

Supplementary tables

Supplementary Table 1. Metabolite uptake and secretion rates used as lower and upper bound constraints for exchange reactions.

Days 2-4				
Reaction ID	P10AVG	P12A	P26AVG	P28AVG
EX_ala_L(e)	0.11488785	0.10998864	0.11417243	0.1199731
EX_arg_L(e)	-0.0083719	-0.0174901	-0.0262433	-0.0002193
EX_asn_L(e)	-0.0703625	-0.0606797	-0.0636735	-0.0523843
EX_asp_L(e)	0.01237243	0.01248391	-0.0116814	0.00174746
EX_cys_L(e)	-0.0066332	-0.0060249	-0.0074701	-0.0064527
EX_gln_L(e)	-0.103233	-0.0818512	-0.0975054	-0.0977585
EX_glu_L(e)	0.02875166	0.0201034	0.0272922	0.02597857
EX_gly(e)	0.01674229	0.02119034	0.00825866	0.02581755
EX_his_L(e)	-0.0038299	-0.0031269	-0.0046662	-0.0030272
EX_ile_L(e)	-0.0117691	-0.0075432	-0.0128572	-0.0047623
EX_leu_L(e)	-0.0222804	-0.0145185	-0.0219073	-0.0095245
EX_lys_L(e)	-0.0110609	-0.0064226	-0.0152629	-0.0037475
EX_met_L(e)	-0.0053434	-0.0041598	-0.0069393	-0.003119
EX_phe_L(e)	-0.0063262	-0.0051054	-0.0062903	-0.0044215
EX_pro_L(e)	0.00388689	0.0051221	0.00118397	0.00482055
EX_ser_L(e)	-0.0315439	-0.018359	-0.0268297	-0.0090033
EX_thr_L(e)	-0.0060582	-0.01	-0.0144642	-0.01
EX_trp_L(e)	-0.0024328	-0.001755	-0.0025669	-0.0012119
EX_tyr_L(e)	-0.0092567	-0.0059119	-0.007346	-0.0031408
EX_val_L(e)	-0.0113421	-0.0094403	-0.0116694	-0.0092107
EX_glc(e)	-0.3206525	-0.2124735	-0.2917124	-0.2985122
EX_lac_D(e)	0.18115302	0.17399467	0.26152072	0.25937921
EX_nh4(e)	0.0692352	0.0611262	0.0900898	0.087675
EX_pyr(e)	-1000	-1000	-1000	-1000
EX_orn(e)	-1000	-1000	-1000	-1000
EX_taur(e)	-1000	-1000	-1000	-1000

