1   **Theoretical design of a space bioprocessing system to produce recombinant proteins**

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Abstract

Space-based biomanufacturing has the potential to improve the sustainability of deep space exploration. To advance biomanufacturing, bioprocessing systems need to be developed for space applications. Here, commercial technologies were assessed to design space bioprocessing systems to supply a liquid amine carbon dioxide scrubber with active carbonic anhydrase produced recombinantly. Design workflows encompassed biomass dewatering of 1 L *Escherichia coli* cultures through to recombinant protein purification. Equivalent system mass (ESM) analyses had limited utility for selecting specific technologies. Instead, bioprocessing system designs focused on minimizing complexity and enabling system versatility. Three designs that differed in biomass dewatering and protein purification approaches had nearly equivalent ESM of 357-522 kg eq. Values from the system complexity metric (SCM), technology readiness level (TRL), and degree of crew assistance metric identified a simpler, less costly, and easier to operate design for automated biomass dewatering, cell lysis, and protein affinity purification.

Introduction

28   Liquid amine scrubbing is a promising technology to capture CO_2 produced during

29   crewed extraterrestrial missions[1,2]. In this system, a CO_2-rich gas stream is passed over an

30   organic liquid amine which absorbs the CO_2. Pure CO_2 is recovered by raising the solution

31   temperature, which regenerates the amine for subsequent capture at low temperature[3]. An

32   efficient liquid amine system should have fast absorption kinetics to reduce system size as well

33   as a low desorption temperature to minimize energy inputs. Different liquid amines either have

34   fast absorption kinetics or low desorption temperature but not both simultaneously[4]. Carbonic

35   anhydrase is an enzyme that catalyzes the interconversion of CO_2 and HCO_3^- to increase the CO_2

36   sequestration in some liquid amine systems. Addition of carbonic anhydrase enhances the

37   absorption kinetics of liquid amines with a low desorption temperature enabling both reduced

38   size and improved energy efficiency of CO_2 scrubbing[5–7].

39   Implementing an enzyme-assisted liquid amine CO_2 scrubber requires a time-course

40   supply of carbonic anhydrase. On long-duration space missions close to Earth, this requirement

41   could be met by resupply or long-term storage. However, resupply is not an economical option

42   for many deep space missions like those planned for Mars. Purified enzymes are typically

43   sensitive to room temperature conditions and require ultra low storage temperatures to retain

44   activity long-term. Not all enzymes retain activity in low temperature storage and in situ

45   production of enzymes would mitigate risks of relying solely on low temperature storage of

46   proteins with limited stability. Space biomanufacturing systems have the potential to produce

47   enzymes and other biological materials using in situ resources during a Mars mission[8–13]. Space

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48   systems must minimize cost and crew time, while assuring astronaut safety and addressing

49   effects of increased radiation and reduced gravity[14–17].

50   Previous space biomanufacturing studies and reviews evaluated large-scale mission

51   design[8,18,19], microbial growth kinetics[20], and bioreactor design[20–22]. Extracting products of

52   interest at sufficient quality is equally essential to develop biomanufacturing. Systems such as

53   Wetlab-2 or the Gene Expression Measurement Module (GEMM) illustrate the challenge of

54   adapting biological sample processing for RNA extraction and molecular analysis in the

55   microgravity environment[23,24]. In these systems, the sample mass and volume processed was

56   small with the goal of providing biological inputs for analytical experiments[23,24]. Future space

57   biomanufacturing systems need to address post-growth processing at larger scales to extract

58   products for in situ use.

59   In this study, we compared commercial technologies and potential designs for in space

60   biomanufacturing systems. Our operational scenario was post-growth bioprocessing to produce

61   recombinant carbonic anhydrase from *Escherichia coli* during a Mars mission. Since carbonic

62   anhydrase is unlikely to be the only useful product in deep space missions, the ability to

63   produce a variety of recombinant proteins from multiple chassis organisms was a key

64   consideration for the designs. The designs were compared using equivalent system mass (ESM)

65   analysis[14,25], a system complexity metric (SCM)[16], technology readiness level (TRL)[26], and crew-

66   mediated steps to guide future prototype development efforts.

Results

68   Bioprocessing technology comparisons were based on a production scenario that

69   required thermostable and high-pH tolerant carbonic anhydrase for a liquid amine CO_2 capture

70   system during 600 days of surface operations on Mars[27,28]. In this system, enzyme activity will

71   decay with heating-cooling cycles, and will require intermittent addition of recombinant protein

72   to supply sufficient enzyme activity throughout the surface operations. Protein purification was

73   assumed to be required to reduce side reactions with the liquid amine; however, multiple

74   chromatography steps to produce highly purified protein are not required for this application[5].

75   Active enzyme can be produced in *E. coli* as an intracellular recombinant protein with a His-tag

76   for affinity purification. A prior study reported 180 mg/L of recombinant carbonic anhydrase

77   yield[29]. A total of 14.4 g of active enzyme was estimated to be required to supply a crew of six

78   for 600 days, and 100 cultures at 1 L volume is estimated to be sufficient.

79   *Potential workflows for the operational scenario*

80   Figure 1 shows five potential bioprocessing workflows (a, b, c, d, and e) starting from a

81   common cell growth and production step. Each workflow considers sub-processes of biomass

82   processing, protein extraction, and storage. These sub-processes were split into steps that

83   could have multiple alternate methods. For the sub-process of biomass processing, dewatering

84   and drying steps were the primary options considered. The stated use case will produce

85   recombinant protein intracellularly and will require protein extraction sub-process including cell

86   lysis, protein purification, and buffer exchange/desalting steps. Finally, protein product could

87   have a storage sub-process, either as biomass or as a purified product.

88   Workflow-a was selected as best aligned with the operational scenario of supplying

89   purified carbonic anhydrase on a 6 to 8-day cycle. This workflow moves from growth and

90   production steps to dewatering, cell lysis, protein purification, and buffer exchange to end with

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91   product utilization, cleaning, and disposal. Although storage and drying steps were considered,

92   they were not included and deemed unnecessary based on the selected scenario.

93   *Biomass processing*

94   Dewatering cultures greatly reduces processing volumes for protein extraction or

95   biomass storage sub-processes. However, it may be feasible to complete protein extraction

96   without a dewatering step. We modeled the impact of dewatering on cell lysis and protein

97   purification steps by scaling processing time for flow-through methods including bead beater

98   lysis (L-1), flow cell sonication (L-3), affinity resin column purification (P-1), and crude lysate

99   column purification (P-2) methods (Fig. 2, Supplementary data 1). This analysis showed that

100   processing cultures without dewatering would require multiple days using flow-through

101   methods.

102   Other methods could be completed in batch including enzyme lysis (L-2), large-volume

103   probe sonication (L-4), batch affinity resin purification (P-3), magnetic bead affinity purification

104   (P-4), and affinity membrane purification (P-5). These were scaled by increasing materials to

105   limit impacts on processing duration (Fig. 2). However, most protein purification methods were

106   developed for cell lysates from concentrated biomass suspensions. It is unknown whether

107   cultures could be lysed directly, or if large-scale batch purification would yield sufficiently

108   concentrated purified protein. We concluded that dewatering would help ensure feasibility for

109   batch methods and allow flow methods to be considered as options in the bioprocessing

110   designs.

111   ESM is a metric to estimate the flown mass required to implement a space technology

112   and can help guide selection of alternative technologies[14,25,30]. The metric is a linear model to

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113   estimate mass of the system along with mass equivalences for providing volume and other

114   infrastructure within a spacecraft (Supplementary data 1). Table 1 gives the 2015 and 2022

115   equivalency factors used to calculate ESM [31,32], and Table 2 reports total ESM for the methods

116   and bioprocessing designs. These calculations were highly correlated (r = 0.992), and the 2015

117   equivalency factors are reported in the figures to enable comparisons to prior technology

118   proposals. Due to the variability in calculating ESM for preliminary designs, the metric is only

119   used for selection of competing technologies when ESM estimates approach a 5- to 10-fold

120   difference[25,33]. Figure 3a shows ESM calculations for dewatering methods. Batch and

121   countercurrent centrifugation have ~5-fold higher ESM than a tangential flow filter. Despite the

122   similar ESM, a countercurrent centrifuge is easier to automate and can potentially reduce crew

123   time requirements. Based on these considerations, we removed batch centrifugation from

124   further consideration.

