Supporting Material for

Effect of Biochar on Microbial Growth: A Metabolomics and Bacteriological Investigation in *E. coli*

Rebecca A. Hill, John Hunt, Emily Sanders, Melanie Tran, Griffin A. Burk Todd E. Mlsna, and Nicholas C. Fitzkee[*]

Department of Chemistry, Mississippi State University, Mississippi State, MS 39762

KEYWORDS. ^{1}H NMR spectroscopy, Metabolomics, Biochar, *Escherichia coli*

The supporting information includes 13 pages, 5 figures, and 3 tables.

Supporting Information Method:

Supporting Information Figures:

Supporting Information Tables:

Method S1. Media Treatment Preparation for Bacterial Growth.

An additional set of experiments were performed to test BC adsorption of metabolites. For these experiments, 0.001% (0.3 mg), 0.1% (3 mg), 1% (30 mg), and 10% (300 mg) of Douglas fir, Ultra-Rinsed Black Owl Biochar (BC) or Norit Activated Carbon (AC) were weighed out and placed in 15 mL sterile conical tubes. 3 mL of a metabolite mixture was measured into each tube and placed in the floor shaker at 200 rpm at 25.0 °C for 24 hours. This allowed ample time for BC and AC to adsorb metabolites from the sample in the absence of bacterial growth. During this time, no significant growth was observed based on O.D. measurements. After 24 hours of shaking, the media was filtered through a 25 mm polyethersulfone (PES), 0.2 μm sterile syringe filter and 400 μL of the supernatant was transferred to another clean, sterile microcentrifuge tube. 200 μL of 200 mM phosphate buffer at pH of 7.0 with 1.000 mM trimethylsilypropanoic acid (TMSP) in 50% D_2O was added to the supernatant as the NMR reference and lock solvent. For NMR analysis, the same procedure follows as described in the main text.

As the mass of BC and AC increased, the adsorption of metabolites from the mixture increased. 10% of BC or AC was selected for the filter media because we observed a larger difference in metabolite adsorption to the surface than the lower percent by weight samples. Once the 10% of BC or AC was introduced to unfiltered media, the media became very thick, making it difficult to monitor the growth of *E. coli* in the media. So, 5% of BC or AC added to the media was used for the unfiltered media. The 5% of BC or AC adsorbed enough metabolites from the media to observe a difference in the metabolic profile of *E. coli*.

Method S2. Point of Zero Charge of BC and AC.

Point of zero charge (PZC) of BC and AC were determined using a 0.01 M NaCl solution with pH values ranging from 2 to 12. The pH values were adjusted using either 0.1 M NaOH or 0.1 M HCl solutions. 1 mL of 0.01 M NaCl solution was added to 50 mg of BC or AC in a clean, sterile microcentrifuge tube. Each sample was vortexed for 2 min and centrifuged at 21,000 x g for 4 min. The supernatant was carefully removed from solution and transferred to a separate clean, sterile microcentrifuge tube. The pH of the supernatant was measured using an ORION ROSS perPHect pH electrode. The PZC was obtained from a plot of initial solution pH versus pH of the equilibrated supernatant. Uncertainties are reported as the standard error of the mean of three independent experiments.

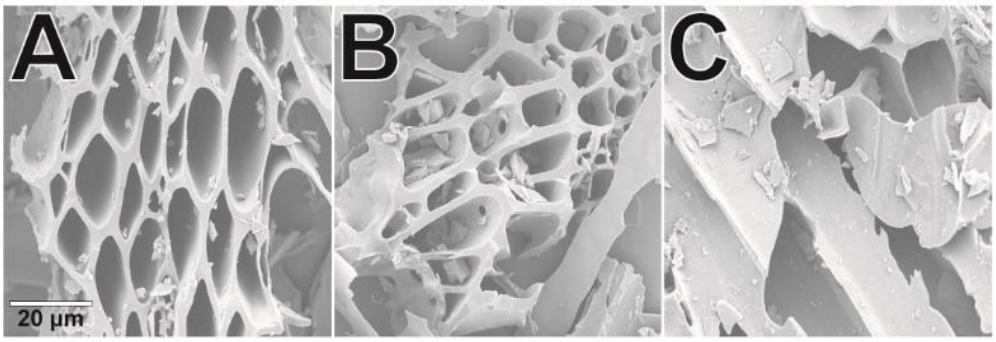

Figure S1. SEM of Biochar. Different views of BC morphology. (A) Black Owl Biochar, (B) BC in RPMI media, and (C) BC in RPMI media with *E. coli*. The surface morphologies were studied at 5 kV acceleration voltage. The 20 µm scale in (A) applies for all three panels.

