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# Feedstock and Process Evidence for Engineering Assessment of Residue-Based Bacillus Surfactin Production

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Agro-industrial and food-processing residues have been investigated as alternative feedstocks for surfactin production by *Bacillus*, but published systems differ in biological configuration, feedstock conversion, medium formulation, reactor operation, oxygen-transfer measurements and product-recovery basis. This targeted engineering synthesis separates directly reported observations from cross-study interpretation. Evidence coverage differed by question: the DGS study directly documented feedstock-conversion dependence, whereas the brewery-trub oxygen-supply study provided reactor-scale oxygen-transfer measurements; direct ranking was not justified because the systems differed in biological, operational and measurement bases. Reported product titers and kLa values therefore required system-specific interpretation. The proposed evidence-to-inference workflow explicitly separates reporting status, comparability and evidence-supported inference, providing a structured basis for cross-system assessment without numerical robustness scores or industrial-performance prediction. It is a conceptual screening procedure rather than a validated predictive tool.

## 1. Introduction

Agro-industrial and food-processing residues have been investigated as alternative feedstocks for surfactin production by *Bacillus*, but the engineering information available for comparing residue-based systems remains heterogeneous. This heterogeneity complicates feedstock assessment and reactor-scale decision making because reported titers may reflect different biological configurations, operating regimes and analytical streams. Because the synthesis is restricted to surfactin-specific evidence, process-level conclusions are not extrapolated automatically to other *Bacillus* lipopeptide products.

Ciurko *et al*. (2022) quantified surfactin production by *B. subtilis* #309 using rapeseed and sunflower cakes, reaching 1.45 and 1.19 g $L^{-1}$, respectively. Zhang *et al*. (2026) used untreated kitchen waste and reported 371.67 mg $L^{-1}$ surfactin after optimization of fermentation conditions. Hu *et al*. (2021) examined a tuna-fish-waste-derived peptone across screening and fermenter scales. These studies illustrate the diversity of feedstock, biological, operational and analytical bases represented in residue-based surfactin research.

The evaluated evidence does not provide a basis for deriving a single transferable process indicator without harmonization of biological, operational and analytical bases, nor does it provide a common dataset jointly quantifying feedstock conversion, formulation, reactor operation, oxygen transfer and recovery under comparable conditions.

The methodological contribution proposed here is to explicitly separate reporting status, comparability and evidence-supported inference so heterogeneous metrics are not converted directly into rankings or scale-up claims. The synthesis is restricted to surfactin-specific evidence from residue-derived feedstocks; recent non-residue bioreactor studies are used only to contextualise oxygen-transfer and scale-up interpretation.

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## 2. Evidence from residue-based *Bacillus* surfactin processes

### 2.1 Feedstock processing and conversion

Using DGS, Zhi *et al*. (2017) showed that low amylase activity of *Bacillus amyloliquefaciens* MT45 limited sugar supply. Addition of the hydrolytic strain X82 increased sugar release, and the MT45:X82 co-culture reached 3.4 g $L^{-1}$ surfactin at 200 g $L^{-1}$ DGS.

Moya Ramírez *et al*. (2016) reported that enzymatic hydrolysis produced the highest surfactin response among the olive-mill-waste pretreatments evaluated. Ciurko *et al*. (2022) quantified 1.45 g $L^{-1}$ surfactin with rapeseed cake and 1.19 g $L^{-1}$ with sunflower cake. Zhang *et al*. (2026) reported 371.67 mg $L^{-1}$ after optimization of untreated kitchen-waste fermentation.

### 2.2 Medium formulation

Nazareth *et al*. (2021a) evaluated brewery-trub medium formulation. Nitrogen-source formulation affected surface tension, while metal supplementation increased volumetric surfactin productivity from 2.98 to 7.43 mg $L^{-1}$ $h^{-1}$.