Table 1. Mars surface equivalency factors for this study		
Parameter	2015 Equivalency factor	2022 Equivalency factor
Shielded Volume (V)	215.5 kg_{eq}/m^3	79.3 kg_{eq}/m^3
Power (P)	87 kg_{eq}/kW	162 kg_{eq}/kW
Thermal Control (C)	146 kg_{eq}/kW	96 kg_{eq}/kW
Cold Storage (CS)	0.79 kg_{eq}/kg	0.21 kg_{eq}/kg
Water Treatment (W)	0.12 kg_{eq}/kg	0.3 kg_{eq}/kg
Waste Storage (WS)	0.83 kg_{eq}/kg	0.93 kg_{eq}/kg

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Table 2. Total ESM for the methods and designs reported in this study.

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Method/design	2015 factors	2022 factors	Designation
Tangential flow filter	50	40	D-1
Countercurrent centrifuge	290	220	D-2
Batch centrifuge	120	90	D-3
Bead beating	14.2	14.4	L-1
Enzyme lysis	4.1	2.6	L-2
Sonicator (flow cell)	189	201	L-3
Sonicator (large volume probe)	182	197	L-4
Affinity resin column	43	32	P-1
Crude lysate column	16	18	P-2
Batch resin purification	46	66	P-3
Magnetic beads	41	46	P-4
Affinity membrane	44	32	P-5
Design 1	460	400	
Design 2	360	340	
Design 3	520	450	

126   *Protein extraction*

127   ESM models for lysis eliminated sonication due to high power and cooling requirements

128   (Fig. 3b). Although enzyme lysis has a low ESM in this analysis, there are several concerns for

129   implementation of an enzyme-only method that could not be easily modeled via ESM.

130   Lysozyme is only effective as a lysis method for a limited number of microbial host species. The

131   enzyme is most effective when combined with chelators, detergent, or sonication to disrupt the

132   outer membrane of gram-negative bacteria like *E. coli*[34]. The chelators and detergents required

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133   for more effective enzyme lysis also create wastewater treatment challenges. Finally, enzyme

134   lysis is not as effective as mechanical lysis[35] and would likely require greater biomass growth to

135   achieve equivalent recombinant protein yield.

136   Mechanical lysis with bead beating is a well-established cell disruption technique for a

137   large variety of organisms and developmental stages including spores[36]. A small footprint, flow-

138   through bead beater has been used on the ISS for biology research, demonstrating technology

139   feasiblity[23]. Based on these factors, flow-through bead beating was selected as the lysis method

140   for design comparisons.

141   ESM estimates for the protein purification step compared five commercial affinity

142   purification methods. ESM estimates were within a 3-fold range for all technologies (Fig. 3c).

143   Like the cell lysis step, we considered feasibility of implementation to select three methods for

144   bioprocessing system design comparisons. The affinity resin column and crude lysate column

145   use equivalent flow-through approaches, but the affinity resin column required a clarified lysate

146   with minimal cell debris. The crude lysate column was selected for increased reliability and

147   reduced complexity. Batch affinity purification using magnetic beads requires a crew-assisted

148   step. By contrast, the batch affinity resin "tea bag" method would be simpler to automate and

149   was selected. The affinity membrane was selected to analyze design requirements for an

150   alternate solid matrix.

151   *Bioprocessing system integrated designs*

152   Figure 4 shows the steps and methods eliminated and retained to design integrated

153   bioprocessing systems. Table 3 summarizes key decisions and the rationale for selection of

154   specific steps and methods. Biomass storage, product storage, and drying steps were

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155   eliminated. For dewatering, the counter current centrifuge and filtration methods were

156   retained. For cell lysis, only a flow-through bead beater was retained. For protein purification,

157   crude lysate column, batch resin purification, and affinity membrane purification were retained.

Table 3. Summary of workflow method decisions and rationales.			
	Decision	Rationale	Issues/concerns
1	No long-term biomass storage	• Reduce complexity	
2	No long-term product storage	• Reduce complexity	• Increases mission risk in case of anomalies
3	No drying	• Reduce complexity	
4	Any component in contact with biological material will be single use	• Reduce crew time for cleaning • Reduce complexity • Reduce chemical safety concerns	
5	Include dewatering	• Shorten processing time	• Adds complexity
6	Use tangential flow filters or countercurrent centrifuge	• Batch centrifuge requires crew assisted steps	• Countercurrent centrifuge may require modification for microbial applications
7	Use bead beating for lysis	• Applicable for multiple chassis organisms • Demonstrated in space	• May require modification to process large volumes
8	Use affinity purification	• Product specific • Produces relatively pure protein after a single purification step	• Must supply affinity binding competitors for elution
9	Exclude affinity column and magnetic beads	• Crude lysate column may increase reliability • Magnets increase complexity	
10	May or may not include a buffer exchange and desalting step	• Insufficient data to either include or eliminate this step	

158   We developed three integrated designs using the methods selected from the trade

159   study (Fig. 5). Design 1 uses a countercurrent centrifugation system to dewater biomass, lyse

160   cells, and purify the protein with a batch resin method. Material flow is mediated by a

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161   peristaltic pump (1) and automated pinch valves (2). Cells from the biomass reservoir (3) are fed

162   into the centrifuge (4) and concentrated. Supernatant media is collected in a spent media

163   reservoir (5). Concentrated biomass is pumped through a bead beater (6) for lysis and the

164   lysate is returned to the biomass reservoir. The lysate is then pumped into the affinity resin

165   reservoir (7) for protein binding. Protein-bound resin is separated from the lysate using the

166   centrifuge, while the spent lysate is collected in a waste reservoir (8). The resin is washed with

167   buffer (9). Wash buffer is separated by the centrifuge and collected into the waste reservoir.

168   The bound protein is eluted from the resin using elution buffer (10), separated by the

169   centrifuge, and collected in the product reservoir (11).

170   Design 2 assumes a peristaltic pump (1) from the growth and production step will pump

171   fluids through a tangential flow filter (12) to dewater and concentrate biomass. Concentrated

172   biomass is returned to the biomass reservoir (3), and clarified media is collected in the spent

173   media reservoir (5). The concentrated biomass is lysed by the bead beater (6), and crude lysate

174   is returned to the biomass reservoir. The lysate is transferred to the purification system by the

175   crew. The purification system uses a separate pump (1) and pinch-valve module (2) to load

176   lysate into a crude lysate column (13). The column is washed with buffer (9), and recombinant

177   protein is eluted into the product reservoir (11).

178   Design 3 uses an identical dewatering and lysis system as in design 2, but with a

179   different protein purification system. Crew will transfer the lysate to the lysate chamber (14) of

180   a continuous loop affinity membrane purification system (15). Rollers (17) move the affinity

181   membrane through buffer chambers for protein binding (14), wash (9), and elution (10). A

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182   second wash chamber equilibrates the membrane for multiple cycles of protein binding. The

183   crew transfers the eluted protein to a product reservoir (11).

184   *Comparative analysis of designs*

185   Each design integrates different methods to complete the same bioprocessing steps

186   (Fig. 5b). Multiple systems parameters including ESM, SCM, TRL, and degree of crew assistance

187   were used to assess the three designs. SCM estimates complexity of life support systems by

188   summing all the components and proposed interconnections of a specific design[16]. Larger SCM

189   values are interpreted as more complex systems with potentially lower reliability. Figure 6

190   depicts the major components and interconnections used to calculate SCM values for each of

191   the three designs. We also analyzed the degree of crew assistance because crew time was not

192   incorporated into ESM calculations. Crew-assisted steps are highlighted in red or pink in Figures

193   5 and 6. TRL assesses technology maturity using a 9-point scale with TRL 9 being spaceflight-

194   proven technologies[26].

195   Figure 7 compares ESM, SCM, and degree of crew assistance for the integrated designs,

196   while Table 4 reports TRL for the dewatering, cell lysis, and protein purification steps

197   (Supplementary data 2). ESM was comparable for all three designs. Design 1 had the lowest

198   SCM due to the multifunctional commercial countercurrent centrifuge that integrates

199   dewatering, cell lysis, and protein purification. The ease of integration and automation in design

200   1 is also reflected in the low degree of crew assistance and higher TRL for dewatering and

201   purification compared to the other designs. Although these metrics suggest design 1 will be

202   more practical to implement, the differences in the metrics between the designs were not large

203   enough to eliminate specific methods without experimental testing.