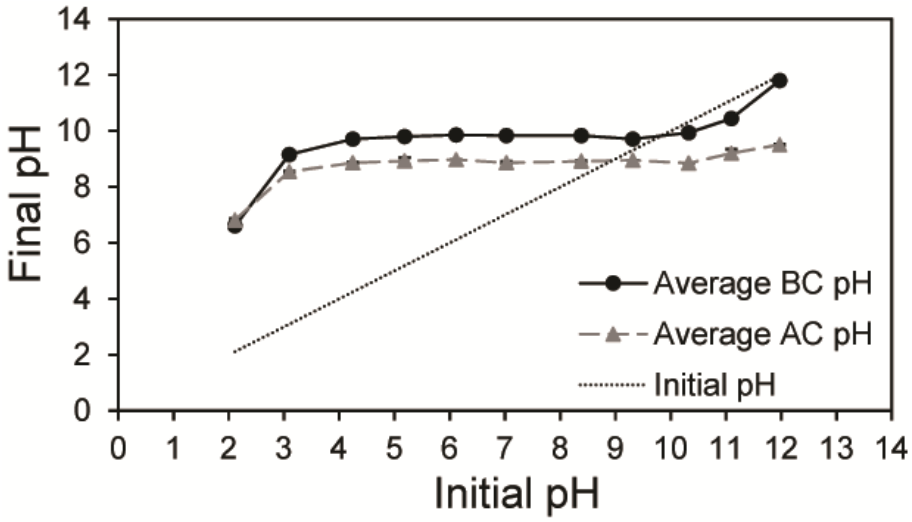

Figure S2. The Average Point of Zero Charge (PZC) of BC and AC. The initial pH (gray dotted line) represents the pH of 0.1 M NaCl ranging from 2 to 12. The PZC is determined as the intesection of the initial vs. final pH curve and the line $y = x$, which assumes no change in pH after addition of BC or AC. At this intersection, the pH does not change upon the addition of AC or BC, suggesting that the material is neutral at this pH. The PZC of BC (solid black line, black dots) was determined to be 9.793 ± 0.005. The PZC of AC (dotted gray line, gray triangles) was determined to be 8.920 ± 0.003. Error bars are the standard error of the mean of three identically prepared samples; however the standard error of the mean was less than 0.020 and is therefore too small to visualize.

