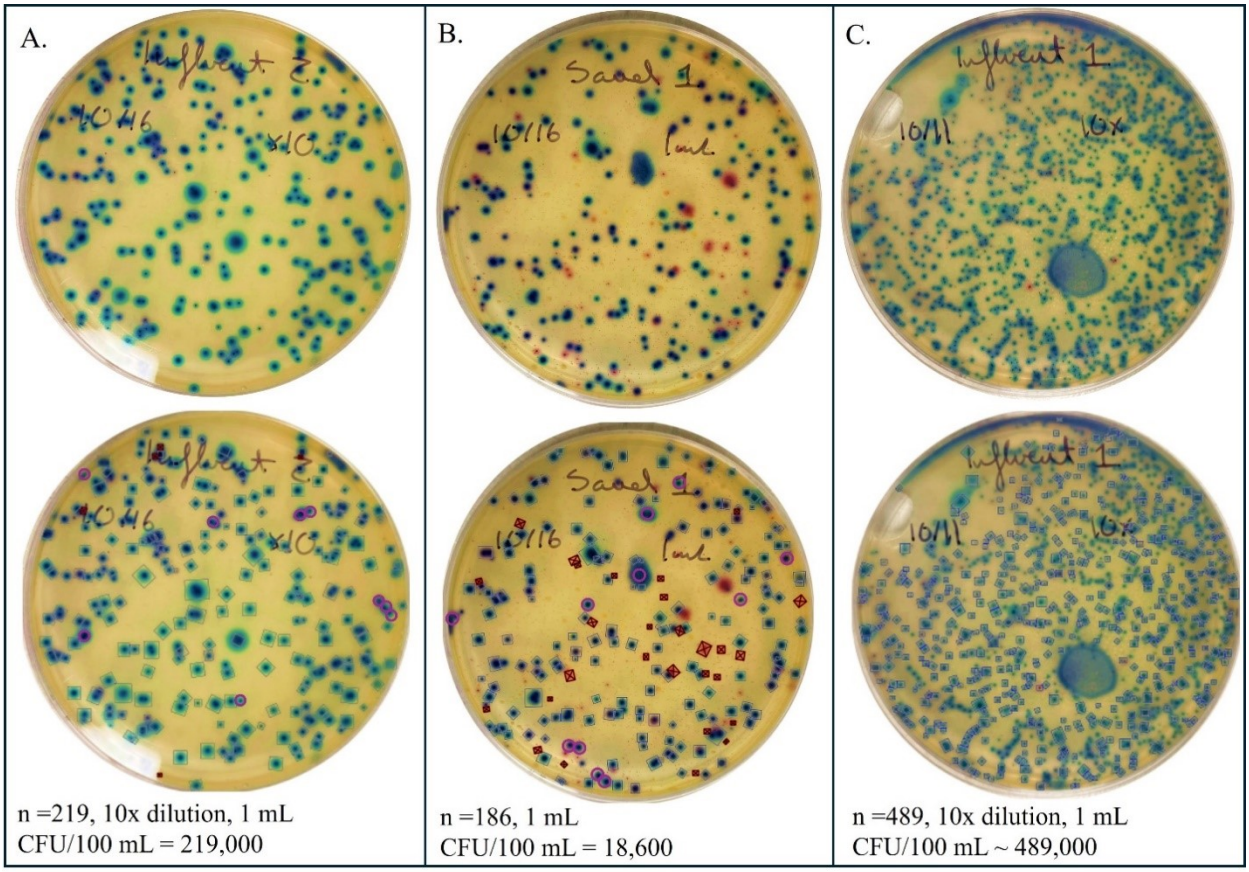


Figure S3. Examples of *E. coli* plates and colony counting methodology, performed according to the manufacturer's recommendations (Micrology Labs). (A.) Colonies that appeared purple, blue, or blue/teal were counted as *E. coli* (open squares indicate colonies identified by Open CFU, circles indicate colonies counted manually), (B.) while colonies that appearred pink or green/teal were excluded (indicated by red squares with x's). (C.) In some cases where *E. coli* concentrations were very high (i.e., > 300,000 CFU/100 mL) very small colonies would form in very high density and the plates would not fully gel properly, potentially causing concentations to be underestimted. However, as these instances were largely isolated to the influent samples, this is unlikely to affect conclusions based on differences between effluent concentrations, which were overall lower than influent concentrations.

