Supplementary Figure Legends

Supplementary Figure S1: Phenolic inhibitors hindered anaerobic xylose consumption. *E. coli* RL3000 was grown in M9 minimal medium, 0.25% (w/v) xylose until the culture reached an early exponential phase (OD_{600} ~0.2-0.3) before it was exposed to 2.75 mM or 5.5 mM feruloyl amide, coumaroyl amide, or ferulic acid. Concentrations of xylose remained in the culture over time were quantified using LC-MS. Growth rates (/hour) and xylose uptake rates (mM/hour/OD) were obtained from nonlinear least-squares (curve-fitting). The extrapolated rates were normalized to those of the controls. Bars represent means of two biological replicates \pm SEM.

Supplementary Figure S2: Feruloyl amide and coumaroyl amide are major contributors to anaerobic growth inhibition of *E. coli*.

(A) Cells were grown in an absence of phenolic inhibitors until early exponential phase ($OD_{600} \sim 0.2$) The cultures were then treated with either of lignin-derived phenolic inhibitor (LDPI) cocktail or feruloyl amide combined with coumaroyl amide. 1X concentrations of LDPI cocktail (see also Figure 1 and Table S1) led to strong anaerobic growth inhibition. 0.5X concentration (2.75 mM) of feruloyl amide and coumaroyl amide (each) was sufficient to account for the same degree of inhibition observed in LDPI cocktail treatment.

(B) Inhibitory effects of LDPIs are pronounced in cells grown in xylose. Anaerobic exponential cultures ($OD_{600} \sim 0.2$ -0.3) in LDPI-free media with either xylose or glucose were introduced to 1.375 mM, 2.75 mM, or 5.5 mM of feruloyl amide, coumaroyl amide, or ferulic acid.

Supplementary Figure S3: Anaerobic growth inhibition by ferulic acid.

(A) Ferulic acid treatment leads to different alterations in intracellular metabolite levels compared to those in feruloyl amide and coumaroyl amide-treated cells.

Exponential *E. coli* cultures grown anaerobically on xylose were treated with 5.5 mM ferulic acid. Intracellular metabolites were extracted at 10, 30, 60, 120, 180, and 240 min after exposure to ferulic acid and measured by LC-MS. Metabolite levels of treated samples were normalized against those of controls at each corresponding time points. Metabolites whose level increases upon phenolic amide treatment are shown in shades of yellow and those that decreases are shown in shades of blue. The data represents the averages of two biological replicates.

(B) Ferulic acid leads to anaerobic growth inhibition of *E. coli*. Anaerobic *E. coli* was grown in an absence of inhibitor before it was introduced to 5.5 mM ferulic acid at early exponential growth (arrow).

(C) PRPP accumulation in the presence of ferulic acid. While PRPP sharply accumulates (at 10 min after treatment) in the presence of feruloyl amide and coumaroyl amide (Figures 1C, 3), the accumulation is not drastic in the presence of ferulic acid. Bars represent means of four biological replicates ± SEM.

(D) Impeded biosynthesis of deoxynucleotide in the presence of ferulic acid. Anaerobic, exponential culture of *E. coli* was exposed to 5.5 mM ferulic acid for 5 min before switched into a medium containing ¹³C-xylose. ¹³C incorporation was allowed for 0, 0.5, 1, 2, 4, 7, 10, and 15 min before quenching. While synthesis of ribose-5-phospate (ribose-5-P), glutamate, and ATP was comparable between the control and ferulic acid-treated samples, biosynthesis of dCTP was impeded in the presence of ferulic acid.

Supplementary Figure S4: Feruloyl amide inhibits nucleotide biosynthesis at both 5 minutes (short-term) and 2 hours (long-term). Anaerobic *E. coli* culture in minimal medium with non-labeled xylose was exposed to 5.5 mM feruloyl amide for either 5 minutes (short-term) or 2 hours (long-term) before it was switched to 1, 2 ¹³C-xylose-containing medium. Samples for intracellular metabolite analysis were the taken at 0.5, 1, 2, 4, 7, 10, and 15 min thereafter. The blue-to-red gradient represents the amount of ¹³C-label incorporation (sum of all ¹³C-labeled forms) into each metabolite as a fraction of its total pool; for each metabolite, the lowest value (non-labeled) was set to blue while the highest ¹³C-label fraction value in control samples was set to red. The data represent the average of two biological replicates.