### 2.3 Reactor operation, oxygen supply and foam handling

Nazareth *et al*. (2021b) varied agitation and aeration during brewery-trub fermentation and measured the volumetric oxygen-transfer coefficient (kLa), OUR, DO and surfactin productivity. The highest volumetric productivity, 9.98 mg $L^{-1}$ $h^{-1}$, occurred at kLa = 45.41 $h^{-1}$; higher oxygen-supply conditions did not uniformly improve productivity, and foam collection enabled product removal and enrichment.

During 3 L cultivation of *B. subtilis* LB5A in cassava wastewater, de Andrade *et al*. (2016) reported kLa = 102.02 $h^{-1}$ with DO at approximately 0% during much of cultivation, followed by foam recovery and ultrafiltration.

Hu *et al*. (2021) used 20 mL screening followed by 7 L and 100 L fermenter operation with fish-derived peptone and *B. subtilis* ATCC 21332. At 100 L, DO was set at 50%, agitation at 120 rpm and aeration at 1 vvm; antifoam was added after foam formation. Surfactin was analytically confirmed, and broth and foam-recovery concentrations were reported as different sample streams.

*Table 1: Representative process evidence used in the engineering interpretation of residue-based Bacillus surfactin* systems

| Evidence domain | Feedstock / system | Directly reported evidence | Reference |
|---|---|---|---|
| Feedstock conversion | DGS; *B. amyloliquefaciens* MT45/X82 | 1.04 g $L^{-1}$ with MT45; low amylase limited sugar supply; 3.4 g $L^{-1}$ at 200 g $L^{-1}$ DGS with MT45:X82. | Zhi *et al*. (2017) |
| Feedstock pretreatment | Hydrolysed OMW; *B. subtilis* | Enzymatic hydrolysis gave the highest surfactin response among tested pretreatments. | Moya Ramírez *et al*. (2016) |
| Medium formulation | Brewery trub; *B. subtilis* ATCC 6051 | N-source affected surface tension; metal supplementation increased productivity from 2.98 to 7.43 mg $L^{-1}$ $h^{-1}$. | Nazareth *et al*. (2021a) |
| Reactor / $O_2$ transfer | Brewery trub; *B. subtilis* ATCC 6051 | Stirred tank; agitation, aeration, $k_La$, OUR and DO measured; highest productivity 9.98 mg $L^{-1}$ $h^{-1}$ at $k_La$ = 45.41 $h^{-1}$; foam collection. | Nazareth *et al*. (2021b) |
| Reactor / $O_2$ transfer | Cassava wastewater; *B. subtilis* LB5A | 3 L; $k_La$ = 102.02 $h^{-1}$ with DO approximately 0 %; foam recovery and ultrafiltration. | de Andrade *et al*. (2016) |
| Scale / recovery | Fish-derived peptone; *B. subtilis* ATCC 21332 | 20 mL screening, 7/100 L fermenters; surfactin confirmed; broth and foam-recovery streams reported separately. | Hu *et al*. (2021) |

## 3. Coupled interpretation of process evidence

The studies summarized in Section 2 report different combinations of feedstock conversion, formulation, reactor operation, oxygen-transfer and recovery measurements. In this synthesis, coupling denotes the need to interpret complementary evidence domains jointly for a defined engineering question; it does not imply that all interactions have been quantified within a common causal model.

### 3.1 Limits of standalone process indicators

The evaluated studies do not provide a basis for treating a single reported indicator as a transferable criterion for cross-system performance comparison. In the DGS system, the 3.4 g $L^{-1}$ surfactin concentration at 200 g $L^{-1}$ DGS was associated with the MT45:X82 co-culture and its hydrolytic function (Zhi *et al.*, 2017). Likewise, the OMW response depended on feedstock pretreatment (Moya Ramírez *et al.*, 2016). Feedstock loading should therefore be interpreted with the conversion strategy and operating context rather than as an independent descriptor or basis for direct titer ranking.