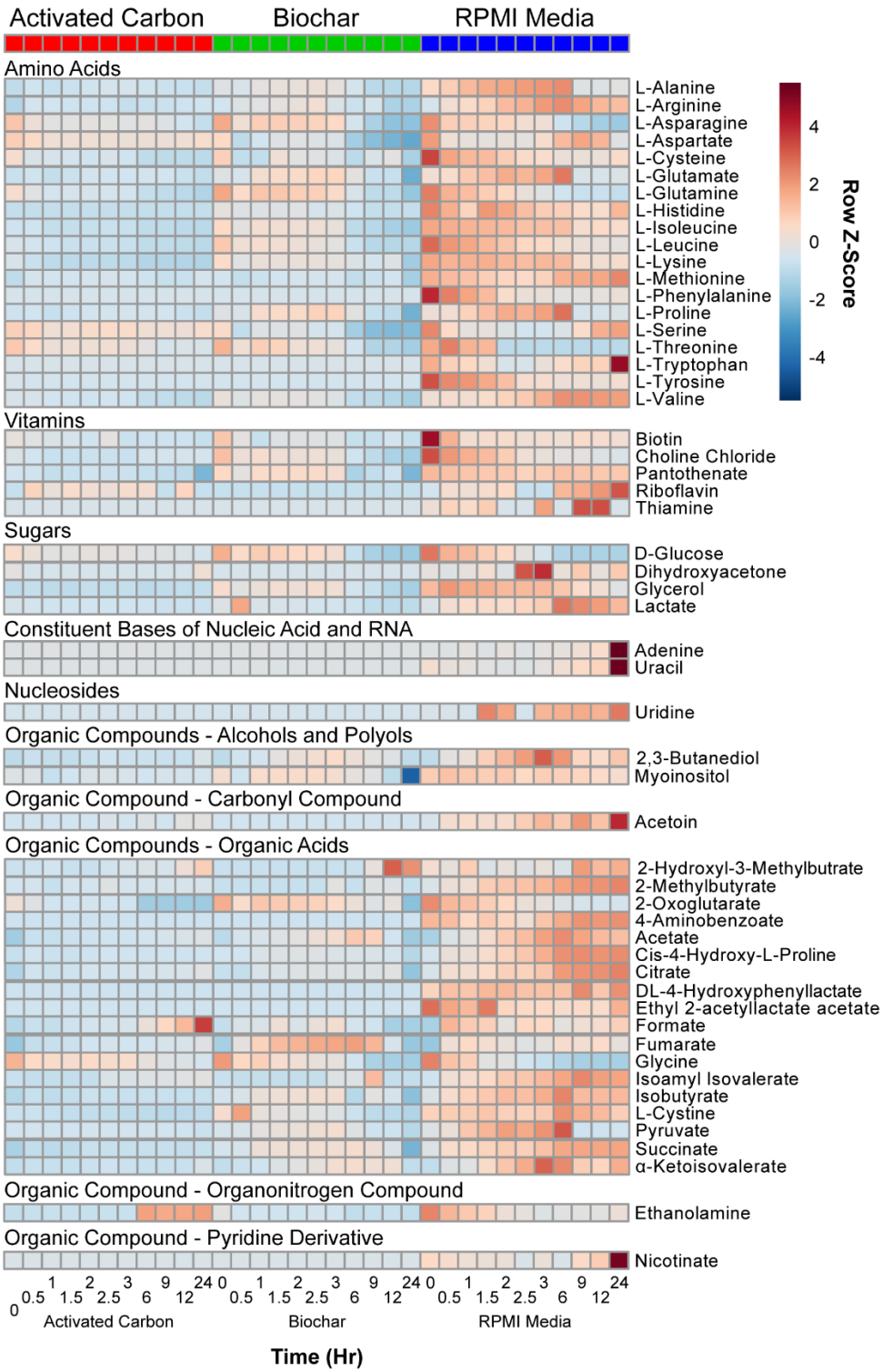

Figure S3. Heatmap of Extracellular Metabolites in Unfiltered RPMI Media Arranged by Chemical Properties. This is the same heatmap presented in the main text, but the metabolites have been arranged by chemical similarities instead of clustered using Ward's linkage algorithm. The row Z-score for each feature was used to color code the map: absent to low concentration metabolites were colored blue and high concentration metabolites were colored red.

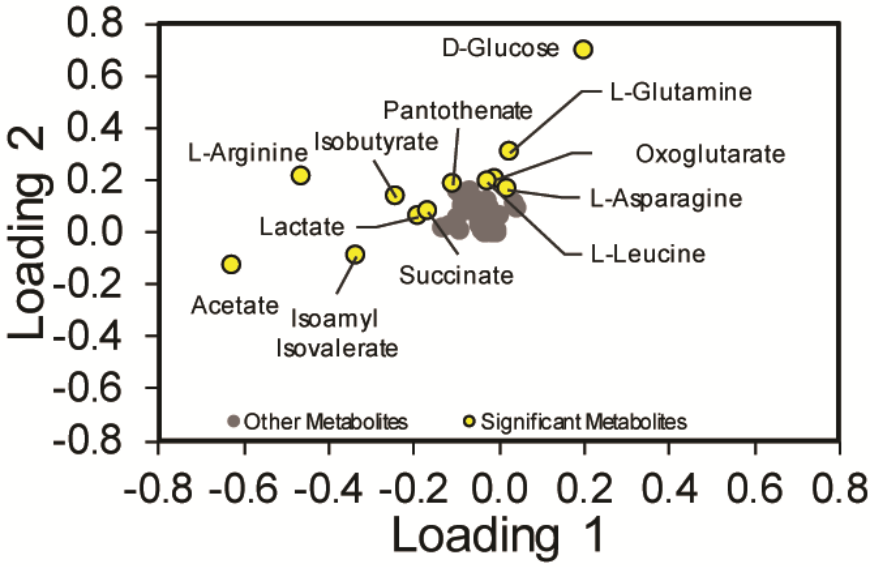

Figure S4. Loading Plot of the Extracellular Metabolites in Unfiltered RPMI Media. The significant metabolites ($p < 0.05$) in the loading plot are represented by the yellow dots. The significant metabolites are those that make the largest contribution to each loading. The other metabolites in the loading plot are represented by the gray dots. The other metabolites are those whose contributions are not significant to each loading; these metabolites are clustered around the origin.