Supplementary Figure S5: Complete inhibition of nucleotide *de novo* biosynthesis in the presence of phenolic amide inhibitors. Fragmentation (LC-MS/MS) analysis from the ¹³C isotopic tracer experiments described in Fig. 2 showed no ¹³C incorporation into nucleobases (adenine of ATP and uracil of UTP) in the presence of feruloyl amide while ¹³C-carbon was incorporated into both phospho-ribose sugar and nucleobases in the control. The same pattern was observed in coumaroyl amide-treated cells. The data represent identical results from two biological replicates.

Supplementary Figure S6: Nucleoside supplementation rescued growth inhibition by phenolic inhibitors. (Top) Feruloyl amide. **(Middle)** Coumaroyl amide. **(Bottom)** Ferulic acid. *E. coli* was grown anaerobically with xylose and supplemented with 0, 0.25, or 0.75 mM of nucleosides (adenosine, guanosine, cytidine, thymidine, and uridine). Exponential culture (OD₆₀₀ 0.2-0.3) was then divided into individual flasks containing 2.75 mM feruloyl amide, coumaroyl amide, or ferulic acid. Cell growth was measured using OD_{600} . This graph is a representation of 2 independent experiments that gave identical results.

Xylose uptake rates (mM/hour/OD)						
	2.75 mM treatment	5.5 mM treatment				
Control	4.593 ± 0.171					
Feruloyl amide	2.196 ± 0.152	1.548 ± 0.248				
Coumaroyl amide	3.119 ± 0.116	1.747 ± 0.110				



2.75 mM Feruloyl amide
2.75 mM Coumaroyl amide
5.5 mM Feruloyl amide
5.5 mM Coumaroyl amide



Figure S3



Figure S4



Figure S5



Feruloyl amide 03 RT: 9.22 AV: 1 NL: 1.24E7 2 505 99ghod30 00 (50.00-535 00 phosphoribose moiety adenine C₅H₇O₉P, 134 C₅H₅N FA1_150200174154 #504 RT: 9.22 AV: 1 NL: 9.7855 F: FTMS - p ESI Full ms2 508.00@hv430 00 M0.00 A04 or +2 134.04695



Feruloyl amide





Supplementary Table S1: Lignocellulose-derived phenolic inhibitors identified in AFEX-pretreated corn stover hydrolysates (Keating, D. H. et al. Front Microbiol 5, 402 (2014))

Lignotoxins	Structures	Concentrations (mM)		
Feruloyl amide		5.5		
Coumaroyl amide	HO NH2	5.5		
<i>p</i> -Coumaric acid	но	1.05		
Ferulic acid	о он	0.36		
Benzoic acid	ОН	0.48		
Syringic acid		0.08		
Cinnamic acid	ОН	0.09		
Vanillic acid	НО ОН	0.09		
Caffeic acid	но он	0.01		
Vanillin	но	0.13		
Syringaldehyde		0.16		
4-Hydroxybenzeldehyde	но-	0.2		
4-Hydroxyacetophenone	но-С	0.02		

5-Hydroxymethylfurfural (HMF) was not included in this study

	Collision			Fragment	Molecular		
Metabolites	energy	Labeled forms	Mass (m/z)	mass (m/z) of	formula	Names of fragments	
	energy			interest	(neutral)		
ATP	30	non labolad	505 09946	134.047	$C_5H_5N_5$	Adenine	
			505.90040	272.957	$C_5H_7O_9P_2$	phosphoribosyl moiety	
			507 00519	134.047	$C_5H_5N_5$	Adenine	
		2 ¹⁶ C-labeled carbons	507.99516	274.964	$C_5H_7O_9P_2$	phosphoribosyl moiety	
				135.050	$C_5H_5N_5$	Adenine	
		3 °C-labeled carbons	506.99654	274.964	$C_5H_7O_9P_2$	phosphoribosyl moiety	
			510 00190	136.054	$C_5H_5N_5$	Adenine	
		4 °C-labeled carbons	510.00169	274.964	$C_5H_7O_9P_2$	phosphoribosyl moiety	
UTP 30		non labolod	492 06124	111.020	$C_4H_4N_2O_2$	Uracil	
			402.90124	272.957	$C_5H_7O_9P_2$	phosphoribosyl moiety	
	30		484 06706	111.020	$C_4H_4N_2O_2$	Uracil	
		2 ¹⁶ C-labeled carbons	404.90790	272.957	$C_5H_7O_9P_2$	phosphoribosyl moiety	
			486 07467	111.020	$C_4H_4N_2O_2$	Uracil	
		4 "C-labeled carbons	400.97407	272.957	$C_5H_7O_9P_2$	phosphoribosyl moiety	

Supplementary Table S2: Target MS/MS parameters used to obtain ¹³C labeling patterns of ATP and UTP

