Supporting information 8: Supporting methods

S8.1 Reconstruction of the metabolic network

From the CMR and KEGG databases, the genomic information of *P. putida* KT2440 was downloaded and the gene information and the E.C. numbers assigned to enzymes encoded by the respective genes were extracted. All metabolic reactions were collected and transferred into the metabolic model reconstruction. Water and hydroxyl ions were not included by assuming that there are other non-enzymatic functions in the cell that uses these molecules and therefore do not need to be balanced in the set composed of enzymatic reactions. Once the set of biochemical reactions has been collected, the list is curated for any inconsistencies or gaps in the network [25].

S8.2 Fermentation conditions

Batch cultures were carried out as follows. Seed cultures were prepared by transferring 500 μL of 10 mL overnight cultures prepared in M9 medium plus 10 g/L of glucose into 250 mL Erlenmeyer flask containing 50 mL of the same medium and incubated in a shaker at 30˚C. Cultured cells were used to inoculate the fermentor containing 2 L of M9 medium containing 20 g/L glucose at 30˚C. Batch culture was carried out in a 6.6 L Bioflo 3000 fermentor (New Brunswick Scientific Co., Edison, NJ). The agitation speed was initially set at 200 rpm and increased accordingly using automatic controlling to maintain a dissolved oxygen concentration (DOC) at 40% of air saturation or greater. The pH was adjusted at 7.00±0.1 using 28% (v/v) ammonia solution. Foaming was controlled by the addition of Antifoam 289 (Sigma, St. Louis, MO). Aeration was done at a flow rate of 0.25 vvm during the whole period of fermentation.

Chemostat cultures were performed to determine the cellular energetic parameters,

growth associated maintenance energy and non growth associated maintenance energy, at dilution rates of 0.15 and 0.20 /h with continuous feeding and draining of a medium and cultural broth. The sterilized M9 medium plus 20 g/L of glucose in a 15 L reservoir was fed into the fermentor while an equal volume of culture broth was drained from the fermentor using a peristaltic pump (Cole-Parmer, Vernon Hills, IL). Steady state in glucose limited chemostat was determined by monitoring the constant concentrations of biomass, glucose, and organic acids in the fermentor for 3 consecutive samples taken at 1 to 3 h intervals. Growth associated maintenance energy (g/gDCW) is represented as the ATP term in the biomass equation and non growth associated maintenance energy (mmol/gDCW·h) is represented in an independent ATP consumption reaction in which the flux is fixed to the value calculated for this parameter. Calculation of the energetic parameters has been detailed elsewhere [25].

Verification of *P. putida* ability or inability in utilizing various compounds was done by culturing cells in a M9 medium supplemented with various other compounds in place of glucose and incubated at 30°C. *P. putida* was unable to utilize a compound for growth if the OD_{600} did not rise above 0.05 after 12 hours of incubation at 30°C.

S8.3 Analytical procedures

Cell growth was monitored by measuring the absorbance at 600nm (OD_{600}) with an Ultrospec3000 spectrophotometer (Amersham Biosciences, Uppsala, Sweden). Cell concentration defined as gram dry cell weight (gDCW) per liter was calculated from the pre-determined standard curve relating the OD_{600} to dry weight (1 OD_{600} = 0.35 gDCW/L). Cell concentration was determined by centrifuging a known volume of culture broth containing cells

of known optical density at 2090 x*g* for 20 min at 4°C. The pellet was then washed three times with 0.15M NaCl. After the final wash, the pellet was re-suspended in 20 mL of NaCl solution and poured into a pre-weighed aluminum plate and dried at 55°C.

The concentrations of glucose and organic acids were determined by high-performance liquid chromatography (Varian ProStar 210, Palo Alto, CA) equipped with UV/VIS (Varian ProStar 320, Palo Alto, CA) and RI (Shodex RI-71, Tokyo, Japan) detectors. A MetaCarb 87H column (300 × 7.8 mm, Varian) was isocratically eluted with 0.01 N H_2SO_4 at 60°C and a flow rate of 0.6 mL/min.

S8.4 *In silico* flux analysis

For the analysis of the genome-scale metabolic model, *in silico* flux analysis was used where the internal metabolites are first balanced under the assumption of pseudo-steady state (Gombert and Nielsen, 2000). This results in a stoichiometric model $S_{ij} \bullet v_j = 0$, in which S_{ij} is a stoichiometric coefficient of a metabolite i in the jth reaction and v_j is the flux of the jth reaction given in mmol $gDCW^{-1}$ h^{-1}. Linear programming (LP), subject to the constraints pertaining to mass conservation, reaction thermodynamics, and capacity, was carried out to determine the fluxes (Varma and Palsson 1994). These constraints are presented in the forms of upper and lower bounds for the fluxes ($v_{j,min} \leq v_j \leq v_{j,max}$) for each reaction j, and used together with an objective function Z, usually the biomass formation rate. The flux of any irreversible reaction is considered to be positive and the negative flux signifies the reverse direction of the reaction.

Flux analysis on the flux distribution through the PHA biosynthesis network was examined by adding two additional exchange reactions for NADH and NAD to PpuMBEL1071_PHAc100 to represent the addition of the two cofactors in the media. Flux

values for these exchange fluxes were set so that the ratio between the two fluxes were between 0.1 and 1.

In silico flux response analysis [12] was performed to examine how the metabolic model responds to constraints applied to specified fluxes. In anaerobic flux response analysis, the minimum and maximum range for the influx of external ATP was determined and biomass formation rate was used as the objective function. A flux profile graph is generated by plotting the objective function (*i.e.* biomass formation rate) against the corresponding flux that is being perturbed in the range of values determined previously (*i.e*. influx ATP).