Table 4. TRL for each bioprocessing step of the integrated designs			
Step	Design 1	Design 2	Design 3
Dewatering	4	3	3
Lysis	9	9	9
Protein purification	3	3	2

Discussion

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205   Carbonic anhydrase improves the efficiency of liquid amine CO_2 capture systems[5–7],

206   which is a candidate technology for deep space missions[1,2]. Application of these liquid amine

207   systems requires a steady supply of carbonic anhydrase that may be met by long term storage,

208   resupply or in situ bioproduction. Long term storage of active enzyme requires low temperature

209   storage conditions, and frequent resupply is not possible for surface missions to Mars.

210   Bioprocessing system designs for space applications need to optimize mass, efficiency,

211   power consumption, complexity, reliability, and ease of operation[37,38]. The initial space design

212   process largely focuses on ESM to ascertain feasibility either in comparison to existing state of

213   the art or other comparable technologies[12,14,25,39–44]. In this study, we employed ESM along with

214   processing duration, SCM, TRL, and degree of crew assistance metrics to estimate reliability,

215   potential for implementation, and ease of operation. Although carbonic anhydrase production

216   was the use case scenario, the designs would be useful to purify any soluble recombinant

217   protein with an affinity tag from a microbial chassis organism.

218   ESM comparisons were able to eliminate batch centrifugation and sonication as

219   methods for integrated designs. However, the integrated bioprocessing designs have a narrow

220   range of 357-522 kg eq ESM. Given these differences are not large enough for system selection,

221   this metric cannot be used to prioritize designs for implementation. The ESM estimates are

222   comparable to a proposed monoclonal antibody production system at 300-1,700 kg eq[45] as well

223   as a plant biomass system for food production at 300-1,300 kg eq per kg[10]. For theoretical

224   designs such as the proposed biomanufacturing systems, a difference of less than 10-fold

225   suggests similar infrastructure costs. On a mission-wide basis, the recombinant protein

226   bioprocessing system may be reasonable for a Mars mission. The bioprocessing system adds

227   only 2-3% to the physio-chemical-biological life support system proposed by ESA for a full Mars

228   mission (~18,000 kg eq)[46].

229   Crew time and system complexity are important factors in space system design [16,47].

230   Design 1 ranked best for number of crew assisted steps and SCM, which primarily reflects the

231   level of integration for automated fluid handling in the commercial counterflow centrifuge.

232   Additional engineering to integrate pumps and controllers would reduce both SCM and crew-

233   assisted steps for designs 2 and 3. Design 1 also has higher TRL components indicating this

234   design may be easier to implement for space, but a limitation of the study is a lack of empirical

235   validation. All components were assumed to operate as intended and to be compatible within a

236   design and many components have not been tested for the specific use case of recombinant

237   protein purification. For example, the counterflow centrifuge in design 1 has not been used for

238   bioprocessing of microbes. Empirical tests are needed to determine the relative efficiency of

239   the selected technologies when integrated.

240   Although closed-loop systems and reusability are attractive long-term goals in space,

241   they require increased crew time and system reliability[38,48,49]. On Earth, biomanufacturing often

242   employs single-use technology for various reasons including improved safety as well as reduced

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243 contamination, footprint, and cost by eliminating cleaning and sterilization of components that

244 contact biological material[50,51]. Single-use materials for bioprocessing is predicted to reduce

245 crew time and to increase reliability. Consequently, the proposed bioprocessing designs use

246 disposable components in the ESM calculations.

247 We also identified several factors that could influence a recombinant protein

248 bioprocessing design that were difficult to quantify. For example, there are safety and shelf-life

249 concerns for commercial affinity purification products. Affinity resins are stored in ethanol to

250 prevent biological contamination. Ethanol interferes with the current Environmental Control

251 and Life Support Systems on the ISS, creating a safety risk when large amounts of ethanol are in

252 the cabin air or wastewater. The ethanol risk could be mitigated with an affinity membrane that

253 is stored dry. Both commercial resins and affinity membranes have 1-2 year shelf lives, while a

254 Mars mission is expected to require a 5-year shelf life for all systems[27]. Removing incompatible

255 chemicals and extending the shelf life of the affinity matrix are both critical to advance space

256 biomanufacturing of recombinant proteins.

257 It is possible that using different strategies to express recombinant proteins could

258 reduce complexity and system mass. For instance, an affinity resin "teabag" can purify proteins

259 secreted into the media without dewatering or lysis steps[52]. Although this strategy would

260 simplify bioprocessing, it limits production to recombinant proteins that have activity after

261 secretion. Moreover, *E. coli* secreted proteins are targeted to the periplasmic space and require

262 an osmotic shock step to release protein into the media. Adding an osmotic shock still requires

263 dewatering to concentrate biomass and the osmotic shock step would replace the cell lysis

264 step.

265 This study illustrates multiple approaches to rank technologies for design of new

266 integrated systems intended for space applications. The extreme environment of space, high

267 cost of launching materials, and limited crew time drive systems to be automated and to

268 minimize mass, power, and volume. Further development of space bioprocessing systems is

269 expected to identify technologies that can be integrated and automated reliably, which could

270 be translated to improved efficiency for industrial biomanufacturing on Earth .

271 **Methods**

272 The operational scenario investigated was a six person crew on a 3-year Mars mission

273 with 600 days of surface operations[31]. A total of 14.4 g of recombinant carbonic anhydrase was

274 estimated to be required to maintain a liquid amine CO_2 scrubber with active enzyme

275 throughout surface operations. Affinity purification of the enzyme was assumed to be needed

276 to reduce liquid amine side reactions with cellular debris. Sufficient carbonic anhydrase could

277 be supplied by approximately 80 bioprocessing runs using 1 L cultures with a yield of 180 mg

278 purified enzyme per run. Supplies and power for a total of 100 production runs were assumed

279 to give sufficient redundancy for production runs that failed to meet expected yield.

280 A review of recombinant protein purification from unicellular microorganisms identified

281 sub-process steps (Fig. 1). Essential steps for the operational scenario, such as lysis or

282 purification, were retained. Biomass dewatering was investigated as an optional step. Non-

283 essential steps, such as storage and drying, were eliminated to simplify potential workflows. A

284 trade study of potential methods for each sub-process step was conducted, and ESM was used

285 to evaluate individual technologies for in-space application. Technologies with comparable ESM

286 were used to develop three designs, which were analyzed using four metrics described below.

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287 *ESM metric*

288   ESM was calculated using Equation 1 and the equivalency factors in Table 1 based on

289 guidelines from Levri, et al. [14]. Parameters for volume (V^{ESM}), power (P^{ESM}), thermal cooling

290 (C^{ESM}), cold storage (CS^{ESM}), water (W^{ESM}), and waste storage (WS^{ESM}) used equivalency factors

291 estimated for a nominal crewed surface mission to Mars[31]. CS^{ESM} assumed that the mass

292 requiring cold storage had a density of 1000 kg/m^3 and was stored in an freezer analogous to

293 that currently used on the International Space Station (ISS)[31]. W^{ESM} and WS^{ESM} values were

294 derived from a prior Mars mission scenario where a crew of six used 30 kg of water per crew

295 member per day, and produced 1.5 kg solid waste per crew member per day[31,55]. W^{ESM} was

296 based on a water processor similar to the ISS water treatment system and WS^{ESM} assumed that

297 waste was stored within the habitat[55]. Crew time was excluded due to the uncertainties in

298 estimating the practical level of automation that could be achieved after integrating

299 technologies from different commercial manufacturers. Detailed assumptions and calculations

300 of ESM are given in Supplementary data 1 and 2. ESM penalties from both 2015 and 2022

301 Baseline Values and Assumptions Documents were used for comparative calculations shown in

302 Table 2[31,32].

303    $$ESM = \sum M_i + \sum M_t * D + \sum (V_i * V_{eq}) + \sum (V_t * V_{eq}) * D + \sum (P * P_{eq}) + \sum (C * C_{eq}) +$$

304    $$\sum (CT * CT_{eq}) + \sum (CS * CS_{eq}) + \sum (W * W_{eq}) * D + \sum (WS * WS_{eq}) * D \ (1)$$