The oxygen-transfer studies show an analogous limitation. De Andrade *et al*. (2016) reported kLa = 102.02 $h^{-1}$ with DO at approximately 0 %, whereas Nazareth *et al*. (2021b) reported the highest volumetric productivity in their brewery-trub system at kLa = 45.41 $h^{-1}$. Martins *et al*. (2025) reported the best performance within a non-residue *B. subtilis* ATCC 21332 system at kLa = 21.88 $h^{-1}$. These heterogeneous observations should not be interpreted as an ordered optimum series. Because kLa determination and liquid-phase bases can differ among studies, the numerical values are used here only as context-dependent observations. Cross-study interpretation therefore requires explicit consideration of the determination method, liquid-phase basis, oxygen state, operating conditions and biological configuration.

### 3.2 Integration of feedstock and process evidence

The proposed interpretation integrates evidence at the level at which it was experimentally reported. Feedstock studies provide loading, hydrolysis and conversion information; formulation studies describe nutrient or supplementation interventions; reactor studies report operating conditions and oxygen-related measurements; and recovery studies document foam handling and the analytical stream from which product concentrations were obtained. Integrating these domains does not convert associations reported in separate experiments into demonstrated causal relationships.

This distinction is illustrated by the brewery-trub literature. Nazareth *et al*. (2021a) investigated medium formulation, whereas Nazareth *et al*. (2021b) examined agitation, aeration, oxygen-transfer variables and surfactin productivity in a related reactor system. Together they provide broader engineering coverage, but not a single experiment in which formulation and transport variables were quantified simultaneously.

The formulation results also depend on endpoint: nitrogen-source effects were reported for surface tension, whereas metal supplementation changed volumetric productivity. Directly reported measurements, within-system interpretation and cross-study synthesis are therefore treated as distinct evidence levels.

### 3.3 Engineering implications for scale-up

Reported product concentration alone is insufficient for scale-up-oriented comparison when biological configuration, reactor scale, operating conditions or analytical sample basis differ. Hu *et al*. (2021) reported both broth and foam-recovery measurements across screening and fermenter scales, which should not be treated as equivalent titers. Hiller *et al*. (2026) evaluated an oxygen-responsive engineered strain and a reference configuration under stepwise DO-setpoint reduction in 30 L stirred-tank cultivation, showing that oxygen-control strategy and strain configuration form part of the process basis required for interpretation. In a non-residue fed-batch system, Hiller *et al*. (2024) showed that growth-rate control through exponential feeding changed surfactin titers and yields, providing additional process context for why operating strategy must be considered before cross-study comparison.

The relevance of a reported variable depends on the engineering question. An oxygen-transfer comparison requires reactor and oxygen-related evidence, whereas a feedstock-conversion comparison may be supported by hydrolysis or substrate-utilization measurements even when kLa, DO or OUR are NR. Such absence limits the inference from the selected publication; it does not establish absence of the phenomenon.

## 4. Evidence-based cross-study assessment

The evidence summarized above indicates that cross-study engineering assessment requires an explicit question and a defined biological, product and analytical basis. The screening procedure does not assign intrinsic performance or robustness to a feedstock; it provides a structured basis for assessing whether reported information for a strain-feedstock-process configuration can support the stated inference.

### 4.1 Screening domains

The comparison basis comprises biological configuration, target product, product metric and analytical sample basis; four evidence domains are screened: feedstock conversion, formulation, reactor/oxygen transfer and foam/recovery. R denotes information reported in the publication and NR information not reported.

Comparability is question-specific: a basis is critical when changing its definition changes the quantity or process attribute being compared. Thus, direct titer comparison requires compatible product, metric and sample basis, while direct kLa comparison requires explicit consideration of the determination method, liquid-phase basis and reactor context.

Incompatibility is recorded rather than normalized or imputed. R/NR describe reporting coverage only, not quality, causality or transferability; R can represent either a quantitative measurement or an interpretation stated by the original authors. Here, evidence-supported means consistent with reported, question-compatible information, not formal evidence grading. The workflow is conceptual and has not been independently tested for inter-rater reproducibility or prospective decision performance.