Other parameters for targeted MS/MS

Resolution: 35,000 AGC target: 5E5 Isolation width: 1.4 (m/z) Default charge: 1 Polarity: negative

Supplementary Table 3: Intracellular metabolite levels after exposure to feruloyl amide and coumaroyl amide

	FeruloyI amide						CoumaroyI amide							
Metabolites\Exposure time 3PG	0_min 0	10_min -0.59898782	30_min -0.506917429	60_min -0.070448779	120_min -0.953475782	180_min -0.553614415	240_min -1.09574809	0_min 0	10_min -0.386337578	30_min -0.70499683	60_min -1.380296819	120_min -0.456253057	180_min -1.043775617	240_min -2.087450862
3-phospho-serine	0	-1.886182023	-2.880006452	-1.769996236	-2.918024288	-2.742565151	-4.321431897	0	-2.252407572	-1.447773206	-4.852652033	-1.453334767	-2.32227962	-3.811537489
acetyl-CoA	0	-0.671685291	-0.981932281	-0.143037379	-1.005443628	-0.955475561	-0.963870413	0	-0.835240966	-0.440193926	-0.781857382	-0.079382381	-0.370139467	-0.67161571
AKG	0	-0.608070824	-0.67652452	-0.026542189	-0.403627659	-0.797574028	-0.663304666	0	-0.98693902	-0.607038758	-1.015102282	-0.061688026	-0.369530951	-0.96354267
aspartate	0	-0.879699073	-1.17081422	-0.593656448	-1.202421564	-0.917891735	-1.137405649	0	-0.803416045	0.134044406	-1.016579667	-0.994883509	-1.358962221	-1.472981282
ATP	0	-0.630375586	-0.177057855	0.378582281	-0.917786245	-1.247771673	-0.502931479	0	0.146880651	-0.235440204	-0.151838751	0.795169863	0.488753576	-0.359039922
citrate/isocitrate	0	-0.181960469	0.305849193	0.442980239	0.100969747	0.38047117	0.278132073	0	0.524859953	-0.353442286	-2.84437926	-1.217884693	-2.476358451	-3.937436739
CMD	0	-0.726735885	-0.388898822	-0.198126774	-1.144461879	-0.949436546	-1.345600193	0	-0.1/80/9281	-0.612614603	-0.279480868	-0.163213957	-0.180714083	-0.3452///2/
CMP	0	0.00649526	1 627942002	-0.367 136595	1 566959242	0.540400492	1 271202721	0	0.221760156	0.302207031	0.779401042	0.417020465	0.125650026	0.620224214
CTP	0	1 00/377262	-0.502/043093	-0.92488701	-0.808073301	-0.31891144	-0.333801304	0	2.002477501	-0.18528667	0.82382117	1 /37288/5	1 10542505	0.029324214
datp	0	-2 750964161	-0.502424737	-1 47904151	-2 550318023	-3 466489929	-0.333601304	0	-0.451113806	-0.10320007	-0.983877978	-0.053529612	-0.950421308	-2 101970885
dCTP	0	0.295027293	-0.383065441	-0.397343375	-1.869116106	-0.902919769	-1.078428322	ő	1.052347072	-0.613740726	-0.167239864	0.025069682	-0.564370052	-1.329084623
deoxyribose-P	0	-2.268392832	-1.909604973	-1.618258642	-1.683541653	-0.896709714	-0.928174275	0	-1.696114704	-1.019439184	-2.292484096	-1.665740483	-1.832161553	-2.182202075
DHAP/GAP	0	-0.590864623	-0.984414423	-0.446651256	-0.547102958	-0.481484363	-0.259955814	0	0.221133863	0.255269517	0.410127188	0.423848504	0.390738094	0.33527536
D-sedoheptulose-1/7-P	0	-0.201353484	-0.590743541	-0.283720223	-0.294913895	-0.277207715	0.158099108	0	-0.507531552	-0.487295986	-1.037788745	-0.648223574	-0.575139619	-0.658830361
dTDP	0	-1.363613946	-1.402745097	-1.230765214	-2.507755722	-1.690524191	-2.155881472	0	-0.333467771	-0.542750318	-0.396657032	-0.18104534	-0.573395518	-0.846452523
dTMP	0	0.087212842	0.137126342	0.465019444	-0.786650702	-1.105302894	-0.881428071	0	0.460430789	-0.315563696	-0.925433316	-1.025652222	-1.638361108	-1.59899078
dTTP	0	0.302550241	0.015338095	-0.337562881	-1.5749491	-1.607474017	-1.709458458	0	1.184571099	-0.197544801	-0.250215408	-0.310575024	-0.347484366	-1.041501427