305 Where,

$$M_i - \text{ Initial mass } (kg)$$

$$M_t - \text{Time dependent mass } (kg)$$

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$$D - Mission\ duration\ (number\ of\ runs)$$

$$V_i - Initial\ volume\ (m^3)$$

$$V_{eq} - Volume\ equivalency\ (kg\ eq/m^3)$$

$$V_t - Time\ dependent\ volume\ (m^3)$$

$$P - Power\ consumed\ (kW)$$

$$P_{eq} - Power\ equivalency\ (kg\ eq/kW)$$

$$C - Thermal\ cooling\ (kW)$$

$$C_{eq} - Thermal\ cooling\ equivalency\ (kg\ eq/kW)$$

$$CT - Crew\ time\ (h)$$

$$CT_{eq} - Crew\ time\ equivalency\ (kg\ eq/h)$$

$$CS - Cold\ storage\ requirement\ (kg\ stored)$$

$$CS_{eq} - Cold\ storage\ equivalency\ (kg\ eq/kg\ stored)$$

$$W - Water\ requirement\ per\ run\ (kg\ water)$$

$$W_{eq} - Water\ treatment\ equivalency\ (kg\ eq/kg\ water)$$

$$WS - Solid\ waste\ generated\ per\ run\ (kg\ waste)$$

$$WS_{eq} - Waste\ storage\ equivalency\ (kg\ eq/kg\ waste)$$

306   *SCM metric*

307   Equation 2 gives the SCM calculation based on the number of components (N) and the

308   number of one-way interactions (I) between components[16].

309         $SCM = Number\ of\ components\ (N) + Number\ of\ interactions\ (I)$ (2)

310   Components are integrated subsystems that perform one or more bioprocessing steps,

311   such as equipment available from commercial vendors. Auxiliary parts such as valves, ordinary

312   filters, and sensors were excluded with the rationale that integration of major components

313   account for most of the system complexity, cost, and failure modes[16]. The number of one-way

314   interactions (I) is derived from a top-level bioprocessing system block diagram based on

315   physical connections between components (Fig. 6).

316   *TRL metric*

317   A TRL was assigned for each of the dewatering, cell lysis, and purification options

318   included in the three designs using existing NASA guidelines[26].

319   *Degree of crew assistance metric*

320   The degree of crew assistance was estimated based on whether the commercially

321   available components were integrated and easily automatable. A crew support step was

322   assumed if commercial technology was not readily available to automate a specific step in the

323   proposed workflow of each design. The sum of crew assisted steps was the degree of crew

324   assistance for the design.

325   *Dewatering technologies*

326   Tangential flow filtration, batch centrifugation, and countercurrent centrifugation were

327   three dewatering methods compared for this analysis. The dimensions and mass of the

328   tangential flow filter were based on Xampler cartridge with 1 mm fiber diameter, 500 kDa pore

329   size and 30 cm path length with a 3M housing (Cytiva, Marlborough, MA, USA). To control fluid

330   flow through the tangential flow filter, an Ismatec Reglo ICC Digital Pump with 4-Channels and

331   8-Rollers (Cole-Palmer, Vernon Hills, IL, USA) was included in the ESM calculations. A modified

332   version of the Drucker model 755VES swinging bucket centrifuge (Drucker Diagnostics, Port

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333   Matilda, PA, USA) has been approved for operation on the ISS, and the specifications of the

334   commercially available centrifuge were used to calculate the ESM. Twenty disposable 50 mL

335   tubes were assumed to harvest the 1 L culture volume during every run. The countercurrent

336   centrifuge data was based on specifications for the CTS Rotea Counterflow Centrifugation

337   System (Thermo Fisher Scientific, Waltham, MA, USA).

338   *Lysis technologies*

339   The methods considered for lysis were bead beating, enzymatic lysis, a flow-through

340   sonicator, and a large-volume probe sonicator. The bead beater analysis was based on the

341   Claremont Biosolutions LLC (Upland, CA, USA) OmniLyse HL beadbeater flow-through lysis

342   device. Although originally developed for small volumes, the OmniLyse HL unit was assumed to

343   lyse large volumes with equal efficiency using extended processing times. The enzyme lysis

344   protocol assumed that the biomass was incubated for 30 minutes at ambient temperature with

345   0.25 mg/mL lysozyme, 0.1 mL/mL Pierce universal nuclease (Thermo Fisher Scientific, Waltham,

346   MA, USA), and 0.1% w/v Triton X at final concentration. Mass and volumes for ESM were

347   calculated assuming the solid reagents had a density equal to NaCl (2.17 g/cm^3), and liquid

348   reagents had a density equal to water (1 g/cm^3). The QSonica (Newton, CT, USA) ultrasound

349   generator was modeled assuming a 1-inch replaceable tip for a large volume batch method and

350   a Q500 FloCell unit for the flow through method.

351   *Protein purification technologies*

352   Five His-tag affinity purification methods were compared using public domain product

353   information available from commercial vendors. The Sigma Aldrich (St Luis, MO, USA) His-Select

354   Ni affinity gel was used to represent affinity columns requiring clarified lysates. The Cytiva

20

355   (Marlborough, MA, USA) HisTrap FF was an exemplar of a crude lysate column that does not

356   require clarification before sample loading. Batch resin purification specifications were

357   estimated for the resin "teabag" method from Castaldo, et. al.[52]. Millipore Sigma (Burlington,

358   MA) HIS-Select® Nickel Magnetic Agarose Beads specifications were used to estimate the

359   amount of resin required for batch purification with magnetic beads, while mass and volume of

360   the beads were assumed to be equivalent to affinity resin. For affinity membrane purification,

361   Capturem large volume filters (Takara Bio USA Inc., San Jose, CA, USA) were used to estimate

362   the quantity of membrane required, while mass and volume of the membrane was assumed to

363   be equivalent to Whatman filter paper (200 g/cm^2).

364   The buffer composition was 20 mM HEPES, 500 mM NaCl and 20 mM imidazole for the

365   binding buffer, and 20 mM HEPES, 500 mM NaCl and 200 mM imidazole for the elution buffer

366   for all the methods. Buffer volumes were modeled using manufacturer protocols or the resin

367   "teabag" method[52].

368   *Bioprocessing system designs*

369   All three post-growth bioprocessing system designs assumed a Claremont Biosolutions

370   LLC (Upland, CA, USA) OmniLyse® HL disposable bead beater for in-line cell lysis from the

371   biomass reservoir. The first design included a Thermo Fisher Scientific (Waltham, MA, USA) CTS

372   Rotea Counterflow Centrifugation System with disposable, single use kits for processing. This

373   counterflow centrifuge includes integrated pinch-valves, a peristaltic pump, and a controller for

374   automation. QIAexpressionist Ni-NTA resin (Qiagen Inc., Valencia, CA, USA) was included for

375   affinity purification.

376   The second design uses a MidGee ultrafiltration cartridge UFP-5-C-MM01A (Cytiva,

377   Marlborough, MA, USA) and an Applikon Biotechnology (JG Delft, Netherlands) "my-control"

378   with built in peristaltic pumps for biomass dewatering using tangential flow filtration and

379   subsequent cell lysis. For protein purification, an Automate Scientific (Berkeley, CA, USA)

380   Perfusion System and ValveLink8.2 Perfusion Controller were assumed to integrate with an

381   Masterflex (Gelsenkirchen, Germany) L/S® Digital Drive peristaltic pump with an Easy-Load® 3

382   Pump Head for Precision Tubing. This fluid control system was assumed to automate affinity

383   purification in a Cytiva 5 mL HisTrap FF crude lysate column (Marlborough, MA, USA).

384   Dewatering and lysis components in design 3 were identical to design 2. Protein

385   purification was based on a non-commercial affinity belt system[57–59]. Rollers were proposed to

386   move an affinity membrane belt continuously through chambers containing the lysate, wash

387   buffers, and elution buffers. The total volume of the system as assumed to be 0.005 m^3

388   including the chambers, walls, and rollers. The mass was assumed to be 500 g. One Transmotec

389   Inc. (Burlington, MA, USA) 12 V, 2A DC motor was included to operate the rollers.

390   Disposable materials such as bags, and sterile filters for all three designs were estimated

391   for the system based on commercial options and material properties, while the additional mass

392   of tubing and luers were assumed to be 0.1 kg for all designs.