Method S1. Detailed description of analytical method for quantification of organic contaminants. Pestanal-grade analytical standards for atrazine, atrazine-d6, diuron, diuron-d6, imidacloprid, imidacloprid-d4, and isoproturon-d6 were obtained from Sigma Aldrich. Analytical standards for 5-H-methylbenzotriazole were also obtained from Sigma Aldrich (>98% purity). Samples for organic contaminant analysis were stored frozen and thawed in the refrigerator overnight prior to preparation for analysis by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) using an isotope dilution method. Aliquots (180 µL) of aqueous samples were spiked with isotope-labelled surrogates (atrazine-d4, diuron-d6, and imidacloprid-d5), diluted 10x in Optima Methanol/Water (80:20), filtered through 0.22 µm glass fiber filters, spiked with isotope-labelled internal standard solution, and stored refrigerated for up to 7 days prior to analysis. Concentrations of atrazine, diuron, and imidacloprid were quantified by isotope dilution according the associated isotope-labelled surrogate standard. Due to budget constraints associated with cost and availability of isotope-labelled analytical standards, methyl-benzotriazole was quantified via an internal

calibration using isoproturon-d6 as an internal standard. Considering that samples were prepared via dilution and direct injection, application of isotope-dilution and internal calibration methods for analyte concentrations are expected to cause minimal variability, particularly as data were interpreted in relation to control conditions. Laboratory blanks consisting of Optima water were also analyzed periodically and no evidence for contamination from the sample preparation process was observed (note that all sample preparation batches contained multiple samples with cases of non-detects for each analyte, as no analytes were detected in the biochar column effluents for the majority of cases).

LC-QTOF-MS analysis was performed with an AB Sciex X500R QTOF coupled to a Sciex ExionLC AD liquid chromatography system equipped a 50 µL sample loop (25 µL injection volume), a reverse phase chromatography column (Luna C18, 5µm, 100 x 3 mm; Phenomenex) and a column oven (40 ˚C). Chromatography was performed at a flow rate of 0.4 mL/min using aqueous and organic mobile phases consisting of Optima grade water and Optima methanol, respectively, both containing 2mM formic acid. The gradient method proceeded as follows: ramp from 5% organic to 60% organic from 0.5 to 5 minutes, ramp from 60% organic to 90% organic from 5 to 10 minutes, hold at 90% organic until 15 minutes, then drop to 10% organic to equilibrate for the final 3 minutes (18 minutes total). Mass spectrometry analysis was performed in positive electrospray ionization mode with a spray voltage at 5500 V, ion source gasses at 55 psi, and curtain gas at 35 psi. A multiple-reaction-monitoring high resolution (MRM^{HR}) acquisition method was used to monitor two transitions per analyte (one quantitative, one qualitative, Table S4). Quantification was performed according to 9-point calibration curves with analyte concentrations ranging from 0.050-53.08 µg/L with an R^2 value of 0.99 as the linearity criteria, an accuracy criterion of ±30%, and a signal to noise (S/N) threshold of 10. Quantitative results are reported for instances where analytes concentrations exceeded the reporting limit, which was designated as the concentration of the lowest calibration level that passed the calibration acceptance criteria (Table S4). Samples with calculated concentrations below the reporting limits were considered as not-detected (ND), as analyte peaks could not be confidently distinguished from the noise. While it is possible that low concentrations of analytes below the reporting limits were present in some samples reported as ND, our conclusions are based only on data above the reporting limit, as these data can be reported with a known accuracy of ±30%.

Table S4. MS method details for analytes, isotope-labelled extraction surrogates*, and isotope-labelled internal standard**

Analyte	Parent ion mass (Da)	Quantitative fragment ion mass (Da)	Qualitative fragment ion mass (Da)	Collision Energy (V)	Declustering Potential (V)	Reporting limit (µg/L)
1-(3,4-	219.01	127.0182	174.0541	30	60	0.025
Atrazine	216.10	104.0013	174.0406	34	40	0.025
Diuron	233.02	72.0449	159.9734	22	45	0.025
Imidacloprid	256.06	126.0103	175.0813	30	40	0.126
Methyl-1H-	134.07	79.0543	77.0386	20	60	0.106
Atrazine-d5*	221.12	179.0866	-	22	45	-
Diuron-d6*	239.06	78.0815	-	20	45	-
Imidacloprid-d4*	260.09	179.1240	-	22	45	-
Isoproturon-d6**	213.19	78.0800	-	22	55	-

Table S5. Characterization data provided by Wakefield biochar (Ultimate and Proximate analysis).