### 4.2 Evidence-to-inference workflow

Figure 1 summarizes the evidence-to-inference workflow. After defining the engineering question and biological/product/analytical basis, relevant information is screened across the four domains. Reporting status alone does not establish comparability: compatibility in variables critical to the stated question is assessed before an evidence-supported inference is stated.

When reported information is insufficient, the workflow indicates additional information required for the stated comparison rather than assigning assumed values or numerical scores. Depending on the question, this may include reactor scale, DO, kLa, OUR, feedstock-conversion data, a harmonized product metric or a matched sample basis.

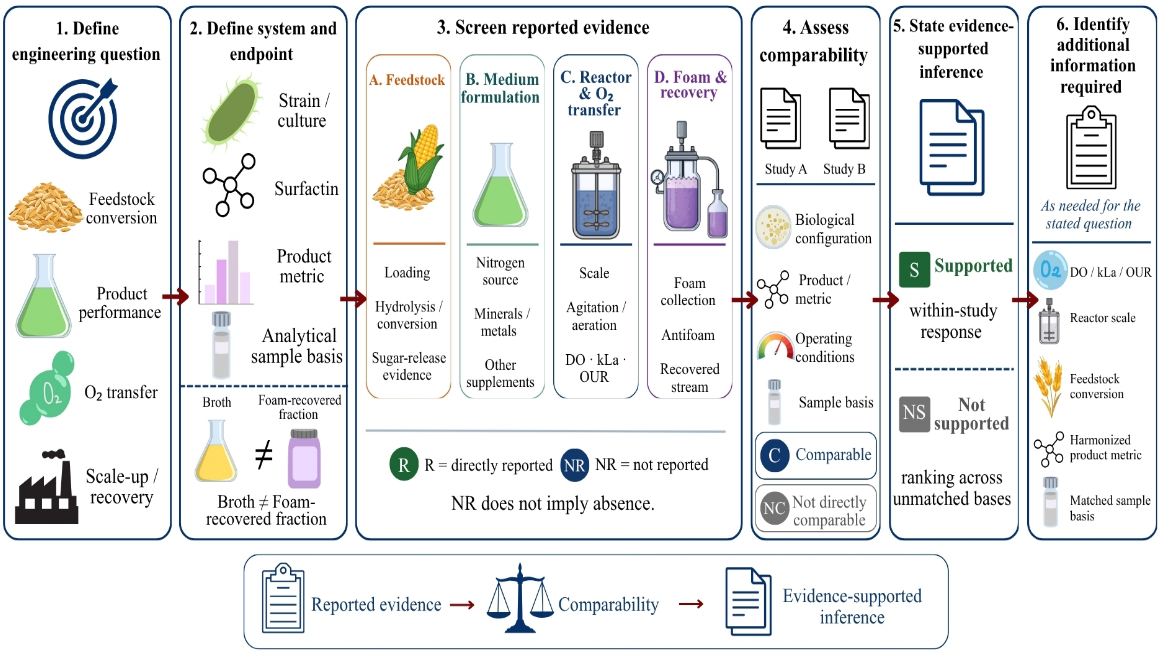


*Figure 1: Evidence-to-inference workflow for cross-study engineering assessment. R = reported; NR = not reported and does not imply absence; reporting alone does not establish comparability.*

### 4.3 Representative application to a scale-up question

As a practical application, the workflow was applied to whether brewery-trub and DGS systems provide sufficient reactor-level evidence for an oxygen-transfer-oriented scale-up comparison. The trub publications provide separate but complementary datasets: Nazareth *et al*. (2021a) evaluated medium formulation and reported bioreactor foam recovery, whereas Nazareth *et al*. (2021b) supplied the oxygen-transfer dataset, including agitation, aeration, kLa, DO, OUR and surfactin productivity.