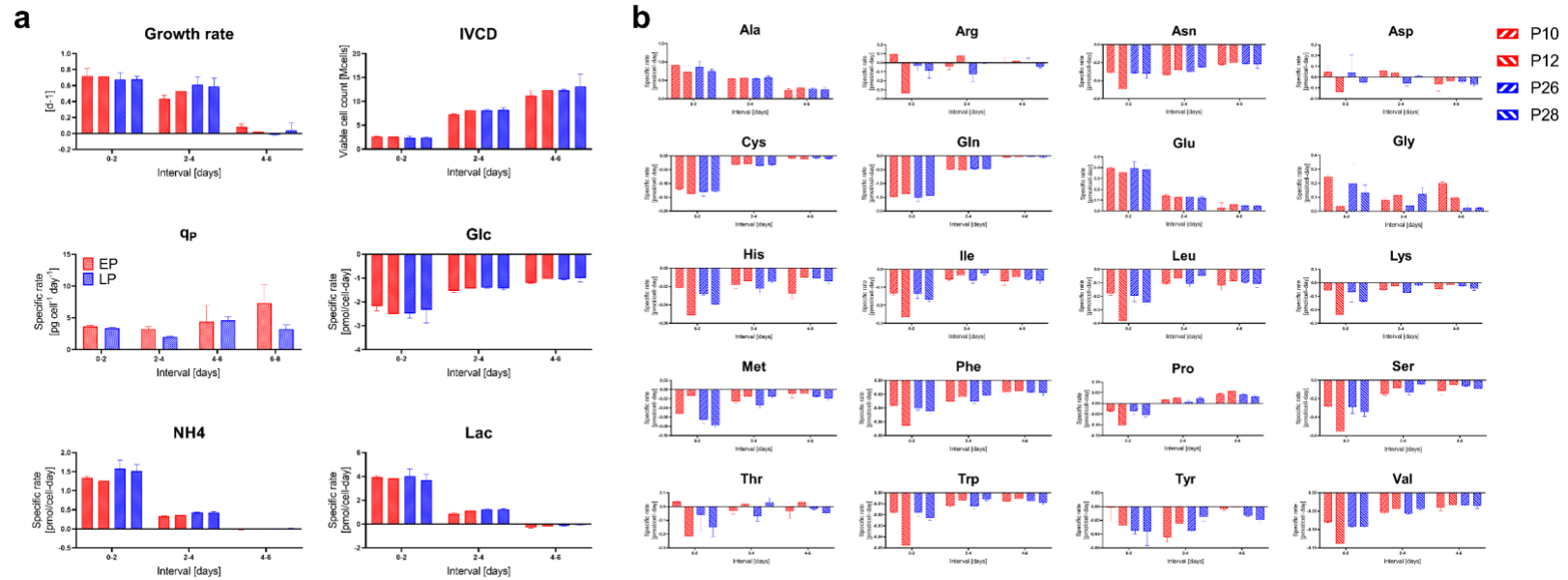
EX_4hpro(e)	-1000	-1000	-1000	-1000
EX_ser_D(e)	0	0	0	0
EX_arg_D(e)	0	0	0	0
EX_asp_D(e)	0	0	0	0
EX_ala_D(e)	0	0	0	0
EX_ala_B(e)	0	0	0	0
EX_pro_D(e)	0	0	0	0
EX_isomal(e)	0	0	0	0
EX_gal(e)	0	0	0	0
EX_lac_L(e)	0	0	0	0
Days 4-6				
Reaction ID	P10AVG	P12A	P26AVG	P28AVG
EX_ala_L(e)	0.0502034	0.06274	0.05721371	0.0515617
EX_arg_L(e)	0.00059819	0.00441098	0.00157125	-0.0089452
EX_asn_L(e)	-0.0462769	-0.0424452	-0.0447013	-0.045339
EX_asp_L(e)	-0.0133312	-0.007848	-0.0084169	-0.0139944
EX_cys_L(e)	-0.0018985	-0.0022862	-0.0016414	-0.0019002
EX_gln_L(e)	-0.0087543	-0.0048782	-0.0063517	-0.0081735
EX_glu_L(e)	0.00616159	0.01244785	0.01067798	0.00981372
EX_gly(e)	0.04160733	0.02014248	0.00455351	0.00457327
EX_his_L(e)	-0.0056645	-0.0020798	-0.002271	-0.0029042
EX_ile_L(e)	-0.013813	-0.0090854	-0.0113911	-0.0132176
EX_leu_L(e)	-0.0252711	-0.0184094	-0.0198138	-0.0224806
EX_lys_L(e)	-0.0098619	-0.0034501	-0.0052116	-0.0092608
EX_met_L(e)	-0.0017622	-0.0018413	-0.003084	-0.003872
EX_phe_L(e)	-0.0034854	-0.0031842	-0.0034875	-0.0038023
EX_pro_L(e)	0.00923252	0.01220159	0.00872216	0.00671336
EX_ser_L(e)	-0.0242032	-0.0109486	-0.0134898	-0.0184526
EX_thr_L(e)	-0.0073731	-0.01	-0.0028849	-0.0099664
EX_trp_L(e)	-0.0016468	-0.001136	-0.0015863	-0.0018419
EX_tyr_L(e)	-0.0007219	-0.0001905	-0.0026585	-0.0040578
EX_val_L(e)	-0.0084763	-0.006818	-0.0069691	-0.0073362
EX_glc(e)	-0.2497368	-0.2166217	-0.220959	-0.2076632
EX_lac_D(e)	-0.0567524	-0.0365025	-0.0274639	-0.0164612

EX_nh4(e)	-0.001334	0.00063921	0.00181305	0.00274711
EX_pyr(e)	-1000	-1000	-1000	-1000
EX_orn(e)	-1000	-1000	-1000	-1000
EX_taur(e)	-1000	-1000	-1000	-1000
EX_4hpro(e)	-1000	-1000	-1000	-1000
EX_ser_D(e)	0	0	0	0
EX_arg_D(e)	0	0	0	0
EX_asp_D(e)	0	0	0	0
EX_ala_D(e)	0	0	0	0
EX_ala_B(e)	0	0	0	0
EX_pro_D(e)	0	0	0	0
EX_isomal(e)	0	0	0	0
EX_gal(e)	0	0	0	0
EX_lac_L(e)	0	0	0	0