393   **Data Availability**

394   All source data and ESM calculations needed to replicate the study are provided in the

395   manuscript and Supplementary data 1 and 2.

396   **Acknowledgements**

400   **Author Contributions**

401   M.S. and M.B.P. completed the trade study investigation, formal analysis, and wrote the

402   manuscript. M.D. completed data collection, formal analysis, and edited the manuscript. H.W.J.

403   provided systems engineering expertise for formal analysis and edited the manuscript. J.A.H.

404   conceived and supervised the project and edited the manuscript. F.M.D., J.M.G., and A.M.S.

405   contributed to methods development, validation, and visualization of the results, and edited

406   the manuscript.

407

408   **Competing Interests statement**

409   The authors declare no competing interests.

410

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557   **Figure Legends**

558   **Fig. 1.** Flow diagram of potential bioprocessing strategies. Dashed boxes group the primary sub-

559   processes of biomass processing, protein extraction, and product storage. Solid boxes give

560   individual steps with bulleted lists of common methods to complete the step. Methods

561   compared in this analysis have parenthetical designations that are used in Fig. 2 and Fig. 3.

562   Lettered arrows (a, b, c, d, e) give examples of possible workflows for processing *E. coli* cells

563   expressing recombinant carbonic anhydrase.

564

565   **Fig. 2.** Estimated process duration for each run of the lysis (L) and purification (P) based on

566   recommended manufacturer protocols or published literature. Methods included: flow through

567   bead beater (L-1), enzyme lysis (L-2), flow cell sonicator (L-3), large volume probe sonicator (L-

568   4), affinity resin column (P-1), crude lysate column (P-2), batch affinity resin (P-3), batch affinity

569   magnetic beads (P-4), and affinity membrane (P-5). Black bars are process times of 1 L culture

30

570   and gray bars are process times of 200 mL concentrated biomass. See supplementary data 1 for

571   detailed calculations.

572

573   **Fig. 3.** ESM models assuming 100 bioprocessing runs. **(a)** Dewatering methods included

574   tangential flow filtration (D-1), countercurrent centrifugation (D-2), and batch centrifugation (D-

575   3). **(b)** Cell lysis methods included bead beating (L-1), enzymatic lysis (L-2), flow through

576   sonication (L-3), and large volume probe sonication (L-4). **(c)** Protein purification methods

577   included clarified lysate column (P-1), crude lysate column (P-2), batch resin purification (P-3),

578   magnetic bead purification (P-4), and affinity membrane purification (P-5). Each bar graph

579   shows mass equivalencies for: total mass (M^{ESM}), volume (V^{ESM}), power (P^{ESM}), cooling (C^{ESM}),

580   cold storage (CS^{ESM}), water treatment (W^{ESM}), and solid waste disposal (WD^{ESM}). See Table 2 for

581   total ESM values and Supplementary data 1 for detailed calculations.

582

583   **Fig. 4.** Flow diagram of bioprocessing strategies showing selected methods for integrated, in

584   space designs. Decisions from the trade study are indicated with pink callouts. The rationale for

585   each decision is given in Table 3.

586

587   **Fig. 5.** Bioprocessing system designs integrating selected methods for dewatering, lysis, and

588   purification. **(a)** Design schematics with the following components: peristaltic pump (1), pinch

589   valve (2), biomass reservoir (3), centrifuge cartridge (4), spent media reservoir (5), disposable

590   bead beater (6), affinity resin reservoir (7), waste reservoir (8), wash buffer (9), elution buffer

591   (10), product reservoir (11), tangential flow filter (12), crude lysate column (13), crude lysate
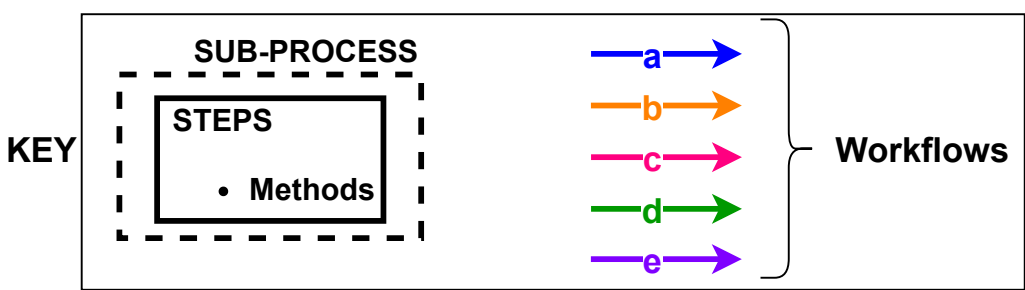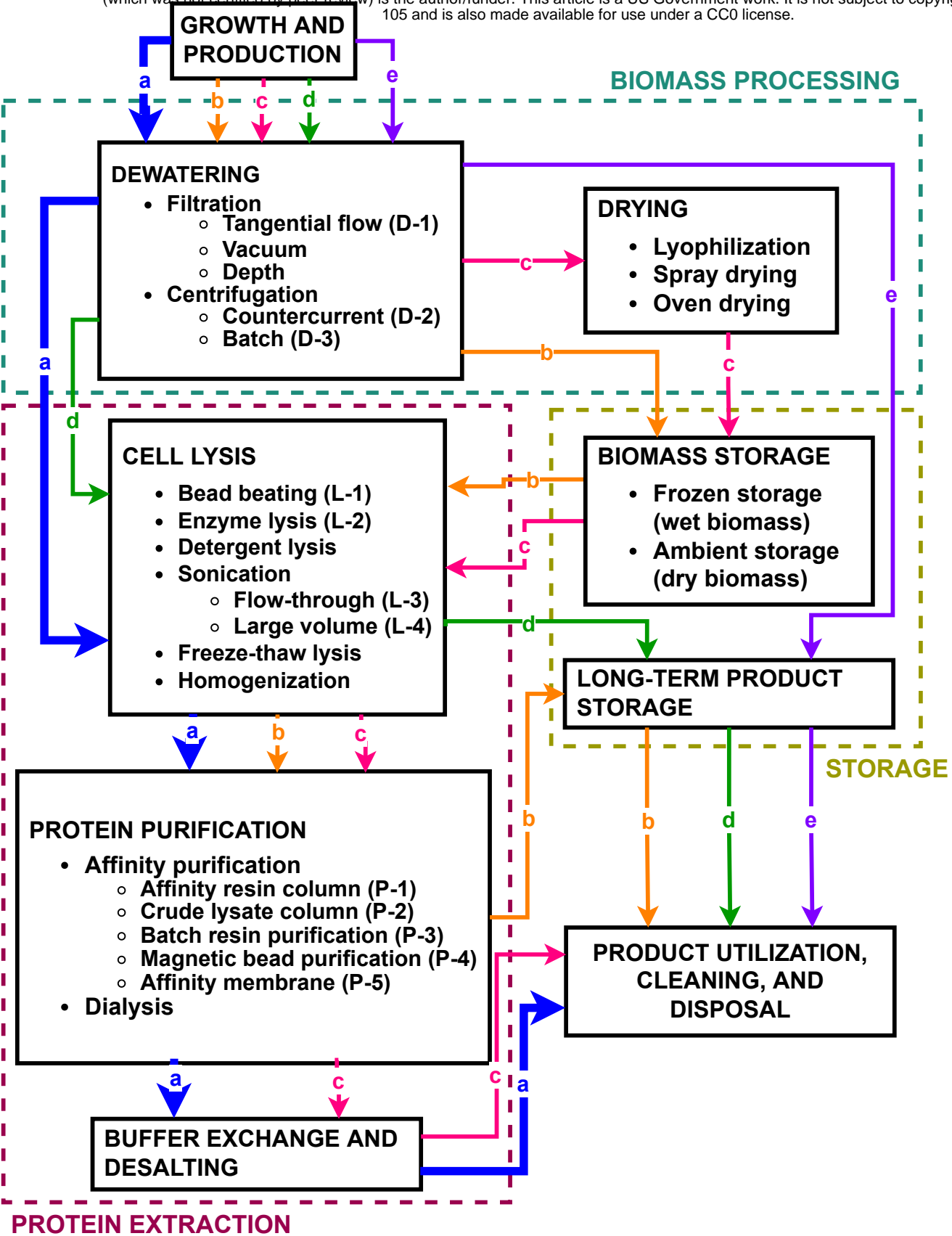
31

592   chamber (14), affinity purification cartridge (15), affinity membrane (16), rollers (17). Yellow

593   arrows indicate crew-assisted steps. **(b)** Flow diagram comparing the bioprocessing designs.