	As Rec'd	Dry	Air Dry
ULTIMATE			
Moisture, %	62.18	0.00	0.57
Ash, %	1.45	3.84	3.82
Sulfur, %	0.007	0.019	0.019
Carbon, %	25.82	68.26	67.87
Hydrogen, %	0.28	0.75	0.74
Nitrogen, %	0.16	0.41	0.41
Oxygen, %*	10.11	26.72	26.57
	100.00	100.00	100.00
PROXIMATE			
Moisture, %	62.18	0.00	0.57
Ash, %	1.45	3.84	3.82
Volatile Matter, %	3.15	8.33	8.28
Fixed Carbon, %*	33.22	87.83	87.33
	100.00	100.00	100.00

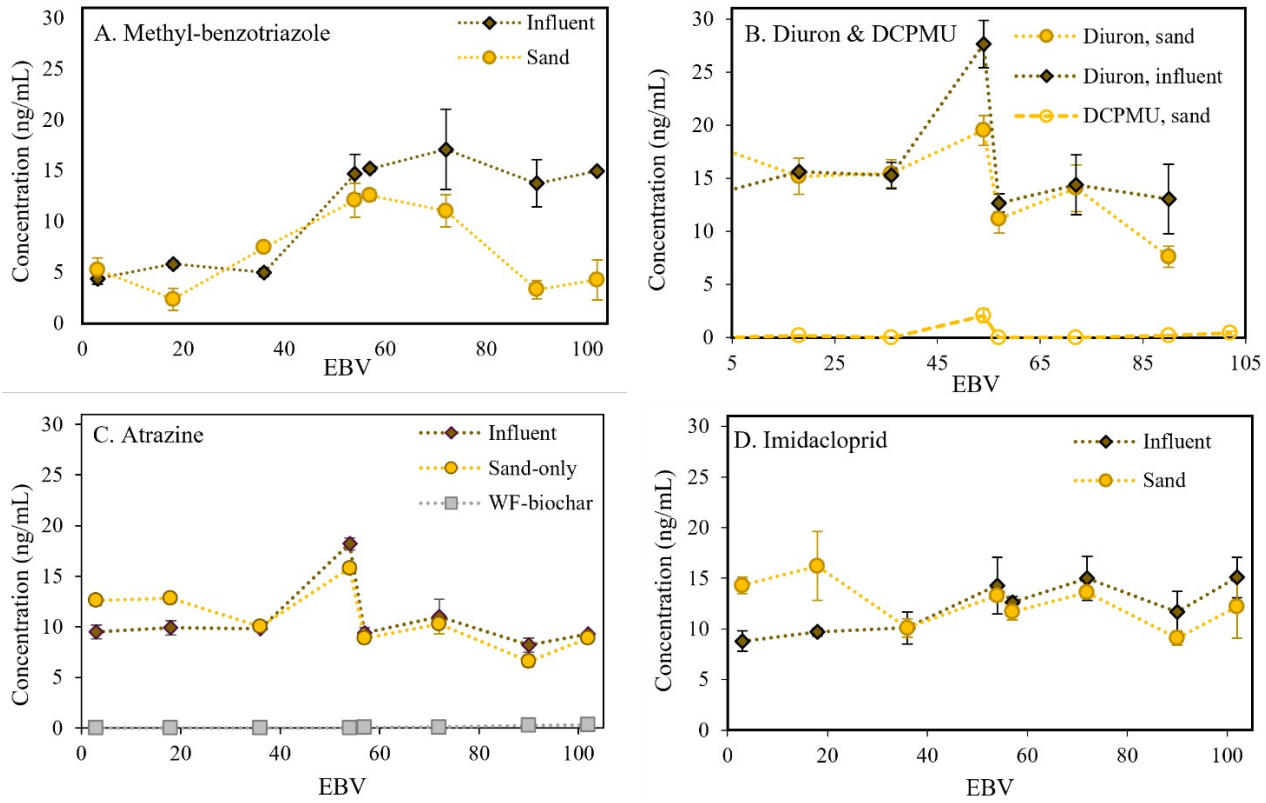


Figure S4. Concentrations of organic contaminants in the influent and effluents from sand-only filters for all cases where any analytes were detected. In the case of DCPMU, data for non-detects are reported as "0" for clarity. Apparent sorption of atrazine and imidacloprid to the influent and effluent reservoirs was observed during the tests conducted at 3 and 20 EBVs, however this appeared to be resolved after sample collection methods were revised during subsequent tests.