FAD	0	-0.552688529	-0.142851531	0.529475703	-0.98894527	-0.371564817	-0.520356472	0	-0.345629392	-0.322829806	-0.50327442	0.378506967	-0.208110692	-0.784457264
FBP	0	-1.328195186	-1.231283845	-0.593885733	-1.442785594	-1.911695137	-1.055011642	0	0.339367009	0.40838806	0.52592838	0.832938285	0.722803728	0.171994578
fumarate	0	-0.108946317	-0.136968325	0.20553425	-0.095477238	-0.07828425	-0.381673658	0	-0.309740119	-0.00138323	-0.689577837	-0.138902505	-0.535057976	-1.066432701
G6P	0	-0.619381603	-1.504236824	-1.368150081	-1.209058054	-1.276995488	-0.517781147	0	-0.273863666	-0.322908413	-0.404451792	-0.30878653	-0.303606669	-0.236091409
glutamate	0	-1.58278072	-1.631342259	-1.427897451	-1.77740545	-1.586558198	-1.764924219	0	-1.922932823	-1.173687038	-2.199621829	-1.722504732	-1.733632979	-2.040599336
glutamine	0	-0.411709916	-1.231352228	-0.492941262	-0.559099872	0.258839172	0.38687285	0	-1.283211895	-0.905820394	-1.78997159	-2.318148271	-1.811942167	-2.027331316
glutathione disulfide	0	0.847329152	1.319080432	1.199321026	0.717623721	0.05020459	0.719031693	0	1./1318191/	1.298859219	1.456549648	1.196887569	1.419693257	1.205004332
glycerate	0	0.001587054	0.318851428	0.576069586	0.491940767	0.855369544	0.937971302	0	1.17415091	0.590720598	1.822196234	1.146584361	1.211007909	0.59012599
GIP	0	-0.8296316	-1.149349761	-0.425022447	-1.552155094	-1.255/865/1	-1.188814834	0	0.378621127	-0.907223901	-0.5//1/8118	0.866977681	0.136142279	-1.240683589
IMP	0	-0.124965003	-1 25583887	0.001735597	-1 301582226	-0.110090473	-0.2906092	0	-1.39300979	-1.510950436	-2.130314007	-0.205930959	-0.530459349	-1.211/541/7
L-arginino-succinate	0	-1 247016795	-0.00808449	0.789044230	-0.31077895	0.035220232	-0.026701378	0	-1.377001222	-0.016307185	-0.561765012	-0.057783246	-0 755327457	-0.911540365
malate	0	-0.417328509	-0.325550448	-0.299165606	-0.900431014	-1.066002318	-0.020731370	0	-0.281553566	-0.010307105	-0.501705512	-0.037703240	-0.733327437	-0.311340303
methionine	0	-2.741134793	-0.111378123	1 044394279	0.036862039	1.151601816	-0.287660819	ő	-1.017595696	-0.302993552	-0.384447473	0.281411236	-0.014550454	0 44965592
N-acetvl-glutamate	0	-2.72652703	0.013579674	-0.205291666	-1.123468862	-1.273583284	-1.735675108	0	-1.77909959	-0.685583331	-2.204569913	-1.949320594	-2.386895923	-2.610614382
N-acetyl-L-ornithine	0	-2.433716247	-0.799444301	-1.133174175	-1.891107356	-1.862247123	-1.681617934	0	-1.447784372	-0.373840484	-1.012010834	-0.924897073	-1.245271593	-1.16496231
NAD+	0	-0.088173424	-0.056098815	0.327999561	-0.34158218	-0.350443739	0.100232055	0	-0.09655746	-0.392732052	-0.668778546	-0.062691412	-0.351307439	-0.621026955
NADP+	0	-0.234174699	-0.251911551	0.380129084	-1.577859806	-1.620806708	-1.342102099	0	-0.726018544	-0.950876194	-1.110845296	-0.341037848	-0.970036735	-1.582543035
octoluse 8/1P	0	-0.359133323	-1.050850117	-0.599830634	-0.969681838	-0.609689943	-0.098854055	0	-0.45113663	-0.494267921	-1.075844045	-0.891367706	-0.738583735	-0.642534062
octulose bisphosphate	0	-1.460247341	-1.227749914	-0.689986123	-1.691935336	-1.274621975	-1.361383503	0	-0.686615845	-0.819836081	-0.77099249	-0.164320051	-0.112801837	-0.752652087
ornithine	0	-1.052629975	-0.598442733	-0.084625475	-1.407413624	-1.236849648	-1.31454358	0	-0.16741834	-0.750290967	-0.349581496	-0.235711224	-0.223347669	-0.265830449