594   Yellow boxes indicate methods that require crew-assistance to initiate or complete the method.
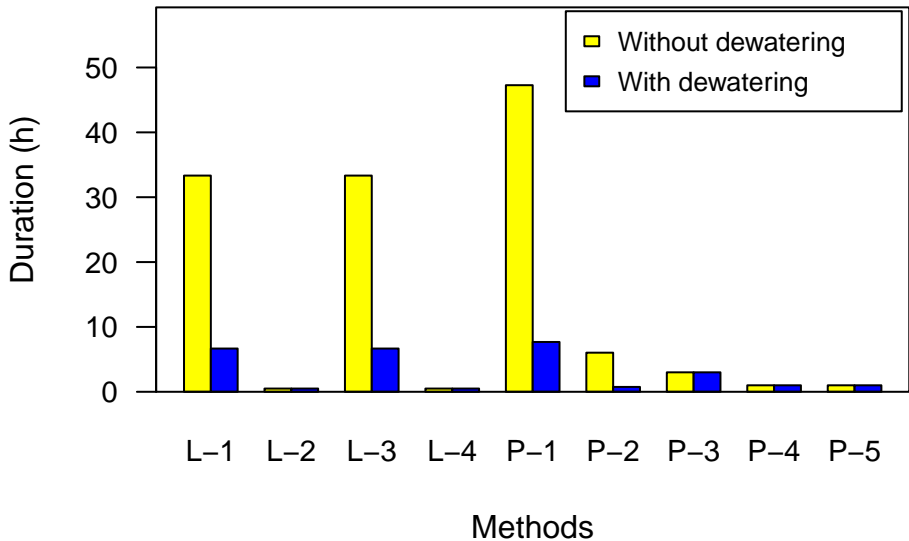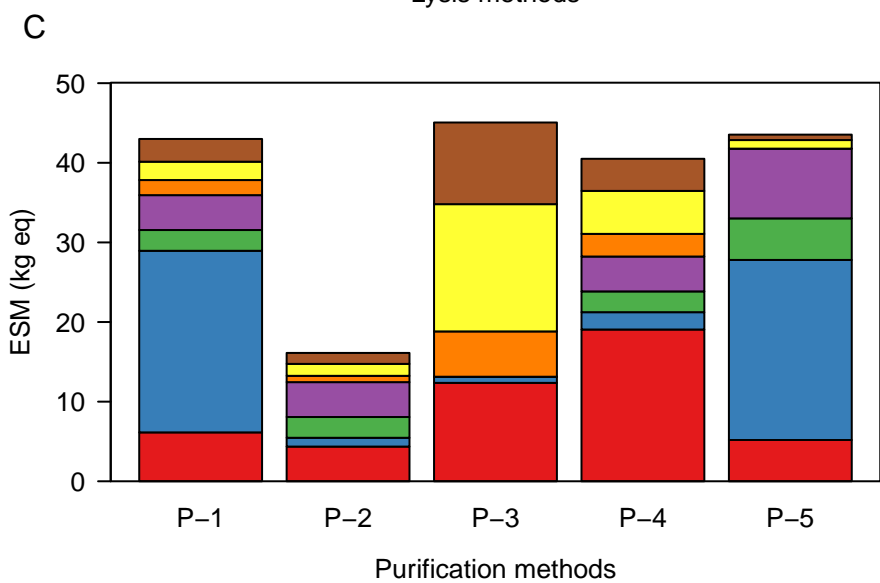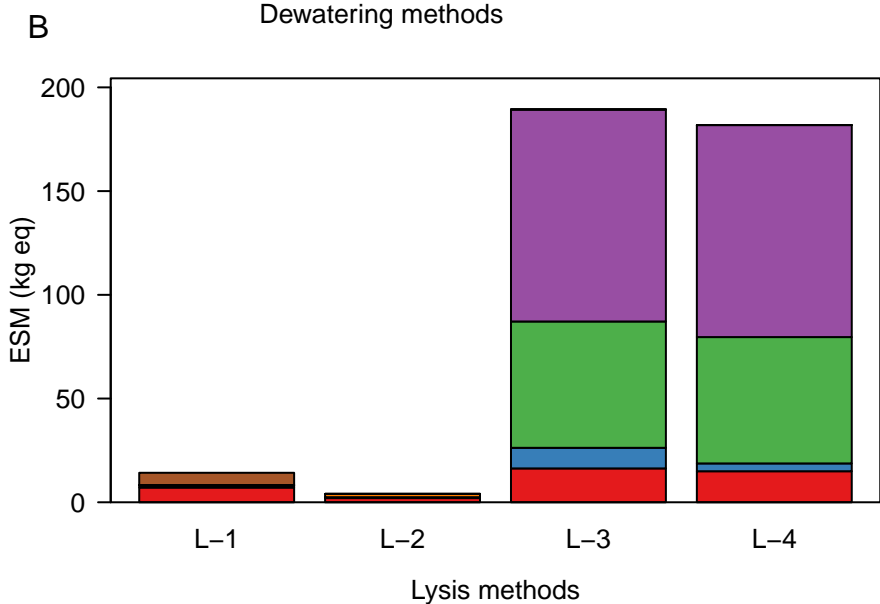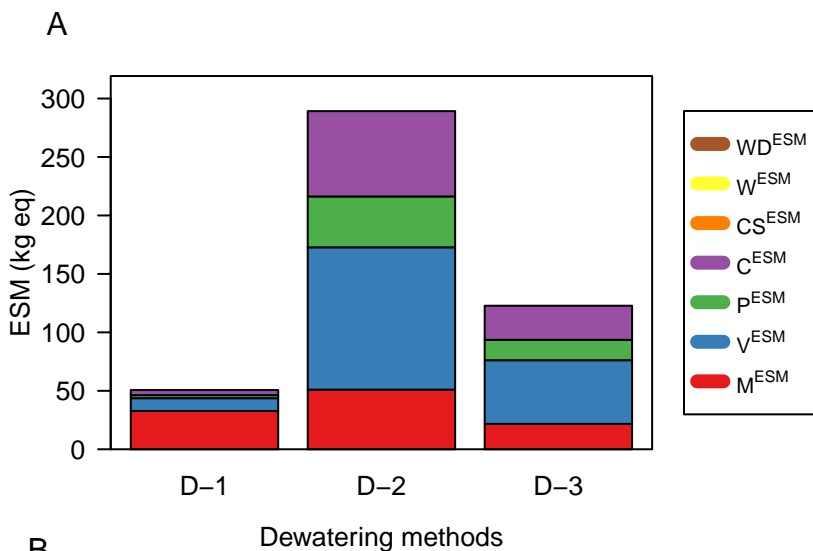
595

596   **Fig. 6.** Top-level block diagram of the three bioprocessing designs used to calculate SCM. Major

597   components (N) are in boxes. Individual interconnections (I) are diagrammed with arrows

598   indicating the direction of material flow. Dashed arrows indicate outputs of the bioprocessing