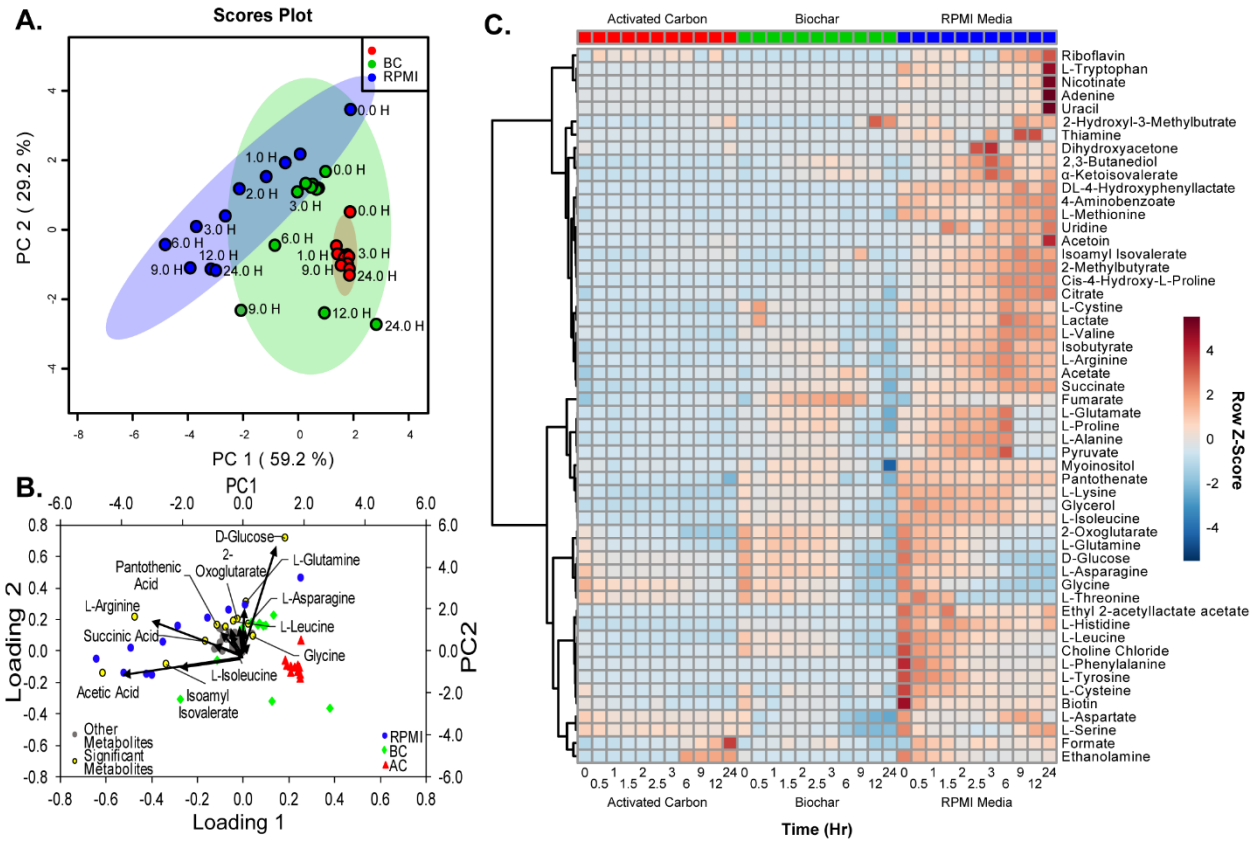

Figure S5. Statistical Analysis of *E. coli* Growth in Filtered RPMI Media. (A) Principal component analysis (PCA) score plot on *E. coli* growth in filtered media was used to identify statistically differences between each of the media types. The shaded oval represents the 95% confidence limit for each media type (RPMI, blue circles; BC-treated media, green circles; AC-treated media, red circles). Time points interval at every half hour are not labeled in this figure for clarity. Data are the mean values of metabolite concentrations of three replicates after being normalized using Pareto scaling. (B) The significant metabolites ($p < 0.05$) in the loading plot overlay the PCA scatter plot on the biplot for filtered media. The yellow dots represent the significant metabolites ($p < 0.05$), i.e. those making the largest contribution to each principal component (RPMI, blue squares; BC-treated media, green diamonds; AC-treated media, red triangles). (C) Heatmap of Extracellular Metabolites of Filtered Media. Heatmap of the extracellular metabolite relative concentrations versus the time. The Pearson correlation of the normalized, relative concentration was used to determine the distance measured. The row Z-score for each feature was used to color code the map. Absent to low concentration metabolites were colored blue and high concentration metabolites were colored red. Ward's linkage algorithm ranked the metabolites in the clustering tree. The first 11 boxes (red) were labeled as AC-treated media with the time increasing from 0 to 24 hours going left to right. The next 11 boxes (green) were BC-treated media and the last 11 boxes (blue) were RPMI media. All data represent the mean values of concentration for 3 replicates after normalization and Pareto scaling.