Zhi *et al*. (2017) showed that the DGS response depended on feedstock conversion and the MT45:X82 co-culture. The reported 3.4 g $L^{-1}$ at 200 g $L^{-1}$ DGS supports interpretation within that configuration, but reactor-level kLa, DO and OUR are NR. Because the systems also differ biologically, titers are not ranked. For this scale-up question, trub provides reactor/oxygen-transfer evidence, whereas DGS provides direct feedstock-conversion evidence. Table 2 restricts R/NR coding to variables pertinent to this comparison.

*Table 2: Engineering evidence screening for scale-up-oriented assessment of selected residue-based surfactin systems*

| Feedstock / system | Biological / product basis | Feedstock / formulation evidence | Reactor / $O_2$ / recovery evidence | Evidence-supported inference / limitation | Reference |
|---|---|---|---|---|---|
| Brewery trub | *B. subtilis* ATCC 6051; surfactin | 2021a R: N-source and metal-supplementation effects. | 2021a R: bioreactor and foam recovery; kLa/OUR: NR. 2021b R: stirred tank, agitation, aeration, kLa, DO/OUR and foam collection. | Shared strain/feedstock context gives complementary coverage; formulation and oxygen-transfer variables come from separate datasets. | Nazareth *et al*. (2021a); Nazareth *et al*. (2021b) |
| Fish-derived peptone | *B. subtilis* ATCC 21332; surfactin analytically confirmed | R: fish-derived peptone; concentration investigated. | R: 20 mL screening, 7/100 L fermenters; 100 L DO setpoint; foam/recovery. kLa/OUR: NR. | Multi-scale operation and DO-control information reported; direct kLa/OUR comparison remains limited; broth and foam-recovery titers are not equivalent. | Hu *et al*. (2021) |
| DGS | *B. amyloliquefaciens* MT45/X82; surfactin | R: hydrolytic co-culture, sugar-supply response, DGS loading. | Reactor-scale kLa, DO and OUR: NR. | Feedstock conversion interpretable; $O_2$ scale-up comparison and cross-configuration titer ranking unsupported. | Zhi *et al*. (2017) |
| Cassava wastewater | *B. subtilis LB5A;* surfactin | R: cassava wastewater substrate; conversion metrics NR. | R: 3 L; $k_La$ and DO monitored; foam recovery and ultrafiltration. | System-specific $O_2$ state interpretable; $k_La$ is not a transferable oxygen-sufficiency threshold. | de Andrade *et al*. (2016) |

Across the four representative systems, evidence coverage permits different inferences. Fish-derived peptone provides multi-scale operation, a 100 L DO setpoint and sample-stream information, but not the kLa/OUR dataset reported for brewery trub; cassava wastewater provides paired kLa/DO evidence at 3 L but no multi-scale comparison.

These are not deficiencies of the original studies; they define which cross-study questions can be answered. The workflow therefore states what can be inferred, what cannot be compared directly and which additional measurements would strengthen a specific comparison.

## 5. Conclusions

The residue-based Bacillus surfactin studies provide uneven coverage of feedstock conversion, formulation, reactor operation, oxygen transfer and recovery. Complementary reactor-context studies further show that oxygen-transfer indicators require interpretation within their measurement and operating bases. Across the evaluated evidence, markedly different kLa-performance states occur under distinct biological, operational and analytical configurations; these observations do not provide a basis for a transferable kLa threshold or direct performance ranking across heterogeneous systems.

The evidence-to-inference workflow defines the engineering question and analytical basis before screening reported information for comparability. It provides a structured basis for question-compatible inference and identification of information required for a specific comparison without robustness scoring. The workflow evaluates reporting coverage and question-specific comparability, not methodological quality or formal evidence strength. Future comparisons should report biological configuration and sample basis explicitly while harmonizing product endpoints, feedstock-conversion metrics and reactor/oxygen-transfer reporting where feasible. Extension to other Bacillus lipopeptide processes requires product-specific evaluation.

## Nomenclature

DGS – distillers' grains
DO – dissolved oxygen
kLa – volumetric oxygen-transfer coefficient, $h^{-1}$
NR – not reported
OMW – olive mill waste
OUR – oxygen uptake rate
R – directly reported

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