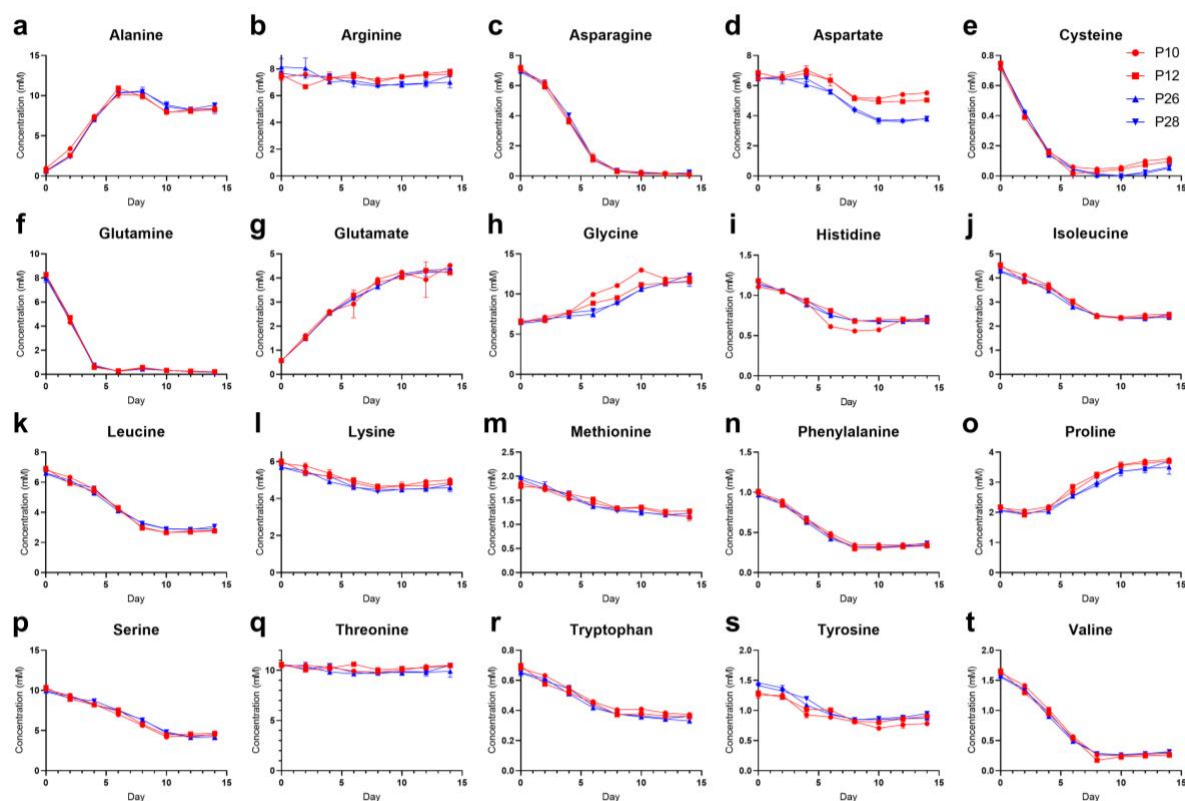
Supplementary Table 2. Performance metrics of machine learning models averaged over 5-fold cross-validation. Values are presented as Mean $\pm$ Standard Deviation.

Model	Full Name	Accuracy	Precision	Recall	F1-score
LR	Logistic Regression	0.600 $\pm$ 0.138	0.640 $\pm$ 0.125	0.600 $\pm$ 0.138	0.599 $\pm$ 0.146
Lasso	Lasso Regression	0.629 $\pm$ 0.191	0.707 $\pm$ 0.149	0.629 $\pm$ 0.191	0.633 $\pm$ 0.176
EN	Elastic Net	0.943 $\pm$ 0.048	0.954 $\pm$ 0.035	0.943 $\pm$ 0.048	0.943 $\pm$ 0.048
CART	Classification and Regression Trees (Decision Tree)	0.629 $\pm$ 0.104	0.701 $\pm$ 0.075	0.629 $\pm$ 0.104	0.627 $\pm$ 0.082
KNN	K-Nearest Neighbors	0.629 $\pm$ 0.138	0.690 $\pm$ 0.130	0.629 $\pm$ 0.138	0.609 $\pm$ 0.165
SVM	Support Vector Machine	0.714 $\pm$ 0.207	0.753 $\pm$ 0.185	0.714 $\pm$ 0.207	0.723 $\pm$ 0.188
GP	Gaussian Process	0.986 $\pm$ 0.029	0.987 $\pm$ 0.025	0.986 $\pm$ 0.029	0.985 $\pm$ 0.029
RF	Random Forest	1.000 $\pm$ 0.000	1.000 $\pm$ 0.000	1.000 $\pm$ 0.000	1.000 $\pm$ 0.000
Extra	Extra Trees Classifier	0.943 $\pm$ 0.052	0.958 $\pm$ 0.034	0.943 $\pm$ 0.052	0.945 $\pm$ 0.049
AdaBoost	Adaptive Boosting	0.957 $\pm$ 0.052	0.967 $\pm$ 0.040	0.957 $\pm$ 0.052	0.958 $\pm$ 0.052
GBM	Gradient Boosting Machine	0.986 $\pm$ 0.029	0.987 $\pm$ 0.025	0.986 $\pm$ 0.029	0.985 $\pm$ 0.029