Table S1. Metabolite Concentration (mM) in RPMI Media. The bold metabolites and highlighted in light green at time point 0 h are the metabolites in RPMI Media.

Time (hours)	0	0.5	1	1.5	2	2.5	3	6	9	12	24
2-Hydroxyl-3-methylbutyrate	0.001	0.001	0.001	0.000	0.001	0.000	0.001	0.000	0.002	0.002	0.002
2,3-Butanediol	0.000	0.003	0.004	0.007	0.010	0.012	0.017	0.013	0.007	0.006	0.008
2-Methylbutyrate	0.020	0.042	0.043	0.075	0.080	0.085	0.096	0.114	0.124	0.126	0.138
2-Oxoglutarate	0.426	0.337	0.317	0.285	0.264	0.209	0.198	0.169	0.151	0.147	0.146
4-Aminobenzoate	0.002	0.002	0.001	0.002	0.001	0.001	0.002	0.002	0.003	0.003	0.003
Acetate	0.381	2.136	2.640	3.307	4.353	4.922	5.954	6.619	5.836	4.980	4.788
Adenine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.013
Cis-4-hydroxy-l-proline	0.057	0.102	0.104	0.127	0.154	0.176	0.216	0.265	0.269	0.261	0.269
Citrate	0.086	0.127	0.129	0.142	0.160	0.158	0.174	0.231	0.238	0.241	0.256
D-Glucose	5.144	3.800	3.524	3.012	2.646	1.845	1.332	0.456	0.341	0.329	0.313
DL-4-Hydroxyphenyllactate	0.021	0.027	0.026	0.031	0.030	0.027	0.027	0.027	0.044	0.026	0.042
Dihydroxyacetone	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
Ethyl-2-acetyllactate acetate	0.008	0.006	0.005	0.008	0.003	0.003	0.003	0.002	0.003	0.003	0.005
Formate	0.003	0.119	0.098	0.092	0.056	0.085	0.083	0.056	0.068	0.073	0.093
Fumarate	0.000	0.015	0.018	0.016	0.014	0.012	0.013	0.016	0.015	0.018	0.013
Glycerol	0.118	0.147	0.133	0.129	0.119	0.120	0.119	0.107	0.085	0.073	0.054
Glycine	0.104	0.069	0.061	0.035	0.035	0.024	0.019	0.009	0.003	0.002	0.003
Isoamyl isovalerate	0.093	0.532	0.630	0.734	0.939	0.987	1.135	1.373	1.658	1.432	1.376
Isobutyrate	0.477	0.684	0.806	0.872	0.952	0.926	1.042	1.269	0.957	0.957	0.963
L-Alanine	0.027	0.031	0.038	0.043	0.046	0.048	0.050	0.052	0.014	0.013	0.015
L-Arginine	0.921	1.721	1.946	2.262	2.798	3.057	3.579	3.847	3.360	2.903	2.775
L-Aspartate	0.139	0.086	0.079	0.076	0.081	0.077	0.091	0.119	0.133	0.123	0.071
L-Asparagine	0.274	0.185	0.181	0.175	0.173	0.145	0.138	0.080	0.052	0.026	0.018
L-Cysteine	0.237	0.156	0.134	0.131	0.113	0.091	0.089	0.075	0.071	0.072	0.090
L-Cystine	0.120	0.126	0.128	0.138	0.121	0.119	0.129	0.196	0.164	0.150	0.123
L-Glutamate	0.295	0.313	0.372	0.399	0.446	0.426	0.464	0.551	0.225	0.217	0.229
L-Glutamine	0.889	0.682	0.628	0.564	0.504	0.381	0.328	0.242	0.217	0.211	0.201
L-Histidine	0.048	0.036	0.024	0.043	0.038	0.031	0.026	0.025	0.020	0.019	0.033
L-Isoleucine	0.233	0.224	0.216	0.212	0.205	0.195	0.198	0.197	0.139	0.152	0.143
L-Leucine	0.347	0.275	0.249	0.219	0.195	0.158	0.149	0.124	0.106	0.102	0.096
L-Lysine	0.136	0.127	0.128	0.129	0.123	0.117	0.122	0.119	0.082	0.076	0.081
L-Methionine	0.048	0.043	0.041	0.037	0.034	0.032	0.035	0.047	0.050	0.051	0.060
L-Phenylalanine	0.060	0.040	0.031	0.024	0.012	0.008	0.008	0.005	0.005	0.006	0.008
L-Proline	0.257	0.271	0.326	0.371	0.417	0.398	0.437	0.528	0.196	0.192	0.204
L-Serine	0.182	0.113	0.085	0.088	0.074	0.062	0.065	0.073	0.108	0.151	0.164
L-Threonine	0.105	0.130	0.098	0.095	0.017	0.016	0.018	0.015	0.016	0.016	0.015
L-Tryptophan	0.006	0.004	0.003	0.002	0.000	0.000	0.002	0.003	0.004	0.004	0.015
L-Tyrosine	0.156	0.116	0.108	0.092	0.073	0.038	0.037	0.029	0.031	0.027	0.035
L-Valine	0.095	0.102	0.099	0.106	0.123	0.137	0.170	0.206	0.203	0.196	0.191
Lactate	0.133	0.251	0.282	0.294	0.289	0.333	0.379	0.720	0.649	0.586	0.466
Myoinositol	0.182	0.190	0.185	0.180	0.185	0.173	0.180	0.179	0.174	0.168	0.163
Pyruvate	0.052	0.071	0.127	0.149	0.176	0.164	0.189	0.249	0.025	0.025	0.031
α-Ketoisovalerate	0.000	0.004	0.004	0.009	0.013	0.016	0.025	0.020	0.012	0.010	0.016
Succinate	0.252	0.344	0.363	0.384	0.405	0.396	0.439	0.509	0.518	0.518	0.519
Uracil	0.003	0.001	0.001	0.001	0.000	0.000	0.001	0.002	0.004	0.005	0.025