599   systems, and red arrows require crew assistance to complete the actions for the
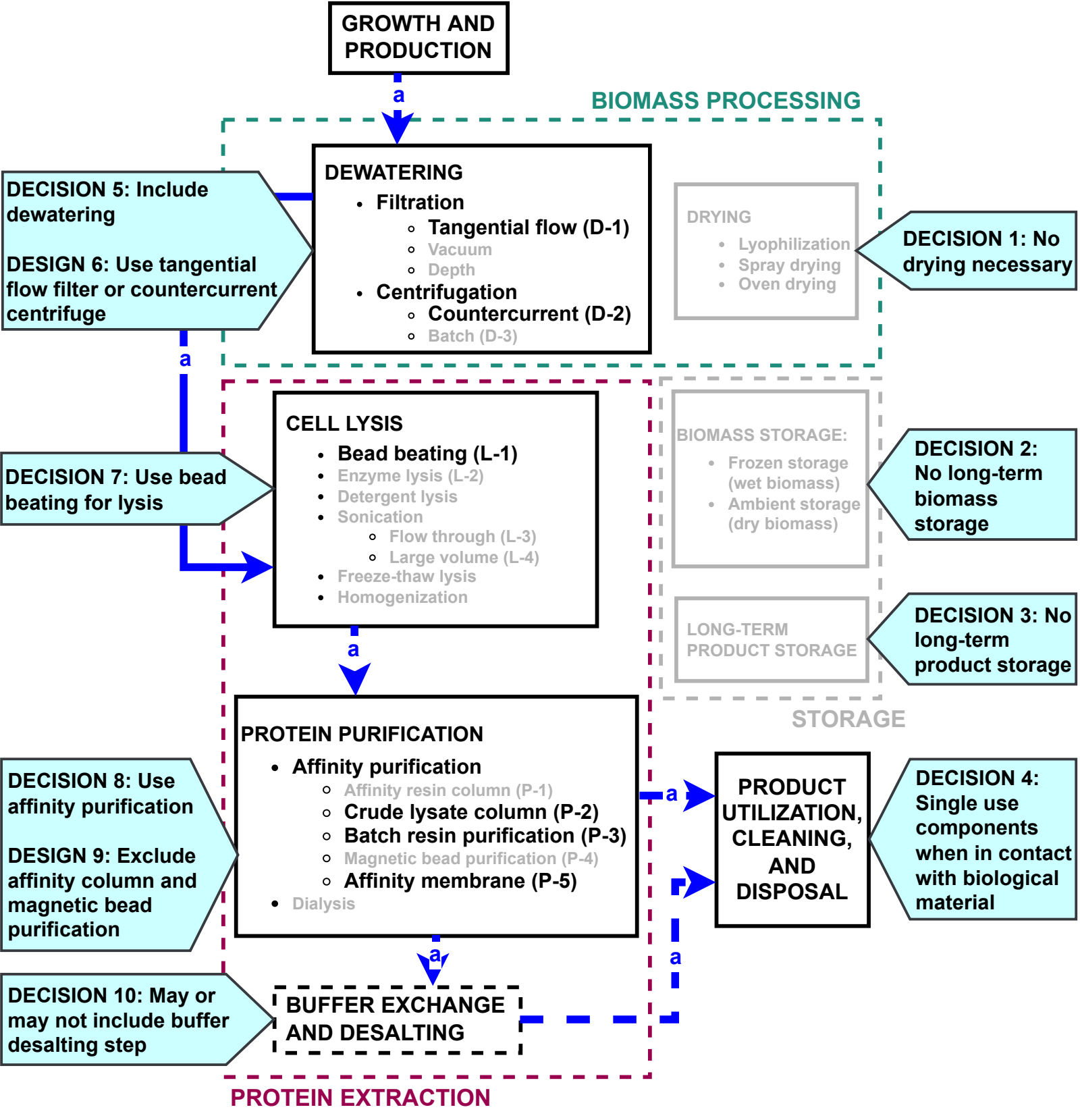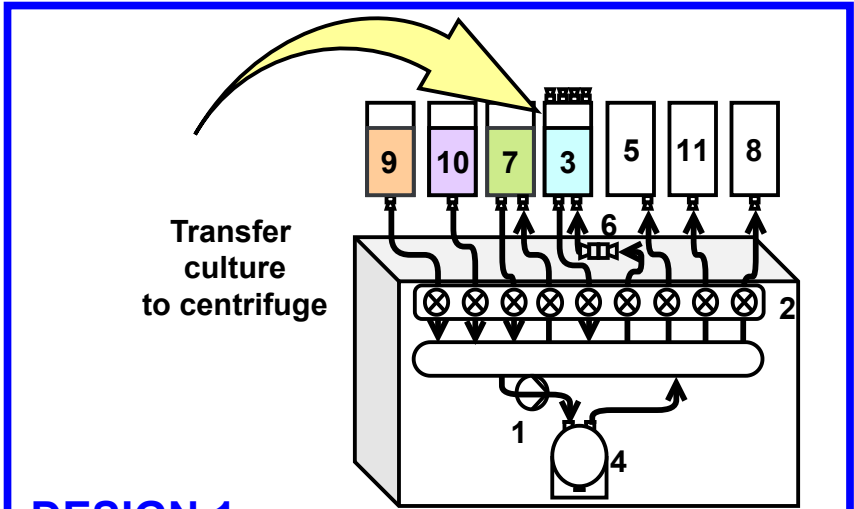
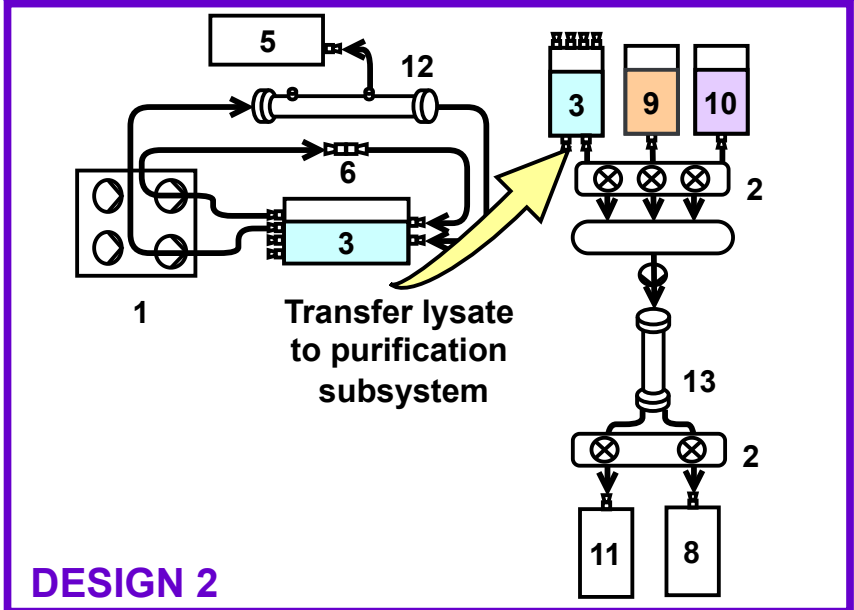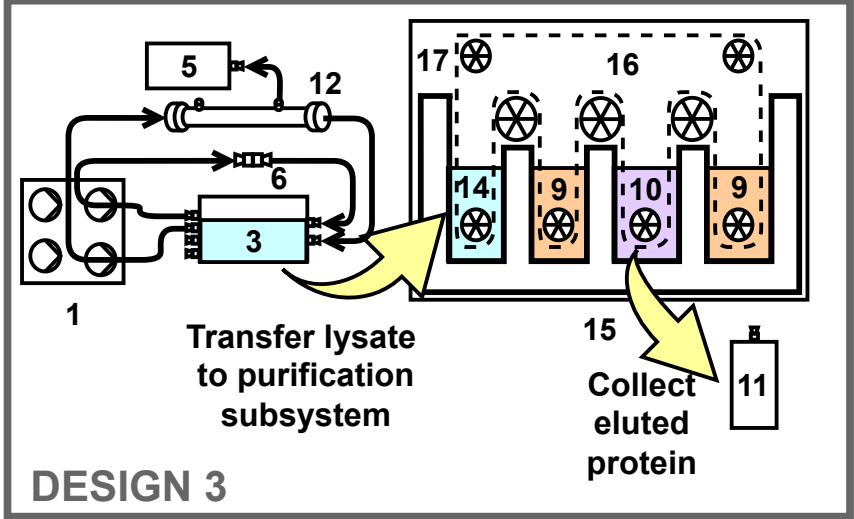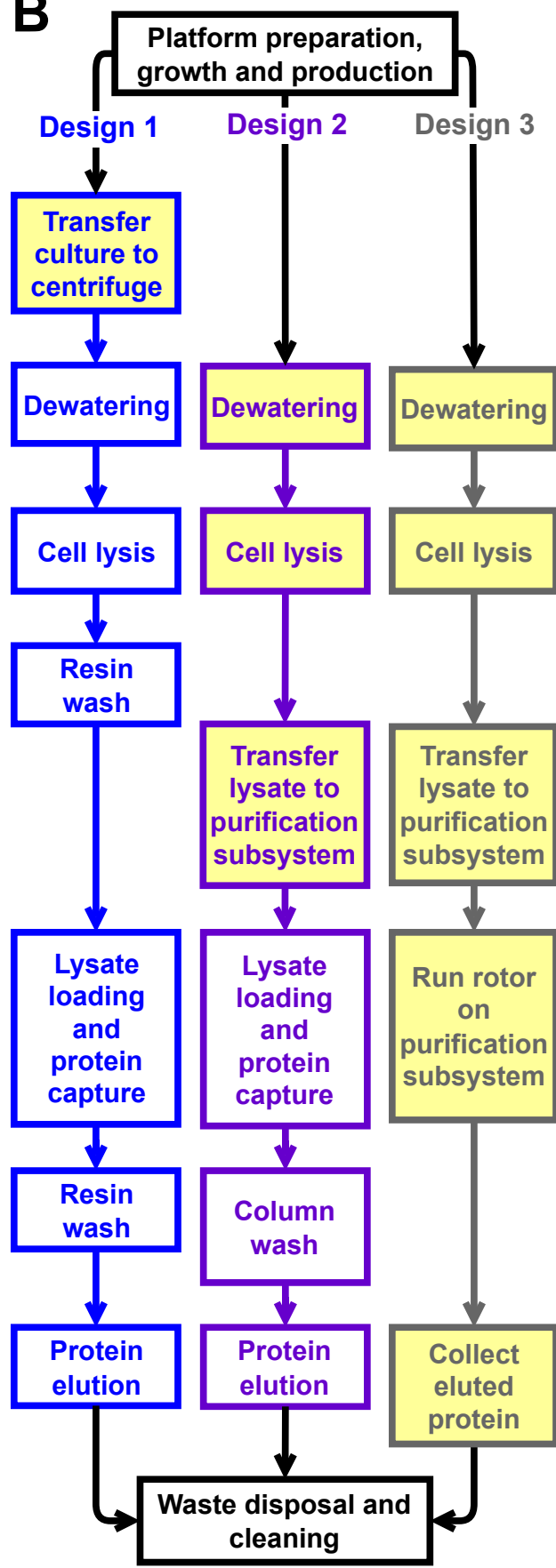600   interconnection.