Table S6. Influent and effluent organic contaminant concentrations from all dosing tests where organic contaminants were measured (ng/mL). ND = not detected (reporting limits in Table S4).

EBV	Influent		Sand-only		WF-biochar		ABC-biochar	
	Mean	St.dev.	Mean	St.dev.	Mean	St.dev.	Mean	St.dev.
Atrazine								
3	9.5	0.7	12.6	0.5	ND	-	ND	-
18	9.9	0.7	12.8	0.4	ND	-	ND	-
36	9.8	0.2	10	0.3	ND	-	ND	-
54	18.2	0.6	15.8	0.5	ND	-	ND	-
57	9.4	0.5	8.9	0.1	0.07	0.06	ND	-
72	11	1.7	10.3	1.0	0.08	0.08	ND	-
90	8.2	0.7	6.6	0.6	0.27	0.14	ND	-
102	9.3	0.1	8.9	0.4	0.29	0.11	ND	-
Diuron								
3	13.7	1.3	17.8	2.6	ND	-	ND	-
18	15.6	0.1	15.2	1.7	ND	-	ND	-
36	15.2	1.2	15.4	1.3	ND	-	ND	-
54	27.6	2.2	19.5	1.4	ND	-	ND	-
57	12.7	0.9	11.1	1.3	ND	-	ND	-
72	14.4	2.8	14.1	2.2	ND	-	ND	-
90	13.0	3.2	7.6	1.0	ND	-	ND	-
102	15.5	5.3	12.2	0.9	ND	-	ND	-
Imidacloprid								
3	8.8	1.0	14.3	0.8	ND	-	ND	-
18	9.7	0.4	16.2	3.4	ND	-	ND	-
36	10.1	1.6	10.1	0.9	ND	-	ND	-
54	14.3	2.8	13.3	0.5	ND	-	ND	-
57	12.6	0.6	11.7	0.8	ND	-	ND	-
72	15	2.2	13.6	0.4	ND	-	ND	-
90	11.7	2.0	9.1	0.7	ND	-	ND	-
102	15.1	2.0	12.2	3.1	ND	-	ND	-
Methyl benzotriazole								
3	4.4	0.5	5.3	1.2	ND	-	ND	-
18	5.8	0.3	2.4	1.1	ND	-	ND	-
36	5	0.4	7.4	0.4	ND	-	ND	-
54	14.7	1.9	12.1	1.7	ND	-	ND	-
57	15.2	0.3	12.6	0.5	ND	-	ND	-
72	17.1	3.9	11	1.6	ND	-	ND	-
90	13.7	2.3	3.3	0.9	ND	-	ND	-
102	15	0.2	4.3	1.9	ND	-	ND	-
DCPMU								
3	ND	-	ND	-	ND	-	ND	-
18	ND	-	0.2	0.1	ND	-	ND	-
36	ND	-	ND	-	ND	-	ND	-
54	ND	-	2.1	0.7	ND	-	ND	-
57	ND	-	ND	-	ND	-	ND	-
72	ND	-	ND	-	ND	-	ND	-
90	ND	-	0.2	0.1	ND	-	ND	-
102	ND	-	0.4	0.2	ND	-	ND	-

(1) MPCA. *Minnesota Stormwater Manual*. https://stormwater.pca.state.mn.us/index.php?title=Main_Page (accessed 2025-04-20).

(2) U.S. Climate Data. *Climate: Duluth - Minnesota*. https://www.usclimatedata.com/climate/duluth/minnesota/united-states/usmn0208?utm_source (accessed 2025-04-20).
(3) Chin, D. A. Estimating Peak Runoff Rates Using the Rational Method. *Journal of Irrigation and Drainage Engineering* **2019**, *145* (6).