Time (hours)	0	0.5	1	1.5	2	2.5	3	6	9	12	24
Acetoin	0.001	0.008	0.009	0.008	0.008	0.013	0.017	0.013	0.022	0.015	0.038
Biotin	0.064	0.028	0.014	0.014	0.014	0.012	0.013	0.012	0.016	0.014	0.013
Choline Chloride	0.033	0.024	0.021	0.020	0.017	0.012	0.011	0.010	0.010	0.009	0.008
Ethanolamine	0.058	0.042	0.034	0.030	0.019	0.016	0.012	0.013	0.014	0.013	0.019
Nicotinate	0.002	0.001	0.001	0.001	0.001	0.000	0.001	0.000	0.001	0.002	0.009
Pantothenate	0.438	0.414	0.408	0.395	0.398	0.362	0.383	0.410	0.401	0.397	0.397
Thiamine	0.001	0.002	0.002	0.002	0.000	0.000	0.004	0.000	0.006	0.007	0.000
Riboflavin	0.000	0.001	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.001	0.002
Uridine	0.000	0.000	0.000	0.006	0.005	0.000	0.004	0.004	0.004	0.004	0.006

Table S2. Metabolite Concentration (mM) in BC Media. The bold metabolites and highlighted in light green at time point 0 h are the metabolites in RPMI Media.