pantothenate	0	2.162674766	1.584593187	1.73373355	1.012083916	1.476944513	0.794280203	0	3.648657839	1.426293304	1.09290469	1.29614786	1.152768614	0.618542457
phenylalanine	0	0.1517583	0.038015922	0.496243924	0.382173908	0.715383116	0.514596314	0	-0.000387084	0.290141044	0.12243114	0.5261247	0.518895207	0.440650905
phenylpyruvate	0	-0.374063883	-0.460245839	-0.179928494	-1.285206965	1.092136504	-0.206152414	0	-0.041088999	0.026756265	-0.124082659	0.312042654	0.270467373	0.082631698
PEP	0	-0.888481	-0.66648388	0.038257718	-0.6698149	0.256971825	-0.375101126	0	-0.284888605	-1.879636608	-1.864951794	-1.474259148	-1.865490388	-1.89863543
PRPP	0	7.017897051	5.644413204	6.332452458	3.753766632	6.106137053	3.285572094	0	4.794875634	2.225095911	3.23156321	3.075182961	2.76046013	0.90092252
ribose-P	0	-0.251446527	-0.448780732	-0.110143177	-0.213372299	-0.45375126	0.042770316	0	-0.162205468	0.232547759	-0.398240973	0.034281189	0.264889317	0.270929395
sedoneptulose bisphosphate	0	-1.351586396	-0.633731364	-0.20615956	-1.543036769	-1.6/684/691	-1.621704693	0	-0.160618689	-0.403137914	-0.31961957	0.293054667	0.344956092	-0.240608048
serine	0	-0.835660035	-0.911403166	0.236451618	-1.052017194	-0.611056178	-1.028469129	0	-0.105262832	0.208365341	0.072349243	0.732789528	0.020936917	-0.284208377
sil-giyceroi-3-P	0	0.022127214	-0.111305617	0.795665903	0.44095691	1.40090/92	0.143201701	0	0.623267429	0.461409010	-0.020120397	-0.230050009	-0.264502105	-0.090220464
succinde	0	-0.742500942	-0.540808312	0.499748737	-0.244220327	0.13/137816	0.230102433	0	0.050710455	-0.176658011	-0.273732406	0.718288347	0.031420080	0.068404428
threenine	0	-0.742300342	-0.270000523	0.205807582	-0.244229327	0.134137810	0.05224100	0	-0.661104056	-0.070121502	-0.2/3/32400	-0.684471628	-0.42733106	-0.487272369
tyrosine	0	-0.454154752	0.270000323	1 027242489	1 232624659	2 052910106	1 724867844	0	0.905270572	1 301224514	1 848772525	2 178774314	2 403833449	2 366164864
UDP	0	1.09208944	-0.563345022	0.035323736	-0.848777964	-0.240420334	-0.326596064	ő	1.083876129	-1 416166721	-0.998075833	-0.05967842	-0.312449574	-0.7859888889
UDP-D-alucose	0	-0.163827418	-1.848858216	-1.319152344	-0.910690031	-0.934371692	-0.701054141	0	-0.020637705	-0.982156006	-0.924450597	-0.700052117	-0.675709648	-0.713097783
UDP-D-alucuronate	0	0.024490082	-0.42408877	-0.000790609	-0.609952893	-0.80417099	-0.783280392	0	0.002347726	-0.731001714	-0.975024289	-0.483432052	-0.735924872	-1.243135271
UDP-GlcNAc	0	-0.034140501	-1.08156606	-0.527603109	-0.658604825	-0.643073283	-0.461703633	0	0.423880357	-0.167109358	-0.476092189	-0.333673398	-0.517019342	-0.461819487
UMP	0	0.299822539	-1.209811403	-0.211828042	-0.253250766	0.124025798	0.288859886	0	-0.402789996	-1.260042905	-1.282986427	-0.892692538	-0.586679995	-0.654970541
UTP	0	0.610055734	-1.565124407	-0.985968338	-1.614126678	-1.428872757	-1.09759562	0	1.214543407	-1.934901301	-1.002903426	-0.139767237	-0.589344135	-1.238561338
valine	0	-0.582214316	-0.51300558	0.200495424	-0.531002783	-0.220833367	-0.682997891	0	-0.438480289	-0.319568966	-0.781201847	-0.699645107	-1.284656438	-1.361043049
xylulose-5-P	0	-0.414002322	-0.480563199	0.031081124	-0.24513169	-0.377882451	0.218505749	0	0.046513569	0.010417967	-0.20180348	0.147688608	0.306142019	0.28925022

For each metabottie, peak intensity was normalized to a "0 min" control. Numbers shown here are log2 of the normalized values.