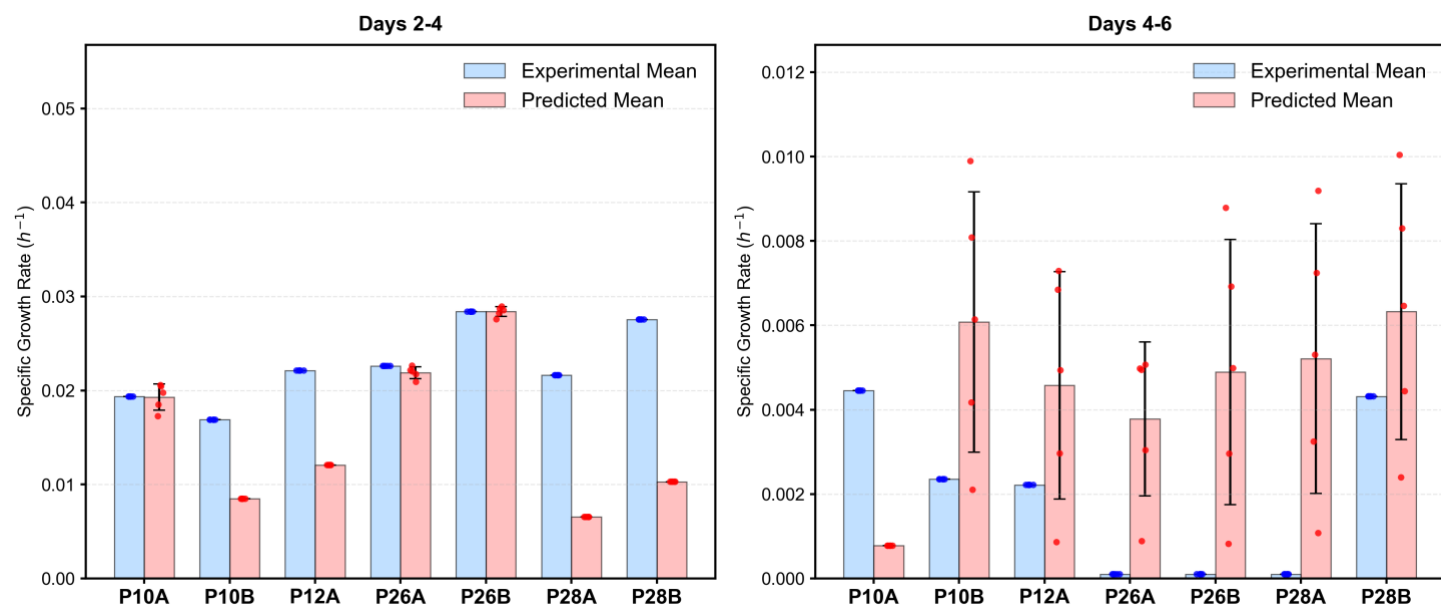
Supplementary figures



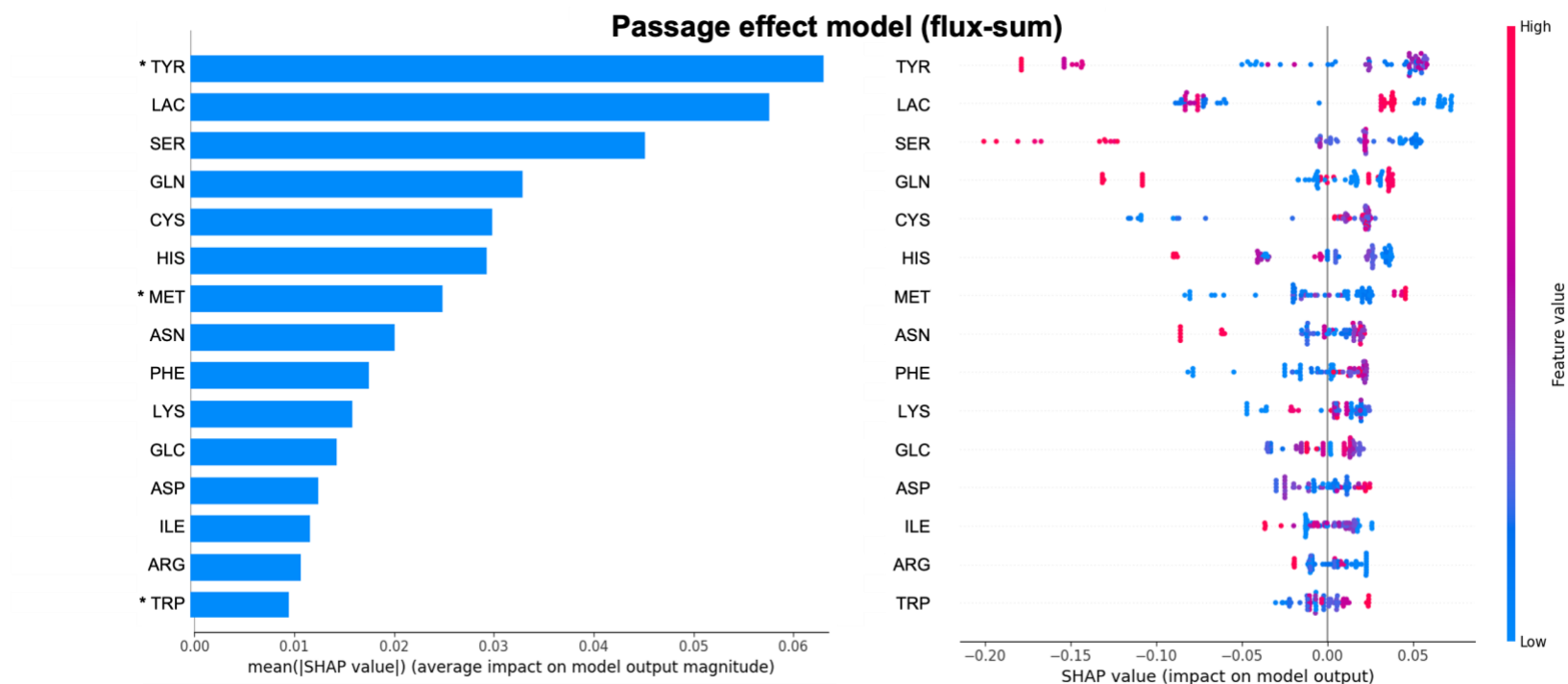
Supplementary Fig. 1 Calculation of specific metabolic rates and metabolic parameters. Growth rate, integrated viable cell density (IVCD), productivity (qP), and secretion rates of glucose, ammonia and lactate are illustrated in (a) across different intervals (days 0-2, 2-4, and 4-6) for early (P10, P12) and late (P26, P28) passages. Secretion rates of 20 amino acids were represented in (b). Here, the negative value of secretion rates indicate their consumption.



Supplementary Fig. 2 Time-course profiles of extracellular amino acid concentrations. Concentration profiles (mM) of 20 amino acids (a–t) were measured bi-daily from day 0 to day 14. Cultures represent early passage (red; P10, circles; P12, squares) and late passage (blue; P26, triangles; P28, inverted triangles) conditions. Here, we represent the mean and standard deviation of biological duplicates.



Supplementary Fig. 3 Comparison of experimental and predicted specific growth rates. The bar plots display the experimentally measured specific growth rates (blue) and the mean predicted growth rates from the top five selected ecFBA solutions (red) for each biological replicate across intervals of days 2-4 and days 4-6. Error bars represent standard deviations.



Supplementary Fig. 4 Shapley Additive exPlanations (SHAP) analysis of the machine-learning model trained to classify passage group using ecFBA-derived flux-sum features. (Left) Top 15 metabolites ranked by mean absolute SHAP value, representing their overall contribution to passage classification. Asterisk (*) denotes that the metabolites were identified as EP-enriched predictors in the MVDA analysis ($VIP > 1$), thereby representing features consistently highlighted across both analytical frameworks. (Right) SHAP summary plot showing the distribution of SHAP values (flux) for each simulated condition, illustrating how feature magnitude influences the predicted passage group.