Time (hours)	0	0.5	1	1.5	2	2.5	3	6	9	12	24
2-Hydroxyl-3-methylbutyrate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.003
2,3-Butanediol	0.000	0.001	0.001	0.001	0.002	0.003	0.003	0.004	0.003	0.002	0.000
2-Methylbutyrate	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.017	0.014	0.000
2-Oxoglutarate	0.337	0.309	0.301	0.307	0.292	0.300	0.285	0.235	0.151	0.190	0.000
4-Aminobenzoate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Acetate	1.849	2.274	2.320	2.428	2.433	2.643	2.691	4.779	6.525	2.930	0.000
Adenine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cis-4-hydroxy-l-proline	0.058	0.068	0.062	0.072	0.071	0.080	0.071	0.069	0.061	0.041	0.001
Citrate	0.063	0.075	0.082	0.091	0.097	0.104	0.104	0.100	0.080	0.086	0.000
D-Glucose	3.669	3.265	3.208	3.241	3.120	3.177	3.022	1.885	0.474	0.032	0.003
DL-4-Hydroxyphenyllactate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dihydroxyacetone	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ethyl-2-acetyllactate acetate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Formate	0.028	0.050	0.041	0.034	0.035	0.039	0.040	0.056	0.066	0.000	0.000
Fumarate	0.005	0.013	0.015	0.018	0.019	0.022	0.023	0.038	0.035	0.008	0.000
Glycerol	0.084	0.072	0.066	0.076	0.072	0.081	0.074	0.054	0.036	0.006	0.001
Glycine	0.090	0.081	0.082	0.073	0.071	0.064	0.064	0.047	0.006	0.000	0.000
Isoamyl isovalerate	0.051	0.000	0.000	0.000	0.000	0.000	0.582	0.653	1.778	0.000	0.000
Isobutyrate	0.503	0.535	0.572	0.606	0.618	0.657	0.653	0.479	0.578	0.360	0.000
L-Alanine	0.016	0.015	0.017	0.022	0.024	0.027	0.026	0.020	0.001	0.000	0.000
L-Arginine	1.363	1.405	1.426	1.462	1.456	1.553	1.578	1.244	0.882	0.000	0.000
L-Aspartate	0.083	0.083	0.089	0.094	0.103	0.105	0.106	0.058	0.019	0.000	0.000
L-Asparagine	0.220	0.192	0.196	0.196	0.198	0.196	0.192	0.152	0.074	0.000	0.000
L-Cysteine	0.054	0.023	0.023	0.098	0.097	0.090	0.082	0.034	0.042	0.065	0.000
L-Cystine	0.105	0.082	0.080	0.075	0.080	0.075	0.072	0.048	0.014	0.000	0.000
L-Glutamate	0.251	0.254	0.296	0.337	0.362	0.405	0.407	0.332	0.175	0.146	0.000
L-Glutamine	0.676	0.615	0.597	0.619	0.586	0.609	0.574	0.402	0.154	0.158	0.006
L-Histidine	0.024	0.013	0.013	0.012	0.014	0.010	0.013	0.006	0.000	0.000	0.000
L-Isoleucine	0.169	0.143	0.137	0.142	0.141	0.148	0.138	0.089	0.037	0.000	0.000
L-Leucine	0.204	0.153	0.141	0.143	0.134	0.133	0.123	0.067	0.002	0.000	0.000
L-Lysine	0.090	0.076	0.075	0.073	0.077	0.080	0.078	0.062	0.035	0.053	0.000
L-Methionine	0.017	0.015	0.015	0.018	0.021	0.022	0.022	0.016	0.010	0.014	0.000
L-Phenylalanine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Proline	0.224	0.234	0.272	0.316	0.339	0.388	0.384	0.307	0.151	0.124	0.001
L-Serine	0.085	0.059	0.047	0.050	0.045	0.028	0.027	0.001	0.005	0.001	0.002
L-Threonine	0.098	0.086	0.082	0.086	0.093	0.090	0.082	0.061	0.001	0.001	0.000
L-Tryptophan	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Tyrosine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Valine	0.090	0.089	0.084	0.089	0.088	0.093	0.084	0.059	0.031	0.014	0.000
Lactate	0.174	0.152	0.145	0.147	0.163	0.147	0.142	0.116	0.049	0.005	0.005
Myoinositol	0.162	0.163	0.162	0.166	0.162	0.169	0.160	0.148	0.137	0.081	0.001
Pyruvate	0.046	0.049	0.067	0.082	0.094	0.106	0.109	0.084	0.028	0.022	0.000
α-Ketoisovalerate	0.000	0.001	0.001	0.002	0.003	0.004	0.004	0.006	0.004	0.004	0.000
Succinate	0.259	0.274	0.278	0.288	0.285	0.299	0.294	0.353	0.317	0.205	0.001
Uracil	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Time (hours)	0	0.5	1	1.5	2	2.5	3	6	9	12	24
Acetoin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
Biotin	0.033	0.016	0.000	0.014	0.014	0.014	0.014	0.000	0.000	0.000	0.000
Choline Chloride	0.020	0.015	0.015	0.014	0.014	0.014	0.013	0.008	0.006	0.001	0.000
Ethanolamine	0.011	0.007	0.007	0.007	0.007	0.010	0.009	0.001	0.002	0.002	0.000
Nicotinate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pantothenate	0.355	0.331	0.328	0.337	0.326	0.335	0.319	0.211	0.245	0.163	0.000
Thiamine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Riboflavin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Uridine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table S3. Metabolite Concentration (mM) in AC Media. The bold metabolites and highlighted in light green at time point 0 h are the metabolites in RPMI Media.