601

602   **Fig. 7.** Design metrics for the three integrated bioprocessing designs. See Table 2 for total ESM

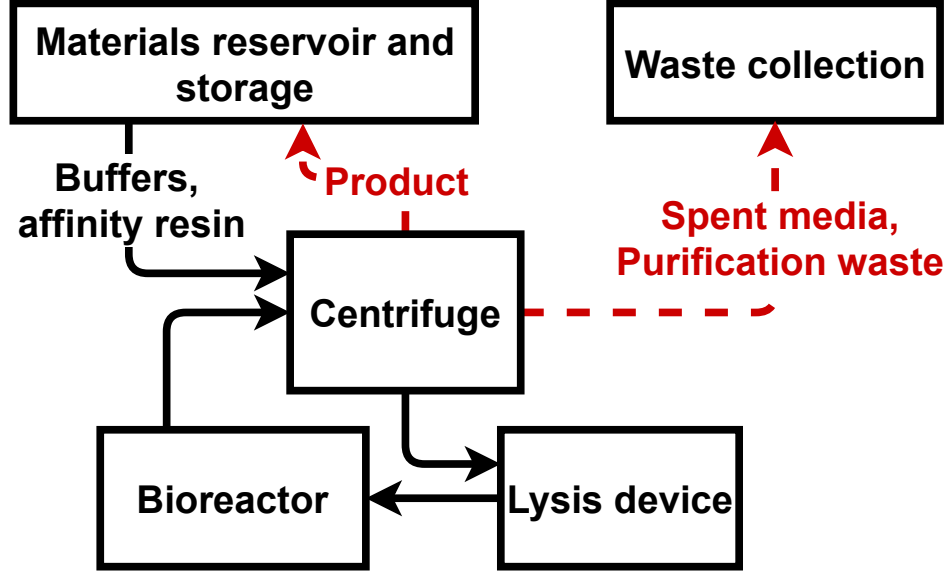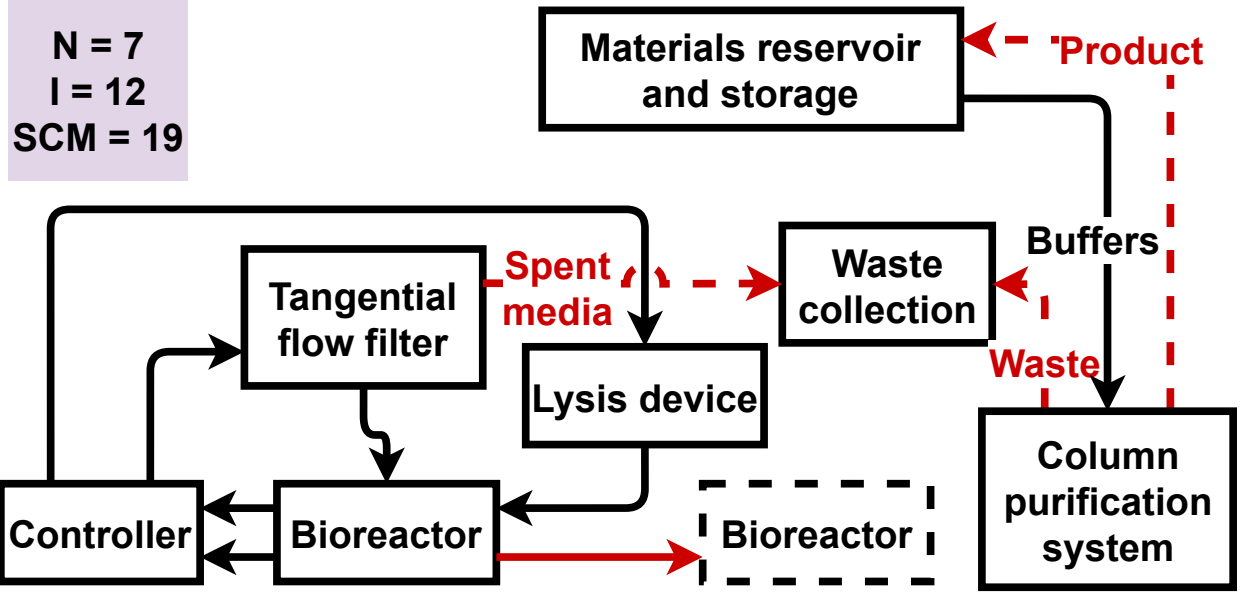603   values and Supplementary data 2 for detailed ESM calculations.

A

B

C

GROWTH AND PRODUCTION

a

BIOMASS PROCESSING

DEWATERING

- **Filtration**
  - **Tangential flow (D-1)**
  - Vacuum
  - Depth
- **Centrifugation**
  - **Countercurrent (D-2)**
  - Batch (D-3)

DECISION 5: Include dewatering

DESIGN 6: Use tangential flow filter or countercurrent centrifuge

DRYING
- Lyophilization
- Spray drying
- Oven drying

DECISION 1: No drying necessary

a

DECISION 7: Use bead beating for lysis

CELL LYSIS

- **Bead beating (L-1)**
- Enzyme lysis (L-2)
- Detergent lysis
- Sonication
  - Flow through (L-3)
  - Large volume (L-4)
- Freeze-thaw lysis
- Homogenization

BIOMASS STORAGE:
- Frozen storage (wet biomass)
- Ambient storage (dry biomass)

DECISION 2: No long-term biomass storage

LONG-TERM PRODUCT STORAGE

DECISION 3: No long-term product storage

STORAGE

a

PROTEIN PURIFICATION

- **Affinity purification**
  - Affinity resin column (P-1)
  - **Crude lysate column (P-2)**
  - **Batch resin purification (P-3)**
  - Magnetic bead purification (P-4)
  - **Affinity membrane (P-5)**
- Dialysis

DECISION 8: Use affinity purification

DESIGN 9: Exclude affinity column and magnetic bead purification

a

PRODUCT UTILIZATION, CLEANING, AND DISPOSAL

DECISION 4: Single use components when in contact with biological material

a

DECISION 10: May or may not include buffer desalting step

BUFFER EXCHANGE AND DESALTING

a

a

PROTEIN EXTRACTION

	ESM (kg eq)	Degree of crew assistance	System complexity metric
Design 1			
Design 2			
Design 3			