Time (hours)	0	0.5	1	1.5	2	2.5	3	6	9	12	24
2-Hydroxyl-3-methylbutyrate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2,3-Butanediol	0.000	0.001	0.000	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2-Methylbutyrate	0.009	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000
2-Oxoglutarate	0.254	0.175	0.139	0.138	0.139	0.121	0.130	0.025	0.019	0.000	0.000
4-Aminobenzoate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Acetate	0.900	1.569	1.503	1.615	1.604	1.474	1.653	1.467	1.658	1.695	1.734
Adenine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cis-4-hydroxy-l-proline	0.045	0.041	0.037	0.040	0.038	0.038	0.041	0.034	0.037	0.035	0.037
Citrate	0.064	0.052	0.045	0.051	0.054	0.051	0.058	0.053	0.054	0.054	0.055
D-Glucose	2.669	1.902	1.682	1.774	1.863	1.716	1.888	1.732	1.749	1.779	1.722
DL-4-Hydroxyphenyllactate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dihydroxyacetone	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ethyl-2-acetyllactate acetate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Formate	0.013	0.026	0.025	0.032	0.036	0.039	0.045	0.068	0.107	0.143	0.262
Fumarate	0.002	0.007	0.006	0.007	0.007	0.008	0.008	0.010	0.011	0.012	0.014
Glycerol	0.041	0.026	0.019	0.019	0.019	0.016	0.020	0.010	0.011	0.012	0.015
Glycine	0.078	0.058	0.054	0.052	0.057	0.051	0.054	0.041	0.040	0.040	0.031
Isoamyl isovalerate	0.045	0.240	0.000	0.253	0.000	0.000	0.000	0.000	0.234	0.000	0.000
Isobutyrate	0.287	0.295	0.265	0.292	0.321	0.292	0.326	0.307	0.311	0.311	0.313
L-Alanine	0.009	0.006	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.004
L-Arginine	0.698	0.901	0.851	0.732	0.200	0.589	0.000	0.372	0.452	0.000	0.000
L-Aspartate	0.120	0.091	0.084	0.091	0.089	0.088	0.093	0.086	0.094	0.093	0.086
L-Asparagine	0.201	0.147	0.125	0.128	0.126	0.117	0.122	0.097	0.088	0.078	0.048
L-Cysteine	0.085	0.021	0.050	0.048	0.012	0.021	0.011	0.014	0.013	0.000	0.000
L-Cystine	0.063	0.031	0.027	0.022	0.020	0.017	0.018	0.014	0.011	0.014	0.022
L-Glutamate	0.180	0.161	0.149	0.173	0.187	0.175	0.193	0.188	0.192	0.187	0.172
L-Glutamine	0.517	0.349	0.283	0.284	0.286	0.248	0.269	0.200	0.172	0.150	0.107
L-Histidine	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Isoleucine	0.096	0.055	0.043	0.042	0.039	0.036	0.036	0.027	0.026	0.021	0.022
L-Leucine	0.119	0.048	0.035	0.029	0.024	0.019	0.017	0.003	0.001	0.000	0.000
L-Lysine	0.068	0.037	0.025	0.023	0.016	0.014	0.012	0.003	0.002	0.000	0.000
L-Methionine	0.019	0.008	0.007	0.009	0.008	0.009	0.008	0.007	0.004	0.000	0.000
L-Phenylalanine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Proline	0.167	0.149	0.140	0.161	0.175	0.164	0.182	0.176	0.183	0.178	0.163
L-Serine	0.104	0.118	0.098	0.107	0.113	0.110	0.115	0.103	0.108	0.107	0.114
L-Threonine	0.094	0.073	0.062	0.064	0.061	0.058	0.058	0.050	0.049	0.043	0.044
L-Tryptophan	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Tyrosine	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L-Valine	0.072	0.055	0.049	0.048	0.043	0.040	0.042	0.023	0.022	0.015	0.010
Lactate	0.140	0.100	0.089	0.090	0.088	0.080	0.084	0.069	0.068	0.069	0.081
Myoinositol	0.146	0.141	0.128	0.138	0.139	0.131	0.141	0.126	0.133	0.131	0.130
Pyruvate	0.034	0.027	0.025	0.030	0.031	0.029	0.032	0.032	0.032	0.030	0.027
α-Ketoisovalerate	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.001	0.002
Succinate	0.144	0.150	0.134	0.148	0.165	0.150	0.170	0.161	0.163	0.165	0.168
Uracil	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Time (hours)	0	0.5	1	1.5	2	2.5	3	6	9	12	24
Acetoin	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Biotin	0.0228	0	0	0.00789	0	0.0065	0	0	0	0	0
Choline Chloride	0.013	0.008	0.007	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Ethanolamine	0.004	0.000	0.001	0.001	0.000	0.001	0.000	0.001	0.001	0.001	0.000
Nicotinate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pantothenate	0.239	0.186	0.159	0.169	0.177	0.159	0.175	0.152	0.148	0.141	0.000
Thiamine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Riboflavin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